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Real-world data and real-world evidence in lung cancer, 2nd edition

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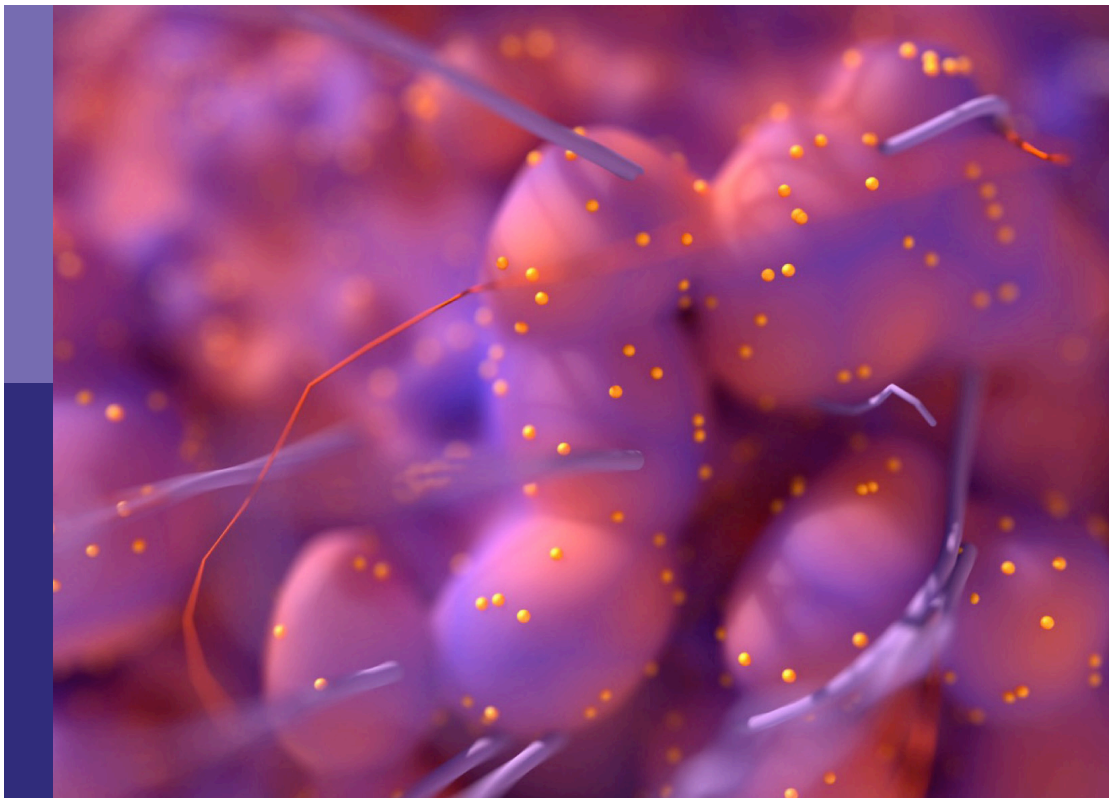

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Real-world data and real-world evidence in lung cancer, 2nd edition

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Editorial: Real-world data and real-world evidence in lung cancer

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Editorial on the Research Topic
Real-world data and real-world evidence in lung cancer

1 Introduction

Lung cancer remains the leading cause of cancer-related death worldwide in both sexes (1). This editorial compels attention to the critical need for real-world data (RWD) and real-world evidence (RWE) to augment lung cancer research. Traditional clinical trials, while essential, represent a highly controlled environment that may not fully translate to the complexities of everyday patient care (2, 3). RWD, in contrast, gathers information directly from real-world clinical needs and settings (4, 5). This unfiltered approach offers invaluable insights into how lung cancer treatments function in a broader patient population, encompassing factors like underlying health conditions and variations in treatment adherence. The editorial argues that RWE can illuminate crucial knowledge gaps, particularly for patient subgroups often excluded from traditional trials due to comorbidities or other factors (3). By incorporating this rich tapestry of real-life data, researchers and clinicians can develop more effective and comprehensive treatment strategies for the fight against one of the most lethal cancers.

2 Composition

The development of prediction models in the clinic to forecast long-term survival in early NSCLC is warranted, mostly considering the upcoming implementation of perioperative and adjuvant chemo-immunotherapy in such a highly heterogeneous disease. In a cohort study including 505 patients diagnosed with stage I-II NSCLC at a tertiary Spanish hospital, Torrente et al. developed a useful prognostic model based on easy-to-obtain clinical risk factors, identifying high- and low-risk patients to tailor adjuvant treatment while eventually adapting surveillance plans and avoiding unnecessary tests or visits. Namely, in patients with T2aN0 stage IB lung adenocarcinoma, adjuvant chemotherapy remains controversial. Lee et al. retrospectively observed an improved

overall and cancer-specific survival only in tumors larger than 3 cm whereas no benefit was seen in smaller tumors even when harboring visceral pleural invasion. In a further analysis by Davey et al., the predictive value of peritumor density and dose variability on local relapse (LR) and regional failure (RF) following stereotactic ablative radiotherapy (SABR/SBRT) of NSCLC were assessed. An internal cohort of 199 patients and an external cohort of 76 patients for validation were analyzed. High peritumor density combined with high dose variability predicted LR but not RF. External validation confirmed the importance of this interaction. These findings suggest the potential use of this model to modify low-dose clinical target volume (CTV) margins for high-risk patients undergoing lung SABR.

Cai et al. examined the prognosis of *p-stage* T3 NSCLC with additional tumor nodules in the same lobe (T3-Add). By interrogating the SEER database and employing propensity score matching (PSM) to account for bias, their results indicated that *p-stage* T3-Add had improved survival vs. other T3 patients and similar survival to T2b patients. While the study suggests reconsideration of the T-category for T3 patients based on additional nodules in the same lobe, previous analyses by the International Association for the Study of Lung Cancer (IASLC) showed a trend toward longer OS in the T3-Add vs. T3 group but the result was not statistically significant (6) and the forthcoming proposal for the ninth edition of the TNM classification for lung cancer maintains the status quo for T3 tumors (7, 8). A further SEER analysis by Hao and Li investigated the metastatic patterns and prognosis of various subtypes of lung cancer: the liver was the most common site of metastasis for SCLC, while BMs were predominant in large cell carcinoma. Squamous cell carcinoma and adenocarcinoma showed a higher likelihood of bone metastasis. In addition, nomograms were developed to predict metastasis and survival probabilities, showing good performance in predicting distant metastasis and overall survival.

Van Dao et al. described the clinical insights into the treatment patterns in stage III non-small cell lung cancer (NSCLC) in the Vietnamese population within the KINDLE-Vietnam cohort study, claiming the firm need for guideline adoption, physician education, and multidisciplinary team in the real-world management of locally advanced NSCLC.

Historically, the 2-year incidence of BMs in stage III locally advanced (LA-)NSCLC has been estimated at 30%. However, recent clinical trials, such as PACIFIC (9) have shown a lower incidence. Although prophylactic cranial irradiation (PCI) decreased the incidence of BMs compared to observation in randomized trials, it did not translate into an overall survival benefit (10, 11). Alhusaini et al. retrospectively analyzed the incidence of BMs in their single-center cohort of 160 stage III NSCLC patients in the contemporary era of imaging. Among them, 23/160 patients (14.4%) underwent MRI surveillance after completing primary treatment while 137/160 patients (85.6%) received brain MRIs at systemic recurrence (restaging) or when neurologically symptomatic. The 2-year cumulative incidence of BMs was 17%, with a higher incidence of BMs observed in patients with adenocarcinoma and those undergoing MRI surveillance.

Velcheti et al. investigated the long-term effectiveness of single-agent pembrolizumab in patients with metastatic NSCLC, PD-L1 expression ≥50%, and good performance status (ECOG PS 0–1), confirming the consistency of RWD outcomes within real-life patients compared to those observed in controlled clinical trials (12). However, ECOG PS 2 has emerged as an independent prognostic factor with a lack of data from randomized phase III trials on the safety and efficacy of immunotherapy in this common and frail real-life setting. Yang et al. retrospectively compared the effectiveness and safety of first-line pembrolizumab vs. sintilimab, a PD-1 inhibitor approved in China, in combination with chemotherapy. The authors retrospectively analyzed data from a Chinese cohort of 164 patients with advanced squamous cell lung cancer treated between 2018 and 2022. The study demonstrated the equipoise of both regimens vis-à-vis effectiveness and toxicity in their patient population.

Liu et al. examined predictive factors and prognosis of immune checkpoint inhibitor-related pneumonitis (CIP) in advanced NSCLC. Logistic regression analysis was employed to evaluate risk factors associated with CIP. In total, 41/222 (18.5%) developed CIP and the study revealed that lower baseline hemoglobin and albumin levels were independent predictors of CIP. Furthermore, the onset of CIP was a prognosticator of overall survival in their patient cohort.

In regards to EGFR-mutant NSCLC, in the adjuvant setting, Liu et al. found out that adding chemotherapy before first-generation EGFR tyrosine kinase inhibitors (TKIs) in a Chinese cohort of stage II-IIIA patients did not improve survival compared with adjuvant EGFR-TKI alone, somewhat mirroring the recent survival data of the third-generation EGFR-TKI osimertinib, namely within the patient cohort that did not receive chemotherapy in the ADAURA trial (13) and possibly suggesting such a chemotherapy-free approach in selected low-risk patients. Moreover, in the EGFR-positive metastatic scenario, Kang et al. provided encouraging effectiveness and safety RWD in favor of the second-generation EGFR-TKI afatinib in the first-line setting of NSCLC patients with brain metastases (BMs), most importantly even in those harboring uncommon EGFR mutations. Likewise, novel prognostic models are needed in the real-world clinic to predict survival in difficult-to-treat settings such as EGFR-positive NSCLC with BMs undergoing targeted therapies (Zhu et al.).

The transformation to SCLC is a known mechanism of resistance against molecularly targeted therapies. *De novo* transformation occurs rarely, and most cases involve transformation from EGFR-mutant adenocarcinomas (14). Ding et al. investigated the effectiveness of etoposide/platinum (EP) and anlotinib plus anlotinib maintenance therapy in 10 patients with transformed SCLC from EGFR TKI-resistant lung adenocarcinomas recruited from 3 Chinese regional hospitals. This combination showed encouraging effectiveness and a low toxicity profile warranting further investigation. Chang et al. aimed to identify factors associated with outcomes after progression on first-line EGFR-TKI in advanced EGFR-mutant NSCLC patients. In total, 206/242 patients progressing on first- or second-generation TKIs and receiving second-line treatment were assessed. Second-line treatment with osimertinib was associated with longer overall survival (OS) compared to chemotherapy and other EGFR-TKIs. These findings align with the latest recommendations from the American Society of Clinical Oncology (ASCO) for patients with EGFR alterations, specifically Exon 19 deletion and L858R mutations (15).

Gow et al. retrospectively tested a large clinical cohort of NSCLC patients with no EGFR or ALK alterations using reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) to detect METex14 mutations. RWD confirmed the presence of such an aggressive oncogene addiction in elderly individuals, never-smokers, with poor performance status and a higher frequency in sarcomatoid carcinoma while showing pemetrexed-based chemotherapy, strong IHC staining, BM, and lung radiotherapy being independent prognostic factors for survival in these patients. Moreover, RWE seemed to confirm the shorter median survival rates of smokers compared to non-smokers among ALK-positive patients, further suggesting the role of predictive testing irrespective of smoking status and age, as reflected by Zheng et al.

Another relevant aspect of this Research Topic addressed the real-life diagnostic setting with the seminal use of cerebrospinal fluid (CSF) as a liquid biopsy tool to complement the genomic profiling of plasma circulating tumor DNA (ctDNA) in a large cohort of NSCLC patients with brain metastases. In such a dismal setting, Shen et al. detected a higher diagnostic accuracy using whole genome sequencing on CSF supernatant compared to plasma, including all genomic alterations, especially the troublesome copy number variations.

Zhou et al. developed a prediction model using serum folate receptor-positive circulating tumor cells ($FR^{+}CTC$) and various other blood biomarkers including tumor markers to non-invasively aid in the preoperative diagnosis of benign vs. malignant solitary pulmonary nodules (SPNs). Age, FR+CTC, thymidine kinase 1 (TK1), and neuron-specific enolase (NSE) were independently associated with malignant SPNs on multivariable analysis. They developed a predictive model incorporating these factors, achieving a sensitivity of 71.1%, specificity of 81.3%, and an area under the curve (AUC) of 0.826, demonstrating a superior performance than any single biomarker which could aid in predicting SPN malignancy.

Further, RWE is required for testing the clinical application of biosimilar drugs to reference originator products. In this vein, Zhao et al. retrospectively confirmed the effectiveness and safety of biosimilar bevacizumab in 946 Chinese patients with locally advanced or metastatic NSCLC with no new safety concerns.

Wang et al. addressed clinical and prognostic features of a rare form of adenocarcinoma, namely pulmonary enteric adenocarcinoma (PEAC) in their cohort of 26 patients recruited between 2014 and 2021. In addition, the authors interrogated the Surveillance, Epidemiology, and End Results (SEER) database which identified 20 patients. Treatment was in line with the management of lung cancer in general across all stages and prognosis was unsurprisingly determined by disease stage.

Robinson et al. addressed the issue of random missing data from a large population-based dataset of NSCLC patients in Ontario, Canada. Characteristics and outcomes of staged vs. unstaged patients were compared. In total, 51,152 patients were analyzed with 5,707 (11.2%) patients unstaged, with evidence that stage data was not missing completely at random. Unstaged patients were more likely to be older, have a higher comorbidity index, and have lower socioeconomic status. In addition, survival analysis suggested that unstaged patients had a proportion of early- and advanced-stage disease with a significant proportion likely being stage IV experiencing rapid death. The study highlights the potential bias in the evaluation of healthcare utilization and outcomes for staged patients, as unstaged patients may represent a distinct subset with different characteristics and prognoses.

3 Conclusions and perspectives

The landscape of lung cancer research is evolving rapidly, with a growing recognition of the crucial role that real-life data and evidence play in enhancing our understanding and management of this devastating disease. Traditional clinical trials, while invaluable, offer controlled environments that may not fully mirror the complexities of real-world patient care. By contrast, RWD directly captures insights from everyday clinical practice, providing a more comprehensive understanding of how treatments perform across diverse patient populations and clinical settings.

The editorial's emphasis on the significance of RWE in augmenting lung cancer research is underscored by various studies presented. From investigating treatment effectiveness in metastatic NSCLC to exploring the clinical insights into treatment patterns in different populations, the evidence consistently highlights the value of RWE in filling crucial knowledge gaps and informing more effective treatment strategies.

Moreover, the editorial and associated studies shed light on several key areas of research and clinical practice, including the development of prediction models for long-term survival, the exploration of treatment approaches in specific patient subgroups, and the identification of factors associated with outcomes and prognosis.

As we move forward, it is imperative to continue integrating RWD and RWE into lung cancer research and clinical practice. This approach not only enhances our ability to tailor treatments to individual patients but also enables us to address disparities in care, optimize treatment strategies, and ultimately improve outcomes for patients battling this formidable disease. By embracing RWE, we can take significant strides toward advancing the fight against lung cancer and reducing its devastating impact on individuals and communities worldwide, often not included in randomized controlled trials.

Author contributions

VG: Writing – original draft, Writing – review & editing. CE: Writing – review & 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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ORIGINAL RESEARCH
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Long-Term Real-World Outcomes of First-Line Pembrolizumab Monotherapy for Metastatic Non-Small Cell Lung Cancer With ≥50% Expression of Programmed Cell Death-Ligand 1

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Objectives: Immune checkpoint inhibitors (ICIs) of programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1) have been rapidly adopted in US clinical practice for first-line therapy of metastatic non-small cell lung cancer (NSCLC) since regulatory approval in October 2016, and a better understanding is needed of long-term outcomes of ICI therapy administered in real-world settings outside of clinical trials. Our aim was to describe long-term outcomes of first-line pembrolizumab monotherapy at US oncology practices for patients with metastatic NSCLC, PD-L1 expression ≥50%, and good performance status.

Methods: This retrospective two-cohort study used technology-enabled abstraction of deidentified electronic health records (EHR cohort) plus enhanced manual chart review (spotlight cohort) to study adult patients with stage IV NSCLC, PD-L1 expression ≥50%, no documented *EGFR/ALK/ROS1* genomic aberration, and ECOG performance status 0–1 who initiated first-line pembrolizumab monotherapy from 1-November-2016 to 31-March-2020 (EHR cohort, with data cutoff 31-March-2021) or from 1-December-2016 to 30-November-2017 (spotlight cohort, with data cutoff 31-August-2020). Kaplan-Meier analysis was used to determine overall survival (OS; both cohorts) and, for the spotlight cohort, real-world progression-free survival (rwPFS) and real-world tumor response (rwTR).

Results: The EHR cohort included 566 patients (298 [53%] men); the spotlight cohort included 228 (105 [46%] men); median age in both cohorts was 71. Median follow-up from pembrolizumab initiation to data cutoff was 35.1 months (range, 12.0–52.7) and 38.4 months (range, 33.1–44.9) in EHR and spotlight cohorts, respectively. Median OS was 19.6 months (95% CI, 16.6–24.3) and 21.1 months (95% CI, 16.2–28.9), respectively; 3-year OS rates were 36.2% and 38.2% in EHR and spotlight cohorts, respectively. In the

spotlight cohort, median rwPFS was 7.3 months (95% CI, 5.7–9.2); 88 patients (38.6%; 95% CI, 32.2–45.2) experienced rwTR of complete or partial response. For 151/228 patients (66%) who discontinued pembrolizumab, the most common reasons were disease progression (70 [46%]) and therapy-related adverse effects (35 [23%]).

Conclusions: Real-world outcomes remain consistent with outcomes observed in clinical trials, supporting long-term benefits of first-line pembrolizumab monotherapy for patients with metastatic NSCLC, PD-L1 expression ≥50%, and good performance status.

Keywords: non-small cell lung cancer (NSCLC), manual chart review, observational study, pembrolizumab, real-world progression-free survival (rwPFS), overall survival (OS), tumor response assessment

1 INTRODUCTION

Pembrolizumab was the first immune checkpoint inhibitor (ICI) of programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1) approved in the United States (US) as a first-line treatment for metastatic non-small cell lung cancer (NSCLC). Supported by findings from the phase 3 randomized controlled trial, KEYNOTE-024 (1), this approval, in October 2016, was for pembrolizumab monotherapy in metastatic NSCLC with no *EGFR* or *ALK* genomic alterations and PD-L1 tumor proportion score (TPS) ≥50%, determined using a companion diagnostic test.

With 5 years of follow-up available for KEYNOTE-024, median overall survival (OS) was 26.3 months (95% CI, 18.3–40.4), and the 5-year survival rate was 32% in the pembrolizumab arm for enrolled patients with metastatic NSCLC (PD-L1 TPS ≥50%) and no sensitizing *EGFR* or *ALK* alterations (2). Moreover, with 4 years of follow-up in recent subgroup analyses of KEYNOTE-042, the median OS was 20.0 months (95% CI, 15.9–24.2), and the 3-year survival rate was 31%, in the PD-L1 TPS ≥50% subgroup of the pembrolizumab arm (3). In another trial, KEYNOTE-598, enrolling a similar patient population (metastatic NSCLC, PD-L1 TPS ≥50%, no *EGFR*/*ALK* alterations), patients who received first-line pembrolizumab monotherapy experienced median OS of 21.9 months (4).

Information from real-world settings is important to understand whether these long-term clinical trial findings apply to the wider population of patients who may not have been eligible or considered for trial participation (eg, because of comorbidities) and who are treated outside the controlled setting of these trials (5, 6). Cohort studies conducted before ICI approvals indicate that patients with metastatic NSCLC who received first-line therapy (most commonly platinum-based chemotherapy regimens) experienced a median OS of 10–11 months (7, 8). In one study, median OS was 9.7 months (95% CI, 9.1–10.3) for patients initiating first-line therapy in 2012–2015, thus before ICI approvals in the US, for stage IV NSCLC with no documented *EGFR* or *ALK* genomic alterations, not restricted to those with good performance status (8).

Since 2016, ICIs have been rapidly adopted in oncology practice as first-line therapies for advanced and metastatic NSCLC (9–11). In a recent retrospective database study of outcomes at US community oncology practices of 7746 patients initiating first-line therapy (March 2015–August 2018) for advanced NSCLC with no documented *EGFR*/*ALK* genomic alterations, 907 patients who initiated first-line ICI monotherapy experienced median OS of 19.9 months (95% CI, 16.6–24.1), superior to median OS with other recorded therapies (9). This cohort included 22% of patients with Eastern Cooperative Oncology Group (ECOG) performance status of 2 or greater (9).

In a prior study, we investigated outcomes for two cohorts of patients clinically similar to those in KEYNOTE-024 who received first-line pembrolizumab monotherapy in real-world US community oncology settings during the first 2 years after approval (2, 12). We observed that patients with good performance status who were prescribed first-line pembrolizumab monotherapy for metastatic NSCLC with PD-L1 expression ≥50% and no known *EGFR*, *ALK*, or *ROS1* alterations experienced clinical outcomes similar to those in the KEYNOTE trials, including OS, real-world progression-free survival (rwPFS), and real-world tumor response rate (rwTRR) for the patient cohort with median follow-up of 15.5 months (12). The objective of the present retrospective two-cohort study was to update those findings with longer follow-up using the same data source derived from electronic health records (EHRs) of patients at US oncology practices.

2 METHODS

2.1 Data Source and Patients

The Flatiron Health database includes deidentified, longitudinal data from EHRs of patients with cancer at ~280 cancer clinics in the US, including approximately 800 sites of care (13). This nationally representative database is used frequently for cohort studies of advanced NSCLC and has previously been described in detail (8, 11, 14, 15). In brief, patient-level structured data, such as diagnosis and clinical visits, and unstructured data, such as physicians' notes, are derived using technology-enabled data processing overseen by trained clinical abstractors. Our study also employed enhanced manual chart review to derive clinical outcomes data for the 'spotlight cohort,' described below.

Ethical approval of the study protocol was obtained from the Copernicus Group Institutional Review Board before study conduct, including a waiver of informed consent for working with deidentified data, and safeguards were in place to maintain

data deidentification. Flatiron Health, Inc. did not participate in the analysis of the data.

Patients included in this study were drawn from the advanced NSCLC EHR database, which requires at least two clinical visits recorded on or after 1 January 2011, and pathologically confirmed evidence of advanced NSCLC on or after 1 January 2011. We selected two analytic cohorts of patients ≥18 years old with ECOG performance status of 0–1 who received first-line pembrolizumab monotherapy for stage IV NSCLC: (1.) The 'EHR cohort' included patients who initiated first-line pembrolizumab monotherapy from 1 November 2016 through 31 March 2020 (index period); data cutoff was on 31 March 2021, thus ensuring at least 1 year of potential follow-up time. (2.) The spotlight cohort included patients randomly selected from patients who initiated first-line pembrolizumab monotherapy from 1 December 2016 through 30 November 2017; data cutoff was 31 August 2020, thus ensuring almost 3 years of potential follow-up time. Other selection criteria for both cohorts were similar, including diagnosis of stage IV or recurrent metastatic NSCLC, tumor PD-L1 expression ≥50%, no known driver alterations (*EGFR*, *ALK*, or *ROS1*) and, for nonsquamous tumors, documented negative test results for both *EGFR* and *ALK* genomic alterations.

We excluded patients without structured database activity within 90 days of the metastatic diagnosis date (i.e., those possibly not receiving active clinical care), as well as patients with a record of clinical trial drug administered after the advanced NSCLC diagnosis. While some patients were likely included in both study cohorts, the rules protecting against patient reidentification prevented us from determining how many and who they were.

2.2 Study Variables

For each cohort, we summarized patient demographic characteristics, in addition to clinical characteristics available in the datasets, including smoking history, NSCLC histology, Charlson comorbidity index (CCI) score (derived from listed comorbidities (16)), and genomic testing results for *EGFR*, *ALK*, *ROS1*, *KRAS*, and *BRAF*. Lines of therapy were identified using Flatiron Health oncologist-defined business rules, with mapping of medication administrations and medication orders to lines of therapy (13).

Clinical outcomes determined for both cohorts included length of follow-up time and OS, the latter determined using death information according to the validated Flatiron Health composite mortality endpoint (17, 18). In addition, we summarized subsequent lines of therapy according to systemic anticancer regimen category.

For the spotlight cohort, manual chart abstraction was used to identify real-world progression (rwP) and real-world tumor response (rwTR) in order to determine rwPFS and rwTRR, respectively. The first episode in which the treating clinician concluded that there had been growth or worsening of NSCLC was identified as rwP, excluding events within 14 days of pembrolizumab initiation and including suspected pseudoprogression, which was defined as an increase in tumor size that the clinician recorded as possibly being an effect of ICI therapy (12, 19). Instead, rwTR was based on changes in NSCLC tumor burden indicated by radiology reports, excluding events within 30 days of pembrolizumab initiation, and mapped as complete response (CR), partial response (PR), stable disease, progressive disease (PD), and other (pseudoprogression, indeterminate, not documented) (12, 20). Both of these endpoints have been recently described and characterized using Flatiron Health advanced NSCLC datasets (12, 19, 20). For the present study, the rwTRR determination was defined as the proportion of patients with at least one CR or PR assessment followed by a subsequent assessment of CR, PR, or stable disease during first-line pembrolizumab monotherapy.

Manual chart abstraction was also used to determine the reasons for pembrolizumab discontinuation when available. Pembrolizumab was considered discontinued when clinical notes explicitly stated discontinuation or with a gap >60 days in pembrolizumab administration. Although adverse events were not actively solicited in this study, we collected any reports of individual adverse events identified during manual chart review, and all were reported as related to pembrolizumab.

2.3 Statistical Analyses

We used summary statistics to describe baseline demographic and clinical characteristics and subsequent lines of therapy for each cohort, in addition to rwTR categories for patients in the spotlight cohort.

The Kaplan-Meier method was used to estimate time-to-event analyses, including OS and rwPFS, beginning from pembrolizumab initiation. We determined OS as (date of death - start date of pembrolizumab) + 1 day, setting the date of death to the 15th of the month because only month and year of death were provided to maintain patient anonymity. Patients with no recorded date of death were censored at the end of the dataset or at their last recorded activity in the dataset, whichever occurred first. Similarly, rwPFS was determined from the start of pembrolizumab to first recorded rwP; patients with no rwP were censored at their last recorded activity or at the initiation of a new line of therapy. In a sensitivity analysis, we excluded suspected pseudoprogression as an event.

The Kaplan-Meier method was also used to estimate median real-world time on treatment (rwToT) for second- and third-line ICI therapy, as previously described (21). In brief, rwToT was calculated as the (date of the last ICI dose - date of first ICI dose) + 1 day and defined as the length of time between first and last administration dates before discontinuation. Patients who had a record of initiating the next line of therapy, or who died while receiving the ICI therapy, were considered discontinued at their last administration date. If none of these events were identified, then having a gap of ≥120 days between the last administration date and last known activity date in the dataset was considered discontinued at the last administration date. If none of the discontinuation criteria were met, patients were considered censored at their last administration date.

A formal calculation of sample size and power was not performed because of the descriptive nature of the study. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC).

3 RESULTS

3.1 Patients

A total of 566 patients who initiated first-line pembrolizumab monotherapy from November 2016 through March 2020 were included in the EHR cohort, and 228 patients initiating first-line pembrolizumab monotherapy from December 2016 through November 2017 were included in the spotlight cohort. The details of patient selection are depicted in **Figures 1** and **2**.

In the EHR cohort slightly over half of patients were men (53%), while in the spotlight cohort 46% were men (**Table 1**). The median age in both cohorts was 71 years, with approximately one-third of patients aged 75 years or older. Most patients in each cohort were White (76–79%), and >90% were current or former smokers. The two cohorts included similar proportions of patients by NSCLC histology, including approximately two-thirds with nonsquamous and one-quarter with squamous histology (**Table 1**). Of the nonsquamous tumors, 26% and 24% in EHR and spotlight cohorts were *KRAS* positive, respectively, and 7% and 6%, respectively, were *BRAF* mutant (**Table 1**).

The geographical distribution of oncology clinics was similar in the two cohorts, and all but 8 and 5 patients in EHR and spotlight cohorts, respectively, were seen at community (rather than academic) oncology clinics (**Table 1**).

3.2 Clinical Outcomes: EHR and Spotlight Cohorts

Median observed follow-up ending at data cutoff in EHR and spotlight cohorts was 35.1 and 38.4 months respectively, while median patient follow-up was 16.5 and 25.7 months, respectively

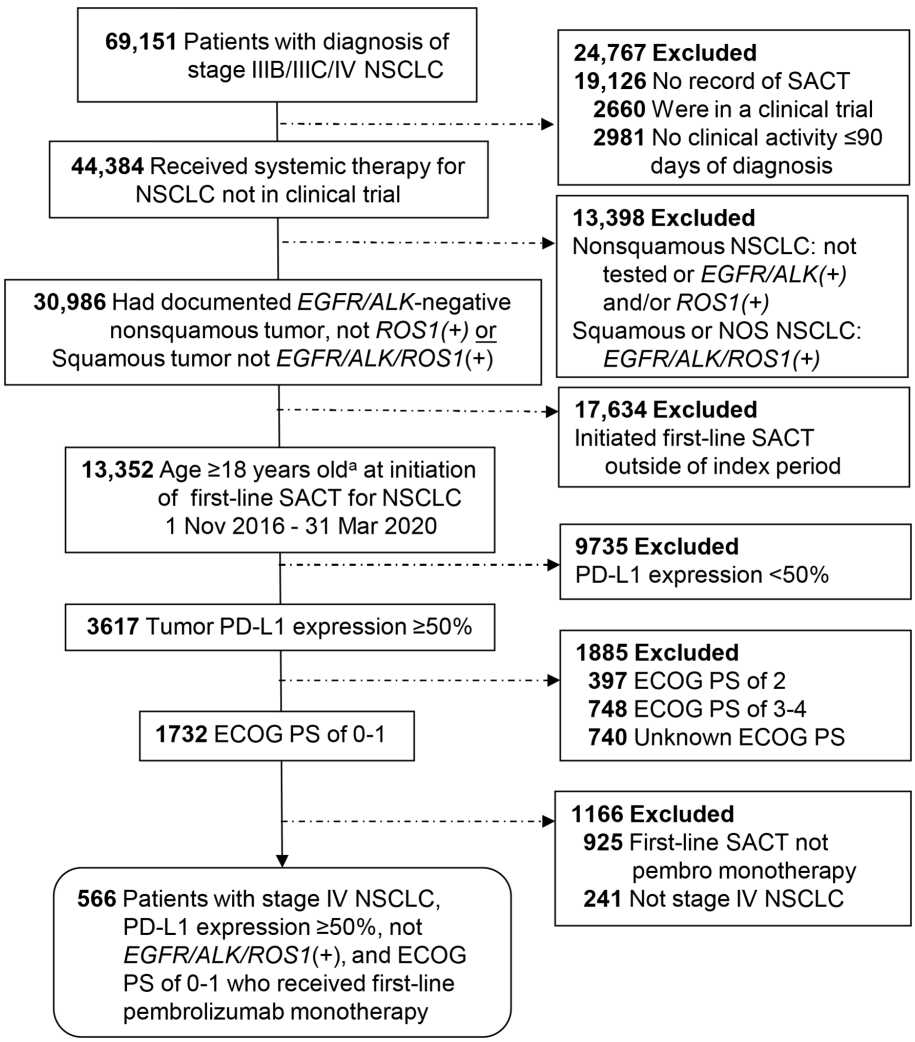

FIGURE 1 | Patient selection for the EHR cohort from the Flatiron Health Database. [a]No patients were excluded for being <18 years of age. ECOG PS, Eastern Cooperative Oncology Group performance status; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death-ligand 1; SACT, systemic anticancer therapy.

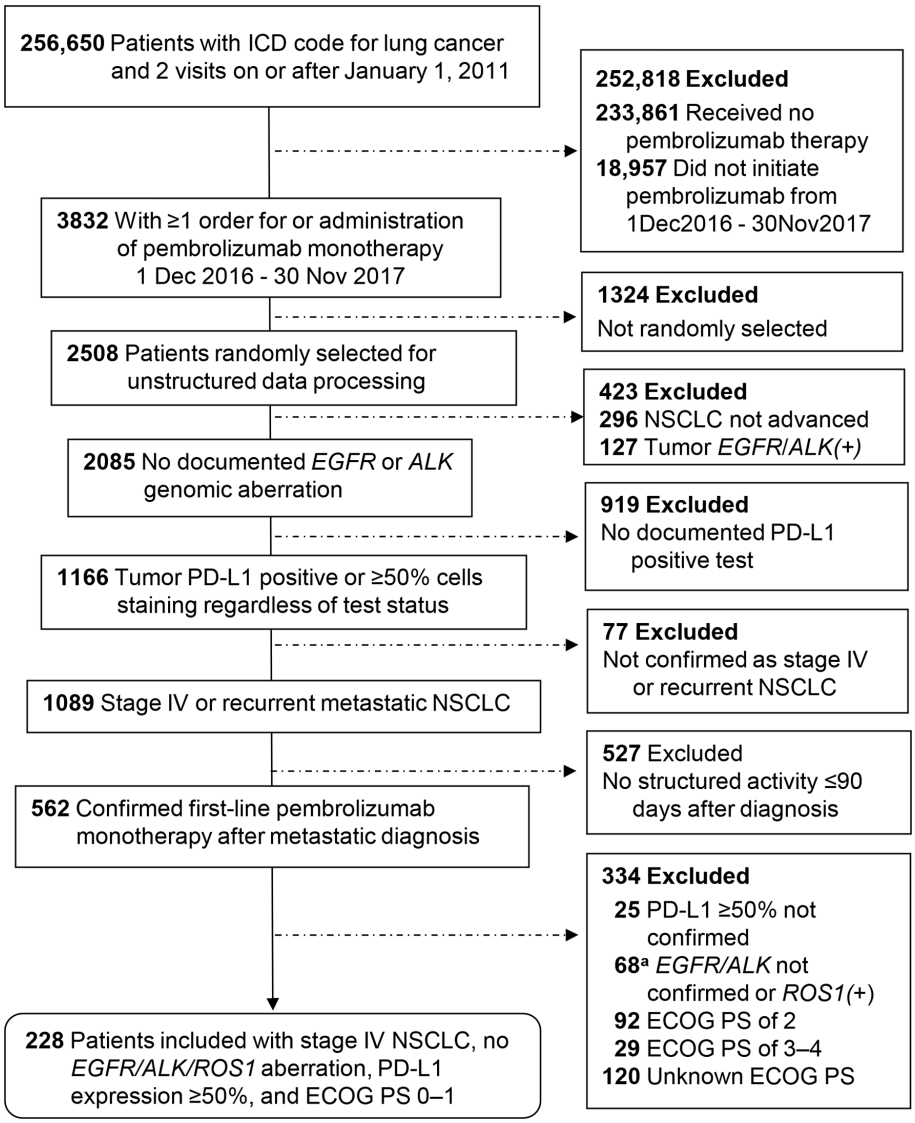

FIGURE 2 | Patient selection for the spotlight cohort from the Flatiron Health Database. [a]Exclusion for lacking confirmation of negative test status for *EGFR/ALK* genomic aberration applied only to nonsquamous tumors. ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed cell death-ligand 1.

(**Table 2**). Patients in the EHR cohort received a median of 9 cycles of pembrolizumab (range, 1–73), while those in the spotlight cohort received a median of 11 cycles of pembrolizumab (range, 1–61).

At data cutoff, 322 patients (57%) had died in the EHR cohort, and 134 patients (59%) had died in the spotlight cohort. Median OS was 19.6 months (95% CI, 16.6–24.3) and 21.1 months (95% CI, 16.2–28.9) in EHR and spotlight cohorts, respectively; Kaplan-Meier estimates of OS at 3 years were 36.2% and 38.2%, respectively (**Table 2**; **Figure 3**).

Second-line systemic therapy, most commonly platinum-based chemotherapy or immunotherapy (ICI monotherapy or ICI-chemotherapy combination), was administered to 182 patients (32%) in the EHR cohort, of whom 63 (35%) received third-line and 16/63 (25%) received fourth-line therapy (details in **Supplementary Table 1**). Immunotherapy was administered in second line to 72/182 patients (40%), including 16 patients (22%) who received pembrolizumab monotherapy, to 19 patients (30%) in third line (4 pembrolizumab monotherapy), to 3 patients (19%) in fourth line, and to 3 (50%) in fifth line.

In the spotlight cohort, 87 patients (38%) received second-line therapy, of whom 26 (30%) also received third-line therapy; only 7 and 3 patients received fourth- and fifth-line therapy, respectively (**Supplementary Table 2**). The pattern of second-line therapies was similar to that for the EHR cohort, with the most common being platinum-based chemotherapy and ICIs,

TABLE 1 | Baseline demographic and clinical characteristics of patients with stage IV NSCLC, PD-L1 expression ≥50%.

Variable	EHR cohort (n = 566)	Spotlight cohort (n = 228)
Male sex	298 (52.7)	105 (46.1)
Age, median (range), y	71 (38-84)	71 (46-82)
<75 years	359 (63.4)	138 (60.5)
≥75 years	207 (36.6)	90 (39.5)
Race data available[a]	505 (89.2)	209 (91.7)
White	383 (75.8)	165 (78.9)
Black	49 (9.7)	18 (8.6)
Asian	17 (3.4)	6 (2.9)
Other race	56 (11.1)	20 (9.6)
Current/former smoker	524 (92.6)	209 (91.7)
No smoking history	42 (7.4)	19 (8.3)
CCI score, mean (SD)	5.1 (3.1)	3.1 (3.2)
Median (range)	4 (0-13)	2 (0-10)
NSCLC histology		
Nonsquamous	405 (71.6)	156 (68.4)
NSCLC histology NOS	28 (4.9)	12 (5.3)
Squamous	133 (23.5)	60 (26.3)
ECOG performance status		
0	201 (35.5)	95 (41.7)
1	365 (64.5)	133 (58.3)
Record of brain metastases[b]	69 (12.2)	17 (7.5)
US CB region, data available[a]	552 (97.5)	219 (96.1)
Midwest	127 (23.0)	51 (23.3)
Northeast	105 (19.0)	58 (26.5)
South	249 (45.1)	84 (38.4)
West	71 (12.9)	26 (11.9)
Community oncology clinic	558 (98.6)	223 (97.8)
Academic oncology clinic	8 (1.4)	5 (2.2)
Index year		
2016	14 (2.5)	7 (3.1)
2017	210 (37.1)	221 (96.9)
2018	159 (28.1)	0
2019	142 (25.1)	0
2020	41 (7.2)	0
BRAF mutation status (nonsquamous only), N	*405*	*156*
Positive[c]	27 (6.7)	9 (5.8)
Wild type	249 (61.5)	68 (43.6)
Indeterminate, unknown, pending, untested	129 (31.9)	79 (50.6)
KRAS mutation status (nonsquamous only), N	*405*	*156*
Positive[c]	107 (26.4)	37 (23.7)
Wild type	125 (30.9)	38 (24.4)
Indeterminate, unknown, pending, untested	173 (42.7)	81 (51.9)
IHC clone, data available[d]	514 (90.8)	201 (88.2)
22C3c	474 (92.2)	187 (93.0)
SP263	17 (3.3)	9 (4.5)
Other	23 (4.5)	5 (2.5)

Data are presented as n (%) of patients unless otherwise indicated. Percentages may not add up to 100% because of rounding.

[a]*Percentages for race and US CB region represent the percentages of patients with available data.*

[b]*Information about prior treatment of brain metastases was not available.*

[c]*Positive biomarker results at any time ("ever positive") were included.*

[d]*Of the 22C3 IHC assays, 455/474 (96.0%) and 182/187 (97.3%) in EHR and Spotlight cohorts, respectively, used the PD-L1 IHC 22C3 pharmDx (Agilent Technologies, Carpinteria, CA; pembrolizumab companion diagnostic).*

CCI, Charlson comorbidity index; ECOG, Eastern Cooperative Oncology Group; EHR, electronic health record; IHC, immunohistochemistry; index year, year of pembrolizumab initiation; NSCLC histology NOS, non-small cell lung cancer histology not otherwise specified; US CB, United States Census Bureau.

the latter administered in second line to 28/87 patients (32%), including 8/28 patients (29%) who received pembrolizumab monotherapy. Eight patients (31%) in third line (1 pembrolizumab monotherapy), and 4 patients (57%) in fourth line, were treated with ICIs. The median rwToT for second-line ICI therapy was 5.8 months (95% CI, 1.4–12.9) and for third-line ICI therapy was 5.6 months (95% CI, 0 to not assessable).

3.2.1 Spotlight Cohort: Outcomes Captured Using Enhanced Manual Chart Review

Additional outcomes for the spotlight cohort are summarized in **Table 2**. Median rwPFS was 7.3 months (95% CI, 5.7–9.2 months), and 184 patients (81%) experienced an episode of rwP or death from any cause (**Figure 3C**). In a sensitivity analysis excluding suspected pseudoprogression, 182 patients

TABLE 2 | Outcomes with first-line pembrolizumab monotherapy in the real-world oncology and clinical trial settings.

Variable	Real-world cohorts		Clinical trials	
	EHR cohort[a] (n = 566)	Spotlight cohort[a] (n = 228)	KEYNOTE-024 (2)[b] (n = 154)	KEYNOTE-042 (3)[b] (n = 299)
Observed follow-up, median (range), mo[c]	35.1 (12.0-52.7)	38.4 (33.1-44.9)	59.9 (55.1-68.4)	46.9 (35.8–62.1)
Patient follow-up, median (range), mo[c]	16.5 (<0.1-52.6)	25.7 (1 day-44.3)	–	–
Real-world TRR (rwTRR)/ORR, n[d]	–	88	71	–
% (95% CI)	–	38.6 (32.2-45.2)	46.1 (38.1-54.3)	–
Time to response, median (range), mo	–	3.2 (1.5-34.4)	2.1 (1.4-14.6)	–
Duration of response, median (range), mo		22.2 (1.4+ to 37.2+)[e]	29.1 (2.2-60.8+)	
Real-world PFS (rwPFS)/PFS				
Events, n (%)	–	184 (80.7)	126 (81.8)	–
rwPFS, median (95% CI), mo	–	7.3 (5.7-9.2)	7.7 (6.1-10.2)	6.5 (5.9-8.6)
12-month rwPFS, % (95% CI)	–	39.3 (32.8-45.7)	–	–
24-month rwPFS, % (95% CI)	–	25.9 (20.2-31.9)	–	–
36-month rwPFS, % (95% CI)	–	14.3 (9.7-19.7)	22.8 (16.3-29.9)	14.5 (10.5-19.0)
Overall survival (OS), N	566	228	154	299
Events, n (%)	322 (56.9)	134 (58.8)	103 (66.9)	219 (73)
OS, median (95% CI), mo	19.6 (16.6-24.3)	21.1 (16.2-28.9)	26.3 (18.3-40.4)	20.0 (15.9-24.2)
12-month survival, % (95% CI)	59.8 (55.5-63.7)	64.2 (57.5-70.2)	–	–
24-month survival, % (95% CI)	45.7 (41.2-50.0)	49.4 (42.5-55.8)	–	–
36-month survival, % (95% CI)	36.2 (31.5-40.9)	38.2 (31.4-45)	43.7	31.3 (26.1-36.6)

[a]*EHR cohort data cutoff, 31 March 2021; spotlight cohort data cutoff, 31 August 2020.*

[b]*Investigator-assessed tumor response and PFS in KEYNOTE-024 are reported. Results from KEYNOTE-042 are reported for patients with PD-L1 TPS ≥50% with locally advanced or metastatic NSCLC, the majority of whom had metastatic NSCLC (n=275; 92%).*

[c]*Observed (theoretical) follow-up was defined as the duration of follow-up from pembrolizumab initiation to database cutoff. Patient follow-up was defined as time from pembrolizumab initiation to the date of death or data cutoff, whichever occurred first.*

[d]*rwTRR refers to the real-world tumor response rate, defined as the proportion of patients with at least one complete response (CR) or partial response (PR) assessment followed by a subsequent assessment of CR, PR, or stable disease during first-line pembrolizumab monotherapy. Analysis of time to response is based on patients with a best rwTR of CR or PR.*

[e]*+ indicates ongoing response.*

EHR, electronic health record; mo, months; NR, not reached; ORR, objective response rate; PFS, progression-free survival; TRR, tumor response rate.

(80%) experienced rwP, and median rwPFS was 8.6 months (95% CI, 6.8–11.2).

A total of 17 patients (7%) had a best rwTR assessed as CR, and 71 patients (31%) were assessed as having PR (**Table 3**), for a rwTRR of 38.6% (95% CI, 32.2–45.2), and a total of 122 patients (54%) with disease control (CR + PR + stable disease).

Two-thirds of patients (151; 66%) in the spotlight cohort had discontinued pembrolizumab at data cutoff (**Table 4**). The most common reason for discontinuation was disease progression (70/151; 46%), followed by adverse effect of therapy (35/151; 23%). Of the 33 patients who subsequently received second- and/or third-line ICI therapy, 8 discontinued first-line pembrolizumab because of disease progression, 6 because of adverse effects, and 5 because of disease-related symptoms (**Supplementary Table 3**).

No serious adverse events were identified during manual chart review (**Supplementary Table 4**).

4 DISCUSSION

This study enlarges and extends the observational period for two real-world patient cohorts treated with pembrolizumab monotherapy identified as being clinically similar to patients enrolled in KEYNOTE-024. The minimum potential follow-up after pembrolizumab initiation to data cutoff was 6 months in our prior study (12), and, in the present study, was extended to 1 year for the EHR cohort and almost 3 years for the spotlight cohort. Median OS was 19.6 and 21.1 months in EHR and spotlight cohorts, respectively, while median OS with pembrolizumab monotherapy in KEYNOTE-024, -042, and -598 was 26.3, 20.0, and 21.9 months, respectively (2–4), We observed 3-year Kaplan-Meier estimated OS rates of 36% and 38% in EHR and spotlight cohorts, respectively, whereas 3-year OS rates in KEYNOTE-024 and KEYNOTE-042 (PD-L1 TPS ≥50% population) were 44% and 31%, respectively (2, 3).

There was no comparator included in the present study; however, the median OS for both EHR and spotlight cohorts was substantially higher than the median OS of patients who received chemotherapy in KEYNOTE-024 (13.4 months) (2). Tumor characteristics were similar, but our real-world populations included proportionately more women and older patients relative to the patient populations in KEYNOTE trials (2, 22), as we reported previously (12). There were likely other differences as well that were not captured *via* retrospective EHR review: for example, we could not reliably capture pretreatment of brain metastases or determine whether patients had a minimum life expectancy of 3 months, as required in KEYNOTE-024 and -042 (1, 22). Subsequent therapy use in both EHR and spotlight cohorts (32% and 38%) was lower than in the KEYNOTE-024 5-year data (53%). However, we observed that in these two real-world cohorts, ICIs, as monotherapy or in combination with chemotherapy, were used as a second- or third-line therapy, and administered for a median rwToT of almost 6 months, among 30–40% of patients receiving a subsequent treatment.

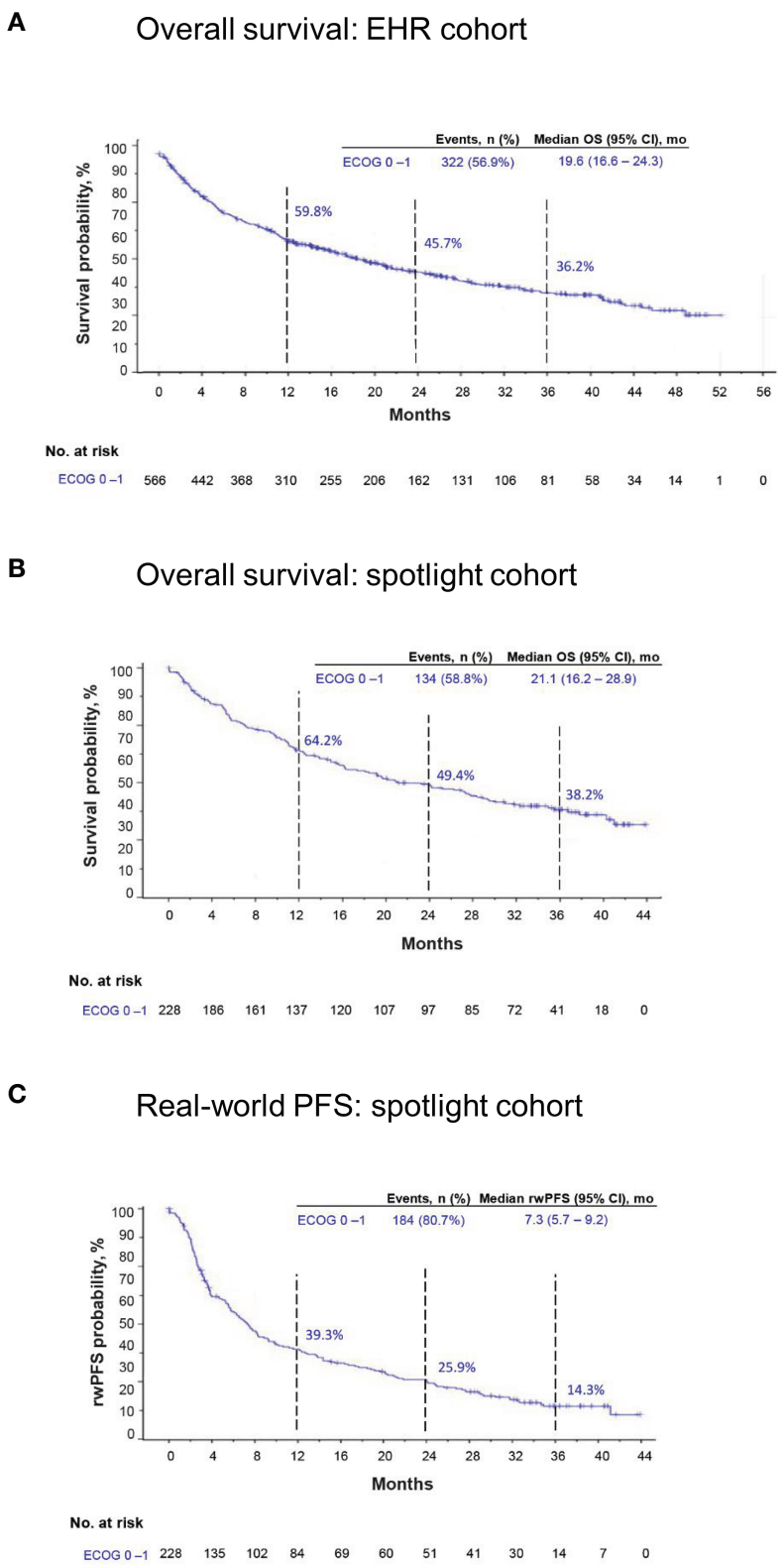

FIGURE 3 | Kaplan-Meier estimates of overall survival (OS) in the EHR and spotlight cohorts and real-world progression-free survival (rwPFS) in the spotlight cohort.EHR, electronic health record; mo, months. **(A)** OS in the EHR cohort. **(B)** OS in the spotlight cohort. **(C)** rwPFS in the spotlight cohort.

TABLE 3 | Best real-world tumor response (rwTR) to first-line pembrolizumab monotherapy: Spotlight cohort.

	Spotlight[a] (n = 228)	KEYNOTE-024[b] (n = 154)
Complete response (CR)	17 (7.5)	7 (4.5)
Partial response (PR)	71 (31.1)	64 (41.6)
Stable disease	34 (14.9)	37 (24.0)
Progressive disease (PD)	50 (21.9)	35 (22.7)
No evaluable assessment	56 (24.6)	0
Indeterminate	1 (0.4)	–
Pseudoprogression[c]	7 (3.1)	–
Not documented/no assessment	51 (22.4)	11 (7.1)

Data are presented as n (%) of patients.

[a]*rwTR determination was based on changes in NSCLC tumor burden indicated by radiology reports. For patients with multiple rwTR assessments, the best response was used to classify the patient (CR>PR>stable disease>PD). Patients without an evaluable assessment (no CR, PR, stable disease, or PD) could be counted >1 time in the subcategories of "no evaluable assessment".*

[b]*KEYNOTE-024 results determined using RECIST 1.1 criteria by investigatory review (2).*

[c]*Pseudoprogression was defined as an increase in tumor size that the clinician recorded as possibly being an effect of ICI therapy.*

TABLE 4 | Summary of reasons for pembrolizumab discontinuation: Spotlight cohort.

	Spotlight (n = 228)
Discontinued, n (%)	151 (66.2)
Reasons for discontinuation, n (%)	*N=151*[a]
Progression	70 (46.4)
Adverse effect of therapy	35 (23.2)
Disease-related symptoms not due to therapy	23 (15.2)
Patient request	6 (4)
Completed treatment	5 (3.3)
No evidence of disease	2 (1.3)
Other[b]	13 (8.6)
Unknown	1 (0.7)

[a]*Patients could have more than one reason for discontinuation.*

[b]*For patients with ongoing treatment until the time of death, the reason recorded was "Other" to comply with data deidentification requirements.*

Several other observational studies from the US, Europe, and Israel have been published recently that evaluated outcomes with first-line pembrolizumab or other ICI monotherapy for similar patient populations with advanced NSCLC, PD-L1 expression ≥50%, and no genomic alterations (23–27). There was some variability in patient populations and length of follow-up time, and survival in these studies for patients with good performance status (ECOG 0–1) also varied, with reported median OS of 18.6 months (26), 22.1 months (28), 22.8 months (23), 28.7 months (25), and not reached (27). In a large European multicenter study of first-line pembrolizumab monotherapy (n=1026), the objective response rate determined by investigators using RECIST (v1.1) criteria (29) was 48.1% for 756 evaluable patients with ECOG 0–1 (23), greater than the 39% rwTRR determined in our study, although the means of rwTR determination *via* manual chart review is not directly comparable with the application of RECIST criteria.

Our study had the advantages of a large patient population and, for the spotlight cohort, long follow-up time. However, while the clinical similarity of our patient population to KEYNOTE trial populations enabled us to examine outcomes relative to those in clinical trials, our ability to investigate potential prognostic factors was limited. Results of a recent study also drawing on the Flatiron Health database indicated that among patients with nonsquamous NSCLC and PD-L1 expression ≥50% treated with first-line pembrolizumab monotherapy, never-smokers had shorter rwPFS and OS than smokers (28), similar to findings in clinical trials and other observational studies (30, 31). Prior cohort studies have identified several independent predictors of shorter OS, including ECOG performance status of ≥2, bone metastases, liver metastases, and baseline steroids (23, 25). Conversely, tumor PD-L1 expression ≥90% was associated with longer OS in multivariable analyses of a large cohort of patients with metastatic NSCLC and PD-L1 expression ≥50% treated with first-line pembrolizumab monotherapy (23). We note that a recent study evaluating outcomes of first-line pembrolizumab monotherapy for a less selective, older US population (≥66 years) with advanced NSCLC reported a median OS of only 11.4 months (32), 15 months less than that in KEYNOTE-024 (2) and considerably shorter than the median OS determined for each of our study cohorts. As the authors of that study observed, the administrative claims data they used lack many important prognostic and predictive baseline factors, including performance status, targetable mutations, and PD-L1 status, to allow stratified analyses by these baseline factors to further explain the results (32). Clearly, further study is needed of potential prognostic factors, including biomarkers, for more heterogeneous populations with metastatic NSCLC treated with pembrolizumab as monotherapy, or in combination with chemotherapy, to inform clinical treatment decisions.

Other strengths of the present study include the use of a well-regarded, well-curated EHR database. Manual chart review for the randomly selected spotlight cohort enabled the determination of rwPFS, rwTR, and reasons for pembrolizumab discontinuation. The curation of rwPFS and rwTRR endpoints from EHR datasets has recently been described and characterized as reliable and clinically relevant (19, 20).

Continued follow-up of these real-world cohorts will be of interest. In addition, as we observed that a non-negligible proportion of patients were treated with an ICI following first-line pembrolizumab, it will be of interest to understand the long-

term real-world outcomes of these patients rechallenged with an ICI in a later line of therapy.

4.1 Limitations

The limitations of retrospective data evaluation are applicable to this study, including the potential for missing or inaccurately recorded data. Attrition in cohort selection was primarily due to missing eligibility-related data, for example, missing ECOG performance status, genomic testing results for *EGFR/ALK* alterations, or PD-L1 testing results. In addition, even with manual chart review for the spotlight cohort, some outcome data, such as information to determine rwTR and reasons for pembrolizumab discontinuation, were missing for some patients.

4.2 Conclusions

With long-term follow-up, real-world outcomes with first-line pembrolizumab monotherapy remain consistent with outcomes observed in phase 3 pivotal clinical trials for patients with metastatic NSCLC, PD-L1 expression ≥50%, no known tumor genomic alterations, and good performance status. These findings support the long-term benefits of first-line pembrolizumab monotherapy for this patient population.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study have been originated by Flatiron Health, Inc. These deidentified data may be made available upon request and are subject to a license agreement with Flatiron Health; interested researchers should contact DataAccess@flatiron.com to determine licensing terms. Requests to access the datasets should be directed to Flatiron Health DataAccess@flatiron.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Copernicus Group Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

VV, XH, LY, and TB developed the study concept and design. LY conducted the statistical analysis. VV, XH, LY, MCP, and TB interpreted the data and critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.834761/full#supplementary-material

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Real-World Treatment Patterns and Clinical Outcomes in Patients With Stage III Non-Small-Cell Lung Cancer: Results of KINDLE-Vietnam Cohort

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Objective: KINDLE-Vietnam was a part of a real-world KINDLE study with an aim to characterise treatment patterns and clinical outcomes of patients with stage III non-small cell lung cancer (NSCLC).

Materials and Methods: Retrospective data from patients diagnosed with stage III NSCLC (American Joint Committee on Cancer, 7th edition) between January 2013 and December 2017 with at least 9 months of follow-up were collected from 2 centres in Vietnam. Descriptive statistics were used to summarise demographics, disease characteristics and treatment modalities. Kaplan-Meier methodology evaluated survival estimates; 2-sided 95% confidence intervals (CIs) were computed. Inferential statistics were used to correlate clinical and treatment variables with median progression-free survival (mPFS) and median overall survival (mOS).

Results: A total of 150 patients (median age: 60 years [range 26-82]) were enrolled; 75.3% were male, 62.0% had smoking history, 56.4% had stage IIIB disease and 62.5% had adenocarcinoma. The majority of the cases (97.3%) were not discussed at a multidisciplinary team meeting. Overall, chemotherapy alone (43.3%), radiotherapy alone (17.0%), sequential chemoradiation (13.5%) and concurrent chemoradiation (12.8%) were preferred as initial therapy. Surgery-based treatment was administered in limited patients (stage IIIA, 10%; stage IIIB, 1.3%). Palliative therapy was the most commonly administered treatment upon relapse in the second-and third-line setting. The mPFS and mOS for the Vietnam cohort were 8.7 months (95% CI, 7.59-9.72) and 25.7 months (95% CI, 19.98-42.61), respectively. The mPFS and mOS for stage IIIA were 11.9 months (95% CI, 8.64-14.95) and 28.2 months (95% CI, 24.15-not-calculable) and for stage IIIB were 7.8 months (95% CI, 6.64-8.71) and 20.0 months (95% CI, 13.01-42.61).

Conclusions: KINDLE-Vietnam offers insights into the clinical findings of stage III NSCLC. There is a high unmet need for identifying patients in the early stages of NSCLC. Strategies for improving clinical outcomes in this patient population include physician education, multidisciplinary management and catering to increased access to novel agents like immunotherapy and targeted therapy.

Keywords: lung cancer, stage III NSCLC, sequential chemoradiation, chemotherapy, chemoradiotherapy

INTRODUCTION

Lung cancer is the most common malignancy and the leading cause of cancer-related deaths worldwide (18.4% of total cancer deaths, GLOBOCAN 2018) (1). The burden of lung cancer is extremely diverse in Asian countries. The highest incidence was observed in Japan followed by countries closer to Europe such as Armenia, Turkey and Kazakhstan as well as South-East Asia and Korea, whereas the lowest incidence was reported in Western Asian countries including Yemen and Saudi Arabia (2). South-East Asia reported 160,068 new cases and 146,990 deaths in 2018 with 23,667 new cases and 20,710 deaths reported from Vietnam (3). Although the incidence of lung cancer in Vietnam is higher than the global incidence (14.4% versus 11.6%), the mortality rate is similar to the rest of the world (18.4%) (4, 5). In the Association of South-East Asian Nations countries, the age-standardised mortality rates (per 100,000) for males and females were highest in Armenia (58.5 and 8.5), Vietnam (35.4 and 11.1) and Singapore (41.5 and 17.2) and lowest in Cambodia (21.6 and 8.7) and Indonesia (19.4 and 6) (6). The age-adjusted death rate was estimated at 24.73 per 100,000 for both genders together, putting Vietnam at the 37th position in the world for lung cancer (7).

Non-small-cell lung cancer (NSCLC) accounts for about 85% of all new lung cancer cases (8). Approximately 25% to 30% of patients with NSCLC are diagnosed at stage III, described as a heterogeneous disease with either locally advanced tumour spread and/or mediastinal lymph node involvement, without clinical evidence of distant spread (9–12). Long-term survival is generally poor in stage III disease, with a 5-year median overall survival (mOS) of 36%, 26% and 13% for stage IIIA, IIIB and IIIC, respectively (13). In Vietnam, more than 80% of cases of lung cancer were NSCLC and the majority of cases (about 89%) were found in the advanced stages (IIIB or IV) (14). A study from 2014 estimated the economic burden of NSCLC in Vietnam to be >3,517 billion Vietnamese Dong, equivalent to $150 million (15). In Asia, epidermal growth factor receptor (EGFR) mutation rates in patients with NSCLC are high with approximately 47.0% in Eastern and South-Eastern Asia. Vietnam has the highest rate of EGFR mutations at 64.0% (8).

Due to heterogeneity, optimum management of stage III NSCLC remains a challenge. Surgical resection, though the preferred treatment, might not be plausible in all patients with stage III disease (16). Hence, a multi-modal management approach involving surgery, radiation and systemic agents is frequently practised. Concurrent platinum-based chemotherapy (CT) and radiotherapy (RT) are the standard of care for unresectable stage III disease with mOS ranging from 15 to 29 months (12, 17–24). Recent studies have combined immune therapy with concurrent CT and RT (cCRT), resulting in the emergence of new multi-modal combination approaches for stage III NSCLC (25, 26). Durvalumab consolidation therapy for patients with unresectable/inoperable stage III NSCLC who have not progressed on ≥2 cycles of cCRT is the new standard of care (12).

Given the significant burden of NSCLC leading to increased economic impact in Vietnam, cost-effective strategies and wide coverage are needed to manage NSCLC cases (15). A systematic and evidence-based approach to cancer care, particularly lung cancer, is a priority for Vietnam's healthcare system (5). The data about prevalent treatment patterns and their associated survival outcomes for stage III NSCLC in the Vietnamese population is limited (27).

Vietnam was a part of the real-world evidence study KINDLE, conducted at 100 centres from 19 countries across Asia, Africa, the Middle East and Latin America (28). The primary objective of the global KINDLE study was to determine the treatment patterns and clinical outcomes of patients with stage III NSCLC, as specified by the American Joint Committee on Cancer (AJCC) criteria (7th edition) in the pre-immuno-oncology era. Here we present data for stage III NSCLC in the Vietnamese population to determine the treatment patterns and clinical outcomes in patients.

MATERIALS AND METHODS

Study Design

Retrospective data were collected over approximately 6 years (2013 to 2018) from the 2 largest cancer hospitals in Vietnam (one each in North and South Vietnam) on patients diagnosed with stage III NSCLC. The study protocol (NCT03725475) was approved by the independent ethics committees/institutional review boards of both the participating centres and the Ethical Review Committee, Vietnam Ministry of Health. The research was carried out in compliance with the Helsinki Declaration, the International Harmonisation Council (ICH), the Good Clinical Practices (GCP), the Good Pharmacoepidemiology Practices (GPP) and the relevant non-interventional and/or observational studies legislation. Adult patients (aged 18 years or older) diagnosed with *de novo* locally advanced stage III NSCLC (as per AJCC 7th edition) between January 2013 and December 2017, with medical records available for a minimum of 9 months from the index date (date of diagnosis of stage III

NSCLC), and signed written informed consent from the patient or next of kin/legal representative (in case of a deceased patient) were included in the study. Patients with an initial diagnosis of stage I to II NSCLC, who progressed to stage III, and those with concomitant cancer at the time of or within 5 years of stage III NSCLC diagnosis (except for non-metastatic non-melanoma skin cancers or *in situ* or benign neoplasms) were excluded.

Medical charts of eligible patients were reviewed and protocol-specified retrospective data were transcribed to the electronic case report forms. Data were collected from the index date until the end of the follow-up period, defined as death, the last medical record available or the end of the data collection. The following data were collected: demographics (age, gender, body mass index and smoking status), clinical characteristics (Eastern Cooperative Oncology Group [ECOG] performance status, NSCLC histology, stage as per 7th edition AJCC, EGFR status and programmed cell death-ligand 1 status) and treatment patterns (modality and line of treatment). The occurrence and date of disease progression were determined from documents within the patient medical records, such as pathology reports, imaging reports, clinical notes and comments on disease progression. Progression-free survival (PFS) was defined as the time from the start of the treatment to documented disease progression or death due to any cause, whichever occurred first. The first progression interval was defined as the period between the index date and the first disease progression, and subsequent progression intervals were defined as the period between sequential progressions. For patients who received treatment, sequential treatment regimens were documented within each progression interval. Overall survival was calculated as the time from the stage III NSCLC diagnosis or start of the treatment to death from any cause.

Statistical Analyses

Descriptive statistics were used to summarise patient demographics, disease characteristics and treatment modalities. Median survival estimates (mOS) and median PFS (mPFS) including rates of the affected patients were evaluated descriptively using the Kaplan-Meier survival curves and median survival estimates are reported along with the 2-sided 95% confidence interval (CI). Inferential statistics as correlation analyses were used to determine the correlation between various clinical and treatment variables and survival outcomes (mPFS and mOS). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

A total of 150 Vietnamese patients were included, of whom slightly more than half (52.0%) were alive at the time of data collection. The mean duration (± standard deviation [SD]) of follow-up was 17.52 (± 13.81) months. The median age (range) of patients was 60.0 (26.0-82.0) years; most (75.3%) were men and 62.0% (75/121) had a history of smoking or were current smokers. In total, 84 (56.4%) patients were diagnosed with stage IIIB disease (7th edition AJCC classification). Adenocarcinoma was the most common histological type (62.5%, 90/144), followed by epidermoid or squamous cell carcinoma (26.4%, 38/144). Most (58.9%, 83/141) of the cases were initially presented to primary care physicians, while 41.1% (58/141) of patients consulted specialist care. The ECOG performance status was not available for the majority of the patients (n = 106); it was ≤1 in 75.0% (33/44) of the remaining patients. Majority of the cases (97.3%) were not discussed at a multidisciplinary team (MDT) meeting. **Table 1** describes the sociodemographic and clinical characteristics of the Vietnam cohort.

Treatment Patterns

Initial therapy comprised of 14 treatment modalities, including CT alone (43.3%, 61/141), RT alone (17.0%, 24/141), sequential chemoradiation (sCRT) (13.5%, 19/141), concurrent chemoradiation (cCRT) (12.8%, 18/141) and targeted therapy (1.4%, 2/141) alone or in combination with other therapies. Among the common treatment modalities reported in initial therapy in stage IIIA and IIIB disease, CT alone was administered in the majority of patients (30.0%, 18/60 and 53.8%, 43/80); RT alone (20.0%, 12/60 and 15.0%, 12/80), sCRT (18.3%, 11/60 and 10.0%, 8/80) and cCRT (18.3%, 11/60 and 8.8% 7/80) were the next common modalities (**Figure 1**). For further analyses, these treatment modalities are broadly grouped into 3 categories as surgery-based therapy, CRT-based therapy and palliative therapy (CT alone, RT alone and targeted therapy). The treatment patterns are summarised in **Table 2**. For stage IIIA and IIIB disease, palliative therapy was administered in the majority of patients (50.0% [30/60] and 71.3% [57/80]), followed by CRT-based therapy (40.0% [24/60] and 27.5% [22/80]) and surgery-based therapy (10.0% [6/60] and 1.3% [1/80]) as initial therapy. After initial therapy was administered in 141 patients (stage IIIA: 60 and stage IIIB: 80), relapse was documented for 44 (73.0%) and 73 (91.0%) patients in stage IIIA and IIIB, respectively. Second-line therapy was administered in 61 patients (stage IIIA: 29 and stage IIIB: 32), while third-line therapy was administered in 26 patients (stage IIIA: 10 and stage IIIB: 16). Palliative therapy was the most commonly administered treatment option upon relapse in second and third-line therapy (IIIA [96.6% and 100%] and IIIB [96.9% and 87.5%]).

Survival Outcomes

The mPFS for the entire evaluable population (N=140) was 8.7 months (95% CI, 7.59 to 9.72): stage IIIA, 11.9 months (95% CI, 8.64 to 14.95) and stage IIIB, 7.8 months (95% CI, 6.64 to 8.71) (**Figure 2**). The mOS for the entire population evaluable in 139 patients was 25.7 months (95% CI, 19.98 to 42.61): stage IIIA, 28.2 months (95% CI, 24.15 to not-calculable [NC]) and stage IIIB, 20.0 months (95% CI, 13.01 to 42.61) (**Figure 3**). The mPFS was numerically higher in patients who underwent surgical resection (n = 18; 9.7 months [95% CI, 6.31 to 13.57]) compared with unresectable patients (n = 91; 8.7 months [95% CI, 6.67 to 12.19]). The mOS was NC in patients who underwent surgical resection (n = 18; NC [95% CI, 13.83 to NC]), while in unresectable patients it was calculable (n = 90; 23.1 months; [95% CI, 14.65 to 39.89]).

The survival outcomes according to the initial therapy are described in **Table 3**. In stage IIIA, the longest mPFS and mOS

TABLE 1 | Baseline sociodemographic and clinical characteristics of patients with stage III NSCLC in Vietnam.

Parameters	Number of Patients (N = 150)
Age (years), median (range)	60.0 (26.0-82.0)
Gender, Male, n (%)	113 (75.3)
BMI (kg/m^2), median (range)	20.5 (16.0-30.0)
Tobacco Smoking[a], n = 121, n (%)	
Current smoker	75 (61.9)
Ex-smoker	12 (9.9)
Never smoker	34 (28.1)
AJCC stage (7th edition), n = 149, n (%)	
Stage IIIA	65 (43.6)
Stage IIIB	84 (56.4)
Histology type, n = 144, n (%)	
Adenocarcinoma	90 (62.5)
Epidermoid or squamous cell carcinoma	38 (26.4)
Large cell carcinoma	9 (6.2)
Other	4 (2.8)
Mixed	3 (2.1)
ECOG performance status, n = 44, n (%)	
≤1	33 (75.0)
≥2	11 (25.0)
T Stage, n = 149, n (%)	
T1a	6 (4.0)
T1b	8 (5.3)
T2a	23 (15.4)
T2b	14 (9.3)
T3	51 (34.2)
T4	45 (30.2)
TX	2 (1.3)
N Stage, n = 150, n (%)	
N0	7 (4.7)
N1	11 (7.3)
N2	70 (46.7)
N3	61 (40.7)
NX	1 (0.7)
EGFR testing, n = 25, n (%)	
EGFR no mutation	19 (76.0)
EGFR-mutated	5 (20.0)
Uncertain	1 (4.0)
To whom did the patient first present, n = 141, n (%)	
Primary care physician	83 (58.9)
Clinical oncologist	17 (12.0)
Others	41 (29.1)
Vital status, n = 150, n (%)	
Alive	78 (52.0)
Dead	72 (48.0)

Percentage was calculated based on the total number of patients available within each level; Unknown and missing data are not included; PD-L1 biomarker testing was performed in 1 patient who had negative status.

[a]*Current smoker is defined as an active smoker; an ex-smoker is defined as having smoked regularly but stopped ≥365 days ago; Never smoker is defined as never smoked regularly. AJCC, American Joint Committee on Cancer; BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death-ligand 1.*

were observed for patients who underwent CRT-based therapy (n = 24; 13.7 months [95% CI, 8.31 to 16.20] and 28.1 months [n = 23; 95% CI, 20.76 to 39.89]), followed by patients who underwent surgery-based therapy (n = 6; 9.7 months [95% CI, 4.14 to 51.98] and 13.8 months [n = 6; 95% CI, 9.10 to NC]). In stage IIIB, the longest mPFS was observed in a single patient who underwent surgery-based therapy (20.7 months [95% CI, NC to NC]), followed by CRT-based therapy in 22 patients (8.6 months [95% CI, 6.70 to 12.39]). In stage IIIB, the longest mOS of 23.1 months (n = 22; 95% CI, 8.21 to 42.61) was observed for CRT, followed by palliative therapy (n = 57; 19.5 months [95% CI, 13.01 to 42.61]).

Univariate analysis for mPFS and mOS favoured stage IIIA (hazard ratio [HR], 0.53, 95% CI, 0.36 to 0.78; p = 0.001) and (HR, 0.53, 95% CI, 0.31 to 0.90; p = 0.016) compared with stage IIIB. The details of univariate analysis as per mPFS and mOS for stage IIIA and IIIB disease for clinico-demographic characteristics and treatment modalities are mentioned in **Table 4**. As per the univariate analyses, for stage IIIA, female gender and surgery as a treatment modality were associated with better mPFS, while adenocarcinoma was associated with better mOS ($p < 0.05$). For stage IIIB disease, adenocarcinoma was associated with better mOS ($p < 0.05$).

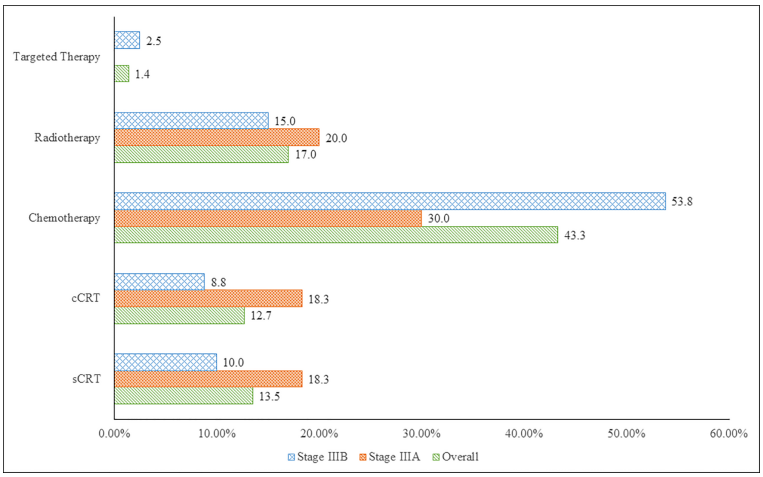

FIGURE 1 | Top 5 Treatment Modalities for Stage IIIA and IIIB NSCLC in Vietnam (7th Edition AJCC). cCRT, concurrent chemoradiation; sCRT, sequential chemoradiation.

DISCUSSION

To our knowledge, this is the first real-world study reporting the treatment patterns and their associated clinical outcomes in a group of Vietnamese patients with stage III NSCLC. This research offers an overview of treatment and survival trends of unresectable/inoperable stage III NSCLC. MDT discussion in lung cancer is known to be associated with better treatment decisions, which potentially improves outcomes and quality of life for patients with lung cancer (29, 30). In our study, a majority (97.3%) of the cases were not discussed at MDT meetings. Although both the participating centres are multi-speciality cancer care (including medical oncologists, radiologists, pathologists and thoracic surgeons), MDT management of lung cancer was not established as part of routine clinical practice in Vietnam during the period of this study.

A recent study assessing real-world clinical outcomes in Medicare patients reported a higher (61.0%) proportion of patients being treated with CRT in stage IIIA and 39.0% in stage IIIB (22, 31). With almost 14 treatment modalities being used as initial therapy, our study observed a wide difference in the treatment trends. More than half of the patients (61.7%) underwent palliative therapy as initial treatment. The American Society of Clinical Oncology guidelines recommend early palliative care to improve symptoms, quality of life and increase survival. Palliative care for NSCLC is categorised into 2 major groups, namely, supportive care and tumour-directed treatment (includes palliative CT and RT) (32). As initial therapy, CT alone was the dominant therapy (43.3%), showing that patients had limited access to RT, given to only 17.0% of the patients. In 2017, intensity-modulated radiation therapy was sparingly used and only 36 linear machines were available in Vietnam for external radiation therapy with 3D-conformal radiation therapy techniques in the country resulting in a low rate of RT and CRT (33). As second- or third-line therapies upon relapse, palliative therapy was the most preferred option in

TABLE 2 | Treatment patterns in stage III NSCLC in Vietnam as per subgroup (7th Edition AJCC).

Treatment Modality	Initial Treatment, n (%)			Second Line, n (%)			Third Line, n (%)		
	Overall n (%) (N = 141)	Stage IIIA n (%) (N = 60)	Stage IIIB n (%) (N = 80)	Overall n (%) (N = 61)	Stage IIIA n (%) (N = 29)	Stage IIIB n (%) (N = 32)	Overall n (%) (N = 26)	Stage IIIA n (%) (N = 10)	Stage IIIB n (%) (N = 16)
Surgery-based therapy	8 (5.7)	6 (10.0)	1 (1.3)	1 (1.6)	1 (3.4)	0	0	0	0
CRT-based therapy	46 (32.6)	24 (40.0)	22 (27.5)	1 (1.6)	0	1 (3.1)	2 (7.7)	0	2 (12.5)
Palliative therapy[a]	87 (61.7)	30 (50.0)	57 (71.3)	59 (96.7)	28 (96.6)	31 (96.9)	24 (92.3)	10 (100)	14 (87.5)
Top 5 Treatment Modalities									
sCRT	19 (13.5)	11 (18.3)	8 (10.0)	1 (1.6)	0	0	1 (3.8)	0	1 (6.3)
cCRT	18 (12.7)	11 (18.3)	7 (8.8)	0	0	0	0	0	0
CT	61 (43.3)	18 (30.0)	43 (53.8)	39 (63.9)	16 (55.2)	23 (71.9)	17 (65.4)	6 (60.0)	11 (68.8)
RT	24 (17.0)	12 (20.0)	12 (15.0)	16 (26.2)	11 (37.9)	5 (15.6)	6 (23.1)	4 (40.0)	2 (12.5)
Targeted therapy	2 (1.4)	0	2 (2.5)	4 (6.6)	1 (3.4)	3 (9.4)	1 (3.8)	0	1 (6.3)

[a]*Includes CT alone, RT alone and targeted therapy.*

AJCC, American Joint Committee on Cancer; cCRT, concurrent chemoradiation; CRT, chemoradiation; CT, chemotherapy; RT, radiotherapy; sCRT, sequential chemoradiation.

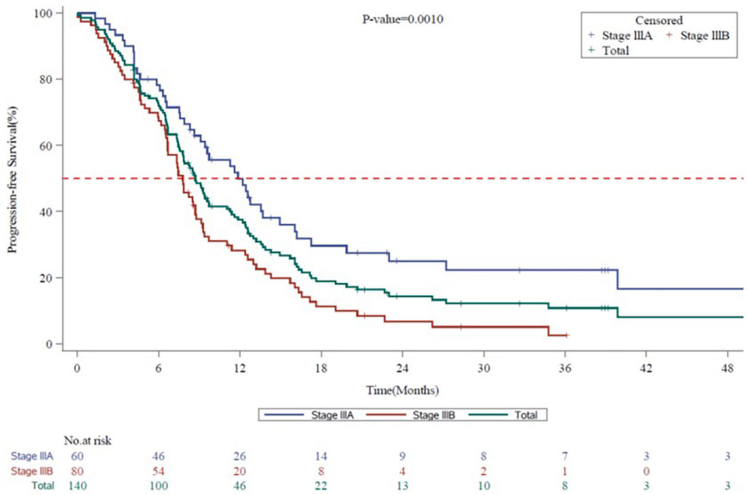

FIGURE 2 | Kaplan-Meier Survival Curves for Progression-free Survival by Disease Stage (7th Edition AJCC) in Vietnam. AJCC, American Joint Committee on Cancer; CI, confidence interval; mPFS, median progression-free survival. Kaplan-Meier Survival curves for progression-free survival for all stage III NSCLC patients are shown in green, whereas stage IIIA and stage IIIB patients are shown in blue or red, respectively. mPFS for the entire Vietnam cohort, 8.7 months (95% CI, 7.59 to 9.72). mPFS for stage IIIA, 11.9 months (95% CI, 8.64 to 14.95). mPFS for stage IIIB, 7.8 months (95% CI, 6.64 to 8.71).

Vietnamese patients and rather the only option administered in the third-line therapy in stage IIIA NSCLC, whereas in KINDLE-global, CT alone was the most favoured second- or third-line therapy after recurrence, followed by RT alone in >20% of patients (28).

Our Vietnam data reports statistically significant longer mPFS (11.9 versus 7.8 months; HR 0.53 [95% CI, 0.36 to 0.78]; p = 0.001) and mOS (28.2 versus 20.0 months; HR 0.53 [95% CI, 0.31 to 0.90]; p = 0.016) in stage IIIA as compared with stage IIIB disease. Similar results were also observed in the KINDLE-global cohort, where longer mPFS (14.3 versus 10.2 months; HR 0.86 [95% CI, 0.77 to 0.96]; p < 0.01) and mOS (43.8 versus 27.7 months; HR 0.78 [95% CI, 0.68 to 0.90]; p = 0.0005) were observed in stage IIIA than stage IIIB [28]. In a Vietnamese study, 51 patients with advanced NSCLC underwent first-line combination CT with pemetrexed and cisplatin followed by pemetrexed maintenance; mPFS and mOS were reported to be 7.8 months and 16.1 months,

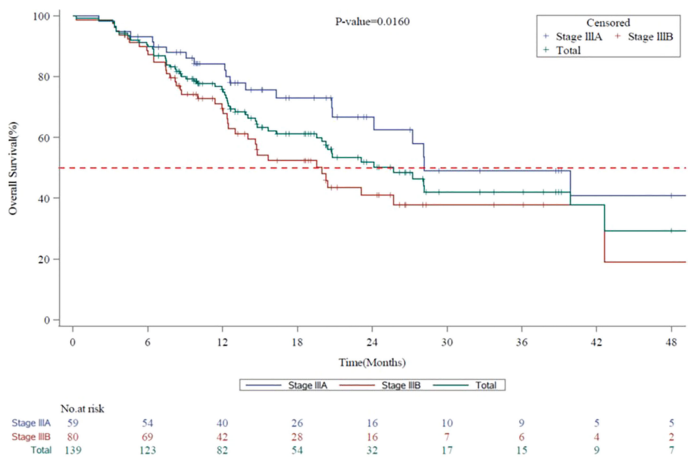

FIGURE 3 | Kaplan-Meier Survival Curves for Overall Survival by Disease Stage (7th Edition AJCC) in Vietnam. AJCC, American Joint Committee on Cancer; CI, confidence interval; mOS, median overall survival. Kaplan-Meier survival curves for overall survival for all stage III NSCLC patients are shown in green, whereas stage IIIA and stage IIIB patients are shown in blue or red, respectively. mOS for the entire Vietnam cohort, 25.7 months (95% CI, 19.98 to 42.61). mOS for stage IIIA, 28.2 months (95% CI, 24.15 to not-calculable [NC]). mOS for stage IIIB, 20.0 months (95% CI, 13.01 to 42.61).

TABLE 3 | Survival outcomes in stage III NSCLC in Vietnam as per Initial treatment regimen and stage (7th Edition AJCC).

	mPFS (95% CI), Months		mOS (95% CI), Months	
	Stage IIIA (N = 60)	**Stage IIIB (N = 80)**	**Stage IIIA (N = 59)**	**Stage IIIB (N = 80)**
Surgery-based therapy	9.7 (4.14 to 51.98)	20.7 (NC to NC)	13.8 (9.10 to NC)	NC (NC to NC)
CRT-based therapy	13.7 (8.31 to 16.20)	8.6 (6.70 to 12.39)	28.1 (20.76 to 39.89)	23.1 (8.21 to 42.61)
Palliative therapy[a]	9.6 (6.31 to 12.58)	7.4 (5.95 to 8.71)	NC (27.24 to NC)	19.5 (13.01 to 42.61)

[a]*Includes CT alone, RT alone and targeted therapy.*

AJCC, American Joint Committee on Cancer; CI, confidence interval; CRT, chemoradiation; CT, chemotherapy; mPFS, median progression-free survival; mOS, median overall survival; NC, not calculable; RT, radiotherapy.

respectively (34). In our study, CRT-based therapy prolonged mPFS (13.7 and 8.6 months) and mOS (28.1 and 23.1 months) irrespective of stage IIIA or IIIB. Similar results were seen in a retrospective Korean study where unresectable stage III patients treated with CRT had better mOS (30.3 months) (95% CI, 26.6 to 34.0) compared with palliative therapy (14.7 months) (95% CI, 13.0 to 16.4) (35).

Real-world data about the adherence to guideline-directed therapies and patient outcomes especially from low-and-middle-income countries are limited. Several randomised clinical studies have also shown a beneficial effect of cCRT compared with sCRT or RT alone in unresectable patients (20–22). In a retrospective study from Vietnam, 5,220 patients with stage III lung cancer were analysed over 3 years of which 70 stage III lung cancer patients having valid survival information were identified (11.7%). The 3-year survival probability of patients with surgery and/or CRT was 34.3% (16.2% to 72.4%), which was higher (10.6%) than CT or RT alone (4.9% to 23.2%; p = 0.055) (15). Another study showed a survival benefit with cCRT having better survival benefits than systemic therapy (14.7 versus 10.9 months) and RT (7.8 months) (36). The preliminary findings of PERTAIN study suggest that implementation of fluorodeoxyglucose-positron emission tomography/CT-guided cCRT results in a substantial improvement in mPFS and mOS for patients with stage III NSCLC in low-and-middle-income countries (37). As a multi-modal treatment, cCRT remains the standard of care for stage III NSCLC with proven benefits over single treatment approaches (38). However, in our study, the use of the cCRT regimen was quite low (12.7%) as initial therapy in comparison to CT alone (43.3%). The most probable reason for this practice was that the MDT meeting approach was employed for discussing <5% of the cases. The MDT (lung cancer tumour boards) requires close collaboration between medical oncologists, radiologists and thoracic surgeons to make an informed decision based on the resectability of the tumour on considering cCRT as an initial treatment modality. Improving the capacity of the radiation approach for stage III NSCLC patients is an important solution for Vietnam. Though the role of MDT is to identify eligible patients who can tolerate cCRT over other approaches, the survival gain of aggressive cCRT may be negated by its severe toxicities (39).

In univariate analysis, adenocarcinoma was a significant positive predictive factor for mOS in both stage IIIA and IIIB disease. The mOS was found to be better in patients with adenocarcinoma (p < 0.046) than in patients with other carcinomas. The female gender was associated with a significantly lower risk of death in both stage IIIA and IIIB. Several studies have shown the female gender as a good prognostic factor (40, 41). However, in one of the studies, no significant difference was found between genders in terms of survival (3-year survival, 29% versus 24%). This was attributed to a smaller proportion of patients being females and which may

TABLE 4 | Univariate analyses for survival outcomes in stage III NSCLC in Vietnam based on clinico-demographic characteristics and treatment regimen (7th Edition AJCC).

Characteristics	**Stage IIIA**		**Stage IIIB**	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Univariate Analyses for PFS				
Age >65 vs ≤65	1.28 (0.73 to 2.22)	0.3833	0.59 (0.33 to 1.06)	0.0762
Male vs Female	1.82 (1.06 to 3.12)	**0.0285**	0.85 (0.48 to 1.48)	0.5675
Adenocarcinoma vs Others	0.85 (0.52 to 1.39)	0.5265	0.70 (0.42 to 1.17)	0.1720
Surgery in initial therapy yes vs no	0.23 (0.09 to 0.57)	**0.0014**	0.20 (0.03 to 1.46)	0.1114
CRT in initial therapy yes vs no	0.63 (0.24 to 1.67)	0.3526	0.43 (0.04 to 4.85)	0.4954
Palliative therapy[a] in initial therapy yes vs no	0.67 (0.22 to 2.09)	0.4947	0.45 (0.04 to 5.28)	0.5234
Univariate Analyses for OS				
Age >65 vs ≤65	1.20 (0.59 to 2.43)	0.6161	0.95 (0.50 to 1.78)	0.8650
Male vs Female	1.57 (0.79 to 3.11)	0.1989	1.23 (0.63 to 2.40)	0.5359
Adenocarcinoma vs Others	0.53 (0.29 to 0.99)	**0.0464**	0.57 (0.32 to 0.99)	**0.0451**

[a]*Includes CT alone, RT alone and targeted therapy.*

AJCC, American Joint Committee on Cancer; CI, confidence interval; CRT, chemoradiation; CT, chemotherapy; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; RT, radiotherapy. Values in bold indicate statistically significant difference (p < 0.05).

have influenced the statistical power (42). Adenocarcinoma has a higher association with smoking in females. The DNA adduct levels are higher among females with adenocarcinoma than their male counterparts after adjustment for smoking dose, and thus females are at higher risk as they are exposed to higher levels of tobacco carcinogens than males (43). Only a small number of patients were available with EGFR test results (n = 25) and the prevalence was found to be 20% (n = 5). Adenocarcinoma is the most frequently encountered histological type of NSCLC, accounting for about half of the cases of NSCLC (44, 45). Adenocarcinoma is also reported to have a better prognosis as compared with other histology subtypes of NSCLC.

With a 1-year survival rate of 42% and a 5-year survival rate of 16%, the survival rate of patients with lung cancer remains low in Vietnam (6). The poor prognosis reflects existing treatment gaps in the management of lung cancer in Vietnam. This KINDLE-Vietnam data provides a benchmark for understanding the treatment patterns, which will be important for evaluating the effectiveness of newer therapies in this population as they become part of clinical practice guidelines. This evidence will also support patient access in Vietnam as a majority (almost 80%) of cancer care (examination and treatment) is covered by health insurance (46).

The limitations of our study include the small sample size and the known challenges of a retrospective study design in real-world settings. Additionally, data collection was limited to the availability of existing health records, resulting in missing data, as many patients could have been lost to routine clinical follow-up. In the initial therapy setting, the study duration covers the era before immunotherapy approval. Thus, the data on the effectiveness of targeted and immunotherapy agents have not been captured in the study.

CONCLUSION

KINDLE-Vietnam study describes the treatment patterns in stage III NSCLC and offers real-world insights into the therapy landscape in the Vietnamese population. Although the study notes adherence to the treatment protocols for CRT-based therapy as the initial therapy in most patients with unresectable disease, there is a definite gap in the optimal selection and sequencing of different treatment approaches. The findings also highlight the need for newer treatment options like immunotherapy in patients with unresectable disease post CRT. Concurrent CRT has been shown to produce better outcomes than sequential CRT. In addition, the PACIFIC trial established CRT followed by 1 year of durvalumab as the standard of care in patients with unresectable stage III NSCLC. Nevertheless, there remains to be a high unmet need for identifying patients in the early stages of NSCLC. Strategies for improving patient outcomes, including guideline adoption, physician education and multidisciplinary management, and catering to increased access to novel agents like immunotherapy and targeted therapy are needed. The data obtained from this study will also contribute to a consolidated framework to help understand the unmet clinical needs in Vietnam in line with their current focus of strengthening the National Cancer Control Programme initiative, in addition to providing baseline data to determine the potential effect of new therapies on the treatment of stage III NSCLC in this region.

DATA PRESENTED AT

KINDLE Global Data Poster Presented at ASCO: Jazieh AR, et al. Contemporary Management and Associated Outcomes of 3,151 Patients With Stage III Non-small Cell Lung Cancer (NSCLC) in a Real-world Setting: Results of KINDLE, a Multicountry Observational Study. J Clin Oncol (2020) 38:15_suppl, 9043-9043. KINDLE Global Data Manuscript Published: Jazieh AR, et al. Real-World Treatment Patterns and Clinical Outcomes in Patients With Stage III NSCLC: Results of KINDLE, a Multicountry Observational Study. J Thorac Oncol (2021) 16 (10):1733-1744. DOI:10.1016/j.jtho.2021.05.003.

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 human participants were reviewed and approved by the Ethic Committee in Biomedical Research - National Cancer Hospital, the Ethic Committee in Biomedical Research - HCM Oncology Hospital, and the National Ethic Committee in Biomedical research - Vietnam Ministry of Health. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TVD, TBD: Conceptualization, Methodology, Writing, Investigation, Editing. TP: Conceptualization, Writing, Methodology, Visualization, Editing. RH, AK: Conceptualization, Methodology, Editing. All authors contributed to the article and approved the submitted version.

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Genomic Alterations Identification and Resistance Mechanisms Exploration of NSCLC With Central Nervous System Metastases Using Liquid Biopsy of Cerebrospinal Fluid: A Real-World Study

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Background: Genomic profiling of cerebrospinal fluid (CSF) can be used to detect actionable mutations and guide clinical treatment of non-small cell lung cancer (NSCLC) patients with central nervous system (CNS) metastases. Examining the performance of CSF samples in real-world settings can confirm the potential of CSF genotyping for guiding therapy in clinical practice.

Patients and Methods: We included 1,396 samples from 970 NSCLC patients with CNS metastases in real-world settings. All samples underwent targeted next-generation sequencing of 1,021 cancer-relevant genes. In total, 100 CSF samples from 77 patients who had previously received targeted treatment were retrospectively analyzed to explore the mechanisms of TKI-resistance.

Results: For NSCLC patients with CNS metastases, CSF samples were slightly more often used for genomic sequencing in treated patients with only distant CNS metastases compared to other patients (10.96% vs. 0.81–9.61%). Alteration rates in CSF samples were significantly higher than those in plasma, especially for copy number variants (CNV). The MSAFs of CSF samples were significantly higher than those of plasma and tumor tissues (all p <0.001). Remarkably, detection rates of all actionable mutations and *EGFR* in

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CSF were higher than those in plasma samples of treated patients (all p <0.0001). For concordance between paired CSF and plasma samples that were simultaneously tested, the MSAF of the CSF was significantly higher than that of matched plasma cfDNA (p <0.001). From multiple comparisons, it can be seen that CSF better detects alterations compared to plasma, especially CNV and structural variant (SV) alterations. CSF cfDNA in identifying mutations can confer the reason for the limited efficacy of EGFR-TKIs for 56 patients (78.87%, 56/71).

Conclusions: This real-world large cohort study confirmed that CSF had higher sensitivity than plasma in identifying actionable mutations and showed high potential in exploring underlying resistance mechanisms. CSF can be used in genomics profiling to facilitate the broad exploration of potential resistance mechanisms for NSCLC patients with CNS metastases.

Keywords: cerebrospinal fluid, resistance mutations, real-world study, non-small cell lung cancer, central nervous system metastases

INTRODUCTION

Lung cancer, one of the most common cancers, remains the most common cause of cancer-related deaths, with high global morbidity and mortality (1). Non-small cell lung cancer (NSCLC), the most frequent (85–90%) cause of malignant lung cancers (2), is also the most common source of central nervous system (CNS) metastases (3). CNS metastases are a frequent and severe complication associated with NSCLC, which occurs in 20–25% of advanced NSCLC patients at initial presentation and is seen in 30–40% of NSCLC patients during their disease (4–6). The median and 1-year overall survival for patients with brain metastases is only 3–7 months and 29.9%, respectively (5–7), and the treatment options for NSCLC with CNS metastases are limited, with most current clinical trials, excluding them. Currently, treatment of NSCLC with CNS metastases is multidisciplinary and involves chemotherapy, radiation therapy, and targeted therapy (5, 8–10).

Next-generation sequencing (NGS)-based genetic testing, which provides abundant genetic information about cancer, has developed therapeutic strategies against driver mutations such as epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), and ROS proto-oncogene 1 (*ROS1*), for NSCLC (11). NGS may also indicate whether NSCLC patients can be treated with immunotherapy. Immunotherapy-based treatments are of greater benefit to non-oncogene addicted NSCLC patients and significantly less effective in the EGFR population (12, 13). In NSCLC with CNS metastases, CNS metastases have distinct genetic information. Thus, performing intracranial biopsy and genetic testing for molecular information and acquired resistance is pertinent (14). Owing to invasive and time-consuming procedures when accessing CNS metastases and the heterogeneity between intracranial and extra-cranial lesions (15, 16), it becomes difficult for NSCLC patients with CNS metastases to access information on genetics or resistance mechanisms. Thus, an urgent need to discover specimens for genetic testing in NSCLC patients with CNS metastases exists.

Liquid biopsy using circulating tumor DNA (ctDNA), which is used in various body effluents, namely, blood, cerebrospinal fluid, ascitic fluid, pleural fluid, and urine instead of tumor tissue, has been widely used in clinical practice to detect genomic alterations in NSCLC (17–19). It can non-invasively identify actionable alterations and overcome both spatial and temporal tumor heterogeneity not addressed by tissue biopsy (17). Liquid biopsy using plasma ctDNA has been widely used in clinical practice, and many studies have demonstrated its feasibility. The new International Association for the Study of Lung Cancer (IASLC) liquid biopsy consensus statement in 2021 noted that liquid biopsy was the preferred method of molecular testing in some clinical settings and proved complementary to tumor tissue testing in others (20). However, owing to the blood–brain barrier, the sensitivity of plasma ctDNA sequencing is limited in NSCLC patients with CNS metastases (21). The 2021 IASLC liquid biopsy statement suggested CSF as an emerging alternative ctDNA source for detecting gene alterations and clonal heterogeneity in patients with CNS metastases (20). Previous research also suggested that cerebrospinal fluid (CSF)-cell free DNA (cfDNA) could reveal unique genetic profiles of intracranial metastases and guide clinical treatment of NSCLC patients with CNS metastases (21–24). However, the potential use of CSF as a liquid biopsy source remains to be examined in a real-world setting.

To provide more implications for the clinical application of liquid biopsy using CSF and explore its potential to identify

Abbreviations: CSF, cerebrospinal fluid; CNS, central nervous system; NSCLC, non-small cell lung cancer; NGS, next-generation sequencing; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; *ROS1*, ROS proto-oncogene 1; ctDNA, circulating tumor DNA; IASLC, International Association for the Study of Lung Cancer; BM, brain metastases; LM, leptomeningeal metastases; WBC, white blood cell; FFPE, formalin-fixed paraffin-embedded; cfDNA, circulating cell-free DNA; SNV, somatic single nucleotide variation; Indel, insertion or deletion of small fragment; CNV, copy number variants; SV, structural variants; MSAF, maximal somatic allele frequency; TKI, tyrosine kinase inhibitor.

actionable mutations and explore underlying resistance mechanisms for NSCLC patients with CNS metastases, we analyzed 1,396 samples from 970 NSCLC patients with CNS metastases (brain metastases [BM] and leptomeningeal metastases [LM]) who underwent NGS in real-world settings.

MATERIAL AND METHODS

Clinical Cohort

In this retrospective cohort study, a cohort of 970 NSCLC patients with CNS metastases (BM and LM) was enrolled at the Geneplus Medical Laboratory (Beijing, China) from May 2019 to July 2021. The diagnosis criteria for BM were based on metastatic lesions detected on brain magnetic resonance imaging, while the diagnostic criteria for LM were based on tumor cells detected in CSF samples or leptomeningeal enhancement on brain magnetic resonance imaging. To analyze the real-world efficacy of CSF in detecting actionable mutations, all patients underwent NGS in a laboratory accredited by the College of American Pathologists. Demographic, clinicopathological, and tumor histopathological results, such as TNM stag, metastatic sites, and cellular differentiation grade, were obtained for each patient. This study was approved by the Institutional Review Board of Shaanxi Provincial People's Hospital. All subjects provided written informed consent before undergoing any study-related procedures. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Sample Processing and DNA Extraction

Within 72 h of collection, peripheral blood samples were centrifuged to obtain plasma and white blood cells (WBCs). The CSF supernatant was centrifuged to separate it from the cell sediment. All tissue samples, including fresh and formalin-fixed paraffin-embedded (FFPE) tissue samples, underwent pathological assessment to confirm histologic classification and adequacy of the tumor tissues, which required a minimum of 20% tumor content. Circulating cell-free DNA (cfDNA) was isolated from the CSF supernatant and plasma using a QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany). Genomic DNA (gDNA) from WBCs and tumor tissues was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Circulating cfDNA from other body fluids were processed into indexed libraries, as discussed in previous studies (25–28).

Library Preparation and Target Enrichment

DNA concentration was measured using the Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and the Qubit dsDNA HS (High Sensitivity) Assay Kit (Invitrogen, Carlsbad, CA, USA). The size distribution of circulating cfDNA was assessed using the Agilent 2100 BioAnalyzer and DNA HS kit (Agilent Technologies, Santa Clara, CA, USA). The SeqCap EZ Library system (Roche NimbleGen, Madison, WI, USA) was used for target enrichment. In total, 1,386 libraries from 970 patients were hybridized to custom-designed biotinylated oligonucleotide probes (IDT, Coralville, IA, USA) covering 1.6 Mbp of the genome, and the captured genomic regions included 1,021 cancer-related genes (**Table S1**). The captured DNA fragments were amplified after hybrid selection and then pooled into several multiplexed libraries. Sequencing was performed using the Illumina Nextseq CN 500 (Illumina, San Diego, CA, USA) or the Gene$^+$Seq-2000 Sequencing System (GenePlus-Suzhou, Suzhou, China), according to the instructions of the manufacturer.

Sequencing and Data Analysis

Sequencing data were analyzed using default parameters. After the removal of terminal adaptor sequences and low-quality reads, the clean reads were aligned to the reference human genome (hg19) using the Burrows–Wheel Aligner (BWA; version 0.7.12-r1039) with default parameters. Base quality recalibration and local realignment were performed using the Gene Analysis Toolkit (GATK; version 3.4-46-gbc02625). Somatic single nucleotide variations (SNVs) and insertion or deletion of small fragments (Indels) were determined by the MuTect2 algorithm. The Contra algorithm (version 2.0.8) was used to detect somatic copy number alterations. All candidate fusion genes were manually mapped to the initial cfDNA fragments using unique barcoding and alignment information. The minimal mean effective depth was 300×, 1000×, and 1,000× in tissue, CSF, and plasma samples, respectively.

Statistical Analysis

Associations between two or more categorical variables were analyzed using Fisher's exact or Chi-square tests. The comparison of means among three or more groups was performed using one-way ANOVA tests. All statistical analyses and presentations were performed using R v3.6.3. All tests were two-sided, and p-values <0.05 represented statistical significance.

RESULTS

Study Design and Patient Demographics

In total, 970 patients (49.90%, male) with stage IV NSCLC and CNS metastases were enrolled in this study. Patient characteristics are presented in **Table 1**. Among patients, adenocarcinoma had the largest proportion (82.27%), followed by squamous carcinoma (2.99%), adenosquamous carcinoma (0.72%), and large cell carcinoma (0.21%). Cellular morphology was unidentified in 134 (13.81%) NSCLC cases. The median age at diagnosis was 57 (range, 18–91) years. Most patients (962/970) were diagnosed with BM, 16 had LM, and eight had both BM and LM.

In this real-world setting, 119 CSF, 416 tumor tissue, 791 plasma, and 70 other samples were collected. The 416 tissue samples included 269 primary tissues, 46 intracranial metastatic tissues, 39 lymph node tissues, 26 other metastatic tissues, and 36 tissues of unknown origin, while the 70 other samples included 59 pleural effusion samples, 5 already extracted DNA, 4

TABLE 1 | Patient Characteristics.

Characteristic	Number	Percentage (%)
Age (years)		
Median	57	–
Range	18–91	–
Gender		
Male	484	49.90
Female	486	50.10
Histology subtype		
Adenocarcinoma	798	82.27
Squamous	29	2.99
Adenosquamous	7	0.72
Large cell	2	0.21
NA	134	13.81
Specimen	N = 1,396*	
Cerebrospinal fluid	119	8.52
Tumor tissue	416	29.80
Plasma	791	56.66
Other**	70	5.01
Previous treatment		
No	240	17.19
Yes	1,156	82.81

**Including 282 patients had multiple samples tested simultaneously or consequently. **Including 59 pleural effusion samples, 5 already extracted DNA, 4 pericardial fluid samples, one brushing washing fluid, and one ascites. NA, not available.*

pericardial fluid samples, one brushing washing fluid, and one ascitic fluid. Most patients (86.7%, 688/970) had a single CSF, tissue, plasma, other body fluid sample, or DNA tested, while 282 patients had multiple samples tested simultaneously or consequently. Meanwhile, 240 samples were treatment-naive, containing 2 (0.83%) CSF, 129 (53.75%) tissue, 98 (40.83%) plasma, and 11 (4.58%) other samples; and 1,156 were treated, comprising 117 (10.12%) CSF, 287 (24.83%) tissue, 693 (59.95%) plasma, and 59 (5.10%) other samples (**Figure 1**).

Among 970 patients included in this study, 428 (44.12%) had distant metastases only from the CNS (CNSM group), and 542 (55.88%) patients had other organ involvement and distant metastases other than CNS metastases (CNSM+ group). Based on whether they were treated samples or distant metastases other than CNS metastases, the 1,396 samples from these 970 NSCLC patients with CNS metastases were divided into four groups: the treatment-naive CNSM group (n = 117), the treatment-naive CNSM+ group (n = 123), the treated CNSM group (n = 438), and the treated CNSM+ group (n = 718).

Comparisons between the four groups revealed the choice of specimen type for genetic profiling in NSCLC patients with CNS metastases in real-world settings (**Figure 2**). Among treatment-naive patients, tissue samples (51.22–56.41%) were the most examined, followed by plasma (40.17–41.46%), other samples (2.56–6.50%), and CSF (0.81–0.85%). Among treated patients, plasma samples (55.02–62.95%) were the most examined, followed by tissue (21.31–30.59%), CSF (9.61–10.96%), and other samples (3.42–6.13%), regardless of other organ involvement (**Figure 2**).

Moreover, plasma samples were slightly more often used in the treated CNSM+ group than in other groups (62.95% vs. 40.17–55.02%). And the treated CNSM group (10.96%) had the largest proportion of CSF samples (**Figure 2**).

CSF in Real-World Setting

To further analyze the efficacy of CSF samples in real-world settings, 970 NSCLC patients with CNS metastases were retrospectively analyzed. All samples were subjected to targeted NGS of 1,021 cancer-relevant genes. **Table S2** provides a detailed list of somatic alterations identified in each patient sample.

Alterations were identified in 114 (95.80%) CSF samples and 416 (100%) tumor tissue samples, compared to 684 (86.47%) plasma and 67 (95.71%) other samples. The detection rate of all alterations in CSF was lower than that in tumor tissues (95.80% vs. 100%, p <0.001) but higher than that in the plasma (95.80% vs. 86.47%, p <0.001) (**Figure 3A**).

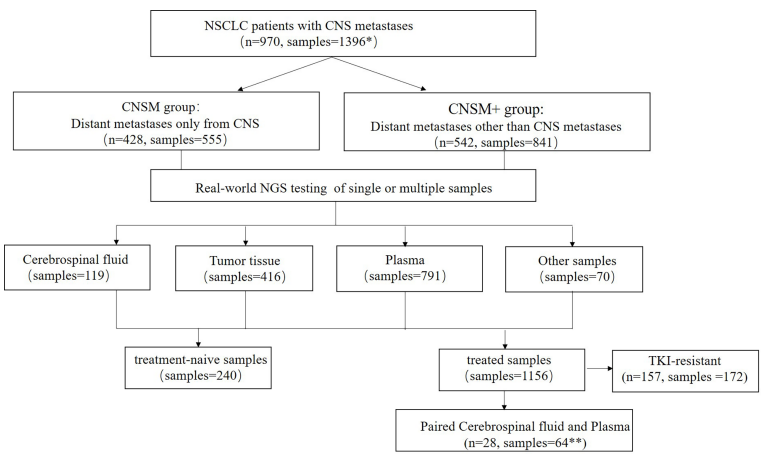


FIGURE 1 | Study design. NSCLC, non-small cell lung cancer; CNS, central nervous system; NGS, Next-generation sequencing. *Among the 970 patients in this study, 688 patients had one sample tested, while 282 patients had multiple samples tested simultaneously or consequently. **Three patients had two or more paired cerebrospinal fluid and plasma samples. CNSM group, patients who had distant metastases only from the CNS, CNSM+ group, patients with other organ involvement, and distant metastases other than CNS metastases.

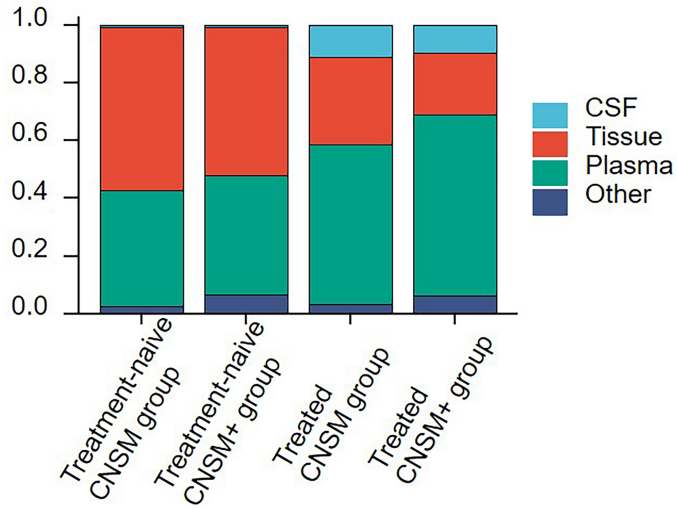


FIGURE 2 | Sample selection for genetic profiling in NSCLC patients with CNS metastases. The 1,396 samples were divided into the following 4 groups according to treatment history and metastasis sites: the treatment-naive CNSM group (n = 117), the treatment-naive CNSM+ group (n = 123), the treated CNSM group (n = 438), and the treated CNSM+ group (n = 718). CNSM group, patients had distant metastases only from the CNS, CNSM+ group, patients with other organ involvement and distant metastases other than CNS metastases.

As shown in **Figure 3A**, 94.96% (113/119), 98.56% (410/416), 84.20% (666/791), and 95.71% (67/69) of CSF, tumor tissue, plasma cfDNA, and other samples, respectively, showed detectable SNV or Indel somatic alterations. The copy number variant (CNV) and structural variant (SV) alteration rates in different samples were also compared. The detection rate of SNVs or Indels in CSF samples was lower than that in tumor tissues (94.96% vs. 98.56%, p = 0.0193). However, the CNV alteration rate in CSF samples was significantly higher than that in tumor tissues (76.47% vs. 61.06%, p = 0.0019), and SV detection rate was not statistically different between CSF and tumor tissues (11.76% vs. 15.63%, p = 0.2952). Furthermore, the SNV or Indel, CNV, and SV alteration rates in CSF samples were significantly higher than that in plasma (SNV or Indel, 94.96% vs. 84.20%, p <0.001; CNV, 76.47% vs. 12.39%, p <0.0001; SV, 11.76% vs. 6.57%, p <0.05). This indicated that compared to plasma, CSF exhibited more CNV alterations (**Figure 3A**).

The maximal somatic allele frequency (MSAF) of the CSF was compared to that of other types of samples (**Figure 3B**). The MSAFs of CSF samples were significantly higher than those of plasma and tumor tissues, with the MSAFs of tumor tissues being significantly higher than those of plasma (all p <0.001).

Genetic profiles of CSF cfDNA, tumor tissue, plasma, and other body fluid samples or DNA from treated patients were compared. In treated-CSF samples, most recurrent mutations were observed in the *EGFR* gene, followed by *TP53*, which is the

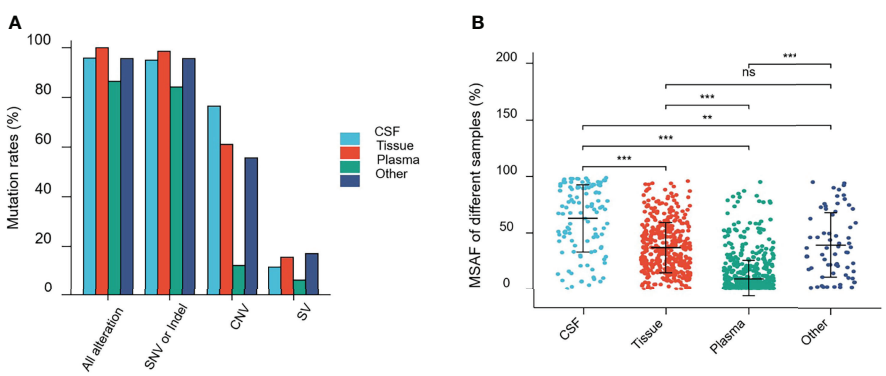


FIGURE 3 | Mutations rates and Maximal somatic allele frequency (MSAF). **(A)** Mutations detected in different samples from patients in this study. **(B)** The MSAFs of CSF, tissue, plasma, and other samples from patients in this study. ns, p ≥0.05; **p <0.01; ***p <0.001.

same as in treated plasma and other samples (other body fluid samples or DNA) types. Among treated-tumor tissues, the most recurrent mutations were observed in *TP53*, followed by *EGFR* (**Figure 4**), which indicated that *EGFR* and *TP53* were the two most frequently mutated genes in all sample types, as previously reported (16).

We performed a subgroup analysis, to more accurately analyze the detectability of different fluid biopsies (CSF and plasma) in patients with different metastatic sites in real-world settings. Due to the small number of treatment-naive patients who opted for CSF testing, this analysis was performed on treated patients. On the basis of distant metastases other than the CNS metastases, samples were divided into two groups as follows: distant metastases only from the CNS group (CNSM group) and distant metastases other than CNS metastases group (CNSM+ group). We analyzed mutation rates and MSAF of CSF and plasma in the two groups of treated patients.

The mutation rate in CSF samples was significantly higher than that in the plasma in both the treated CNSM and CNSM+ groups (CNSM group, 93.75% vs. 80.50, p = 0.0121; CNSM+ group, 97.10% vs. 88.05%, p = 0.0066) (**Figure 5A**). For plasma samples, the mutation rate in the treated CNSM+ group was significantly higher than that in the treated CNSM group (88.05% vs. 80.50, p = 0.0003) (**Figure 5A**). There was no significant difference in the mutation rate between the treated CNSM and CNSM+ groups (93.75% vs. 97.10%, p = 0.3756) (**Figure 5A**). For MSAF of treated-samples, MSAFs of CSF samples were significantly higher than those of plasma in both the CNSM and CNSM+ groups (all p <0.001), and MSAFs of plasma in the CNSM+ group were significantly higher than those of the CNSM group (p <0.001) (**Figure 5B**).

Driver Gene Alterations in CSF

To further analyze the detective capability of actionable mutations of CSF samples in a real-world setting, 1,156 treated samples from NSCLC patients with CNS metastases were retrospectively analyzed. Thus, we compared all actionable mutations and *EGFR*, *ALK*, *ROS1*, *BRAF*, *KRAS*, *MET*, *RET*, and *ERBB2* alterations in patients in the CNSM (distant metastases only from the CNS) and CNSM+ (distant metastases other than CNS metastases) groups. The actionable mutation detection rates of the different groups are shown in **Figures 6A, B**, and the actionable mutation detection numbers of different groups are shown in **Figure 6C**.

Compared to tumor tissue, the detection rates of actionable *EGFR* in CSF were significantly higher than those in tissue samples in both the CNSM and CNSM+ groups (all p <0.001) (**Figures 6A, B**). CSF and tumor tissue had similar sensitivity in detecting all actionable mutations in both the CNSM and CNSM + groups (89.58% vs. 94.77% for the CNSM group, P = 0.2008; 95.65% vs. 97.38% for the CNSM+ group, P = 0.4848) (**Figures 6A, B**). The detection rates of actionable *EGFR* and all actionable mutations in CSF were significantly higher than those in plasma samples in both the CNSM and CNSM+ groups (all p <0.0001) (**Figures 6A, B**).

For CSF, the *EGFR* and all actionable mutation detection rates were not statistically different between the CNSM and CNSM+ groups (p = 0.1977 for the CNSM group; p = 0.1001 for the

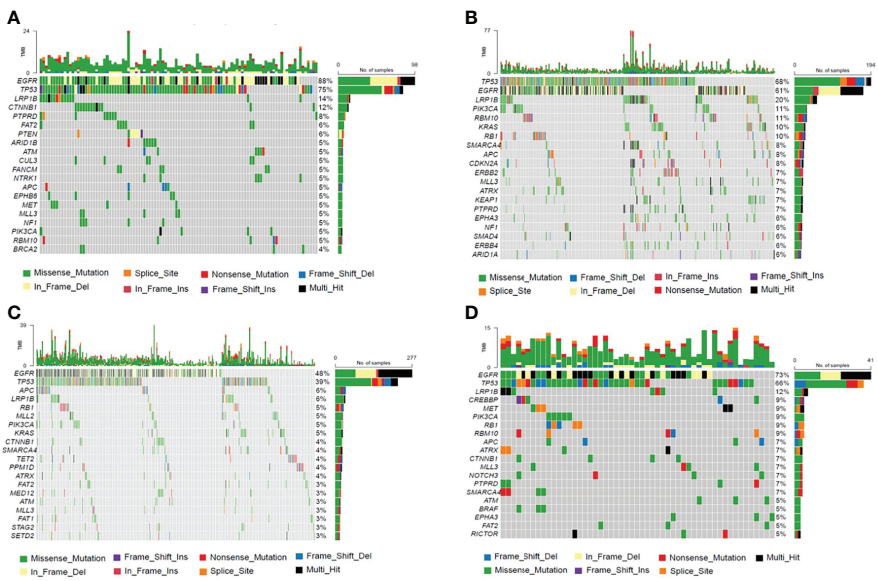


FIGURE 4 | The Frequency Spectrum for treated patients with detectable SNV or Indel mutations. **(A)** Mutation frequency spectrum in treated-CSF samples (six samples were not detected; they only showed the twenty most frequently mutated genes). **(B)** Mutation frequency spectrum in treated-tumor tissue samples (three samples were not detected; they only showed the twenty most frequently mutated genes). **(C)** Mutation frequency spectrum in treated-plasma (117 samples were not detected; they only showed the twenty most frequently mutated genes). **(D)** Mutation frequency spectrum in treated-other samples (two samples were not detected; they only showed the twenty most frequently mutated genes).

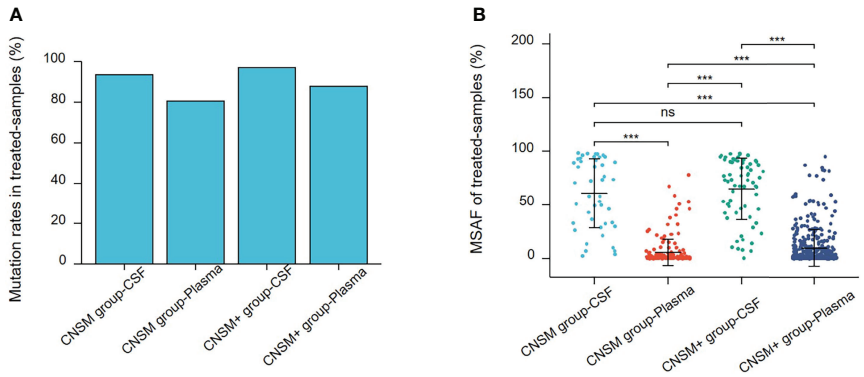


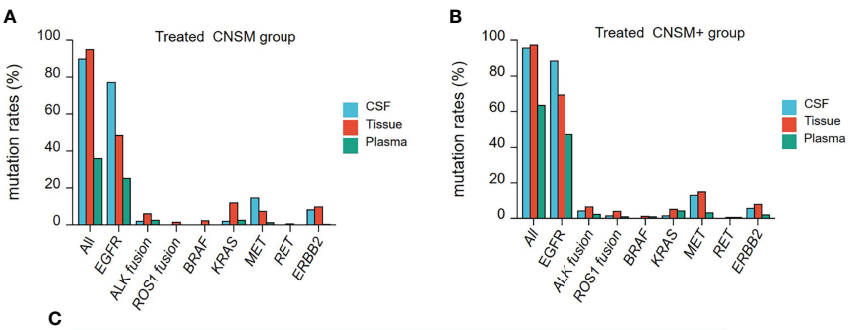
FIGURE 5 | Mutations rates and MSAF of treated samples. **(A)** Mutations detected in different treated samples from patients in the treated CNSM or CNSM+ groups. **(B)** MSAFs of CSF and plasma samples from patients in the treated CNSM or CNSM+ groups. ns, $p \geq 0.05$; ***$p < 0.001$. CNSM group, patients had distant metastases only from the CNS, CNSM+ group, patients with other organ involvement and distant metastases other than CNS metastases.

CNSM+ group) (**Figures 6A, B**). For plasma, detection rates of actionable *EGFR* and all actionable mutations in the CNSM+ group were significantly higher than in the CNSM group (all $p < 0.0001$) (**Figures 6A, B**). CSF showed a potential capability to detect actionable mutations.

Concordance of Paired CSF and Plasma Samples

To verify the results described above, a concordance analysis was performed on 28 treated patients with 32 pairs of paired CSF and plasma samples, which were simultaneously tested for 1,021 cancer-relevant genes. In all 32 paired samples, the detection rate of cfDNA in CSF and plasma was the same (90.62%, 29/32). The MSAF of 32 CSF cfDNAs was compared to that of plasma cfDNA from 28 patients. The MSAF of the CSF was significantly higher than that of the matched plasma cfDNA ($p < 0.001$) (**Figure 7A**).

In the 32 paired plasma and CSF samples, 442 alterations were detected, 377 and 92 of which were detected in CSF and plasma, respectively. As shown in **Figure 7B**, 27 alterations were



C

	CSF		Tissue		Plasma	
	CNSM group Sample=48	CNSM+ group Sample=69	CNSM group Sample=134	CNSM+ group Sample=153	CNSM group Sample=241	CNSM+ group Sample=452
All actionable mutations	43	66	127	149	87	287
EGFR	37	61	65	106	61	213
ALK fusion	1	3	8	10	6	11
ROS1 fusion	0	1	2	6	0	4
BRAF	0	0	3	2	0	4
KRAS	1	1	16	8	6	19
MET	7	9	10	23	3	14
RET	0	0	1	1	0	3
ERBB2	4	4	13	12	1	10

FIGURE 6 | Actionable mutations rates in treated NSCLC patients with CNS metastases. **(A, B)** The actionable mutation detection rates of different groups; **(A)** CNSM group: distant metastases only from the CNS group; **(B)** CNSM+ group: distant metastases other than CNS metastases group. **(C)** Actionable mutations detected in different samples from treated NSCLC patients with CNS metastases. CNSM group, patients had distant metastases only from the CNS, CNSM+ group, patients with other organ involvement and distant metastases other than CNS metastases.

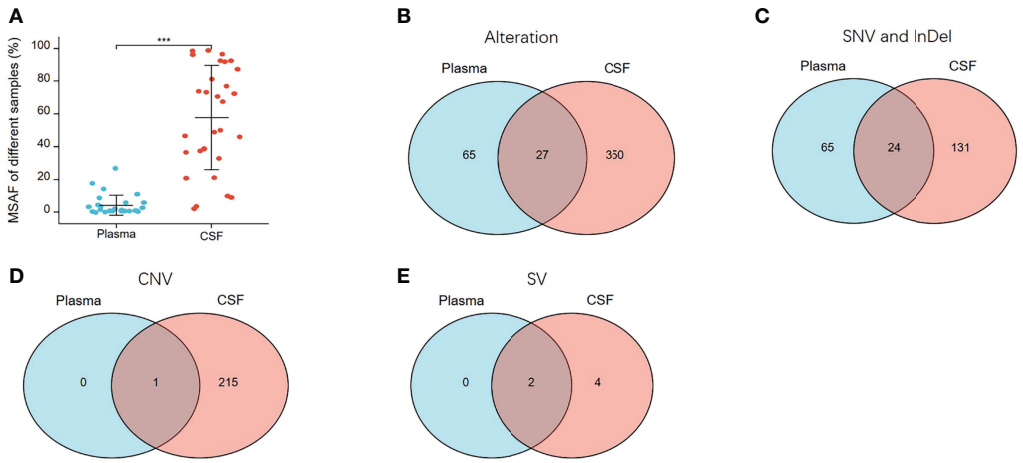


FIGURE 7 | MSAF and concordance of 32 paired CSF and plasma samples. **(A)** MSAF of 32 paired CSF and plasma samples from 28 treated-patients. **(B)** The concordance of CSF and plasma samples for detection of all alterations. **(C)** The concordance of CSF and plasma samples for detection of SNV or InDel. **(D)** The concordance of CSF and plasma samples for detection of CNV. **(E)** The concordance of CSF and plasma samples for detection of SV. ***$p < 0.001$.

detectable in both plasma and CSF, 65 were undetectable in CSF, and 350 were undetectable in plasma. The same alterations were 10.91% (27/442). For SNV or InDel, 220 mutations were detected, of which 155 were detected in CSFs and 89 in plasma. The same mutations were 10.91% (24/220) (**Figure 7C**). For CNV, 216 CNV alterations were detected, of which 216 were detected in CSF and only one in plasma (**Figure 7D**), and for SV, six SV alterations were detected, of which six were detected in CSF and two in plasma (**Figure 7E**). From multiple comparisons, it is evident that CSF can better detect alterations than plasma, especially CNV and SV alterations.

CSF for TKI-Resistance Mechanisms Exploring

In this retrospective cohort, seventy-seven NSCLC patients with CNS metastases who had previously received targeted treatment with EGFR- or ALK-, or ROS1-tyrosine kinase inhibitors (TKIs) were tested for NGS using CSF samples. We successfully tested 100 CSF samples obtained from 77 patients, of which 22 patients were resistant to first or second-generation EGFR-TKIs, 49 were resistant to osimertinib or almonertinib, and 6 were resistant to ALK- or ROS1-TKIs (**Table 2**).

While exploring mechanisms of TKI-resistance, EGFR-TKI sensitizing mutations were undetected in CSF cfDNA in 4.30% (4/93) of patients with EGFR-TKI resistance. Fifty-six patients (78.87%, 56/71) harbored concurrent alterations that might limit the efficacy of EGFR-TKIs, namely, *EGFR* resistance mutation, activation of bypass signaling pathways, *EGFR* amplification, *TP53* exon8 mutation, PI3K-AKT-mTOR gene alterations, and cell cycle gene alterations. In total, known EGFR-TKIs resistance mechanisms, such as PI3K-AKT-mTOR signaling-related genomic alteration, *MET* amplification, and absence of sensitizing mutations were detected in 1, 3, and 1 patient(s), respectively, who were resistant to first- or second-generation EGFR-TKIs. *EGFR* C797S/L792X/G724S/L718Q, PI3K-AKT-mTOR signaling-related genomic alteration, *KRAS* mutation, *ERBB2* amplification, *MET* amplification, and absence of sensitizing mutations were detected in 8, 7, 1, 4, 9, and 3 patients, respectively, who were resistant to osimertinib or almonertinib. Cell cycle gene alterations, *EGFR* amplification, and *TP53* exon8 mutations were identified in 11, 43, and 13 patients with EGFR-TKI resistance, respectively. However, determining whether it would result in EGFR-TKI resistance remains controversial. Co-occurrence of resistance mechanisms was observed in 21 patients, including one patient without EGFR-TKIs sensitizing mutations.

Four patients with ALK- or ROS1-TKI resistance were identified as having *ALK* or *ROS1* fusions. Known ALK-TK resistance mechanisms, such as *ALK* G1269A and absent sensitizing mutations, were detected in one and two of five patients with ALK-TKIs resistance. *TP53* mutations that may limit the efficacy of ALK-TKIs were identified in two patients with ALK-TKI resistance.

This confirmed that liquid biopsy using CSF showed high potential in exploring underlying resistance mechanisms in NSCLC patients with CNS metastases.

DISCUSSION

NSCLC patients with CNS metastases usually have a poor prognosis and limited treatment options. However, following the development of cancer genomics and more effective targeted therapies, new treatments are emerging (4–7, 9). Genotyping can provide genomic information and evolutionary patterns of CNS metastases in NSCLC patients, which may be key in using targeted therapeutic strategies (14, 29). However, because CNS

TABLE 2 | Known resistant mutations detected in CSF samples from NSCLC patients with CNS metastases who had previously received targeted treatment.

	1st/2nd-generation EGFR-TKIs (n = 22, sample = 26)	Osimertinib/almonertinib (n = 49, sample = 67)	ALK- or ROS1-TKIs (n = 6, sample = 7)
EGFR T790M	/	–	–
C797X, L792X G724S, L718Q	–	8	–
ALK/ROS1 SNV	–	–	1
PI3K-AKT-mTOR signaling-related genomic alteration	1	7	/
KRAS mutation	/	1	/
ERBB2 amplification	/	4	/
MET amplification	3	9	/
cell cycle gene alterations	5	6	–
EGFR amplification	9	34	–
TP53 exon8 mutation	6	7	–
TP53 mutation	–	–	2
without sensitizing mutations	1	3	2
Total	25	79	5

tissue collection is difficult and invasive, and plasma insensitivity owing to its inability to penetrate the blood–brain barrier develops, it is clinically challenging to select a suitable sample for genetic testing and genotyping in clinical practice (15, 21).

Among NSCLC patients with CNS metastasis, ctDNA in CSF, which circulates throughout the CNS, can reveal genomic alterations in intracranial lesions (30–32). Previous studies indicated that CSF ctDNA was more exact and complete than plasma ctDNA and could thus be an optimal source of liquid biopsy for genotyping to guide therapy and predict prognosis (21–24). CSF genetic alterations have been associated with the survival of advanced lung adenocarcinoma patients with CNS metastases (24). However, only a limited number of NSCLC patients with CNS metastases were included in these studies; furthermore,the potential use of CSF in real-world settings is yet to be examined.

To date, this real-world study recruited the largest cohort of NSCLC patients with CNS metastases who had CSF or other samples available for NGS testing. All CSF samples in this study were tested using the CSF supernatant, as it was reported that more mutations could be detected in cfDNA from the CSF supernatant than in paired CSF cells because CSF cfDNA was less affected by non-tumor cell components (30, 33). Comparisons between groups divided according to treatment history and sites of metastasis revealed the choice of specimen type for genetic profiling among these NSCLC patients in the real world. For NSCLC patients with CNS metastases, tissue and plasma samples were the most frequently examined in treatment-naive patients and treated patients, respectively. It is worth mentioning that only 11.06% (46/416) of all tissue samples were intracranial lesions, and there was heterogeneity between intracranial and extra-cranial lesions (14); therefore, extra-cranial lesions may not be the optimal sample for NSCLC patients with CNS metastases. CSF was the choice for genetic profiling in all groups, and treated patients who had distant metastases only from the CNS (10.96%) had the largest proportion of CSF samples.

This study also investigated the genetic alterations in CSF samples from NSCLC patients. Alteration rates (including all alterations, SNV or Indels, and CNV), especially CNV alterations, in the CSF samples were significantly higher than those in plasma (all $p < 0.001$), which corresponded to the findings of other reports (16). The MSAFs of the CSF samples were significantly higher than those of plasma and tumor tissues (all $p < 0.001$). We speculated that the lower MSAF in plasma may account for the inferior detection efficacy of plasma compared to CSF and tissues. A comparison of the genetic profiles in different treated-samples indicated that *EGFR* and *TP53* were the two most frequently mutated genes in all sample types, which were the same as previously reported (16). These real-world data suggest that cfDNA isolated from CSF can effectively provide important genomic information about an individual tumor and also that CSF can be used as a substitute in the absence of intracranial tumor tissue.

For driver gene alterations, the detection rate of *EGFR*, *ALK*, *ROS1*, *BRAF*, *KRAS*, *RET*, *MET*, and *ERBB2* alterations in the tissues was consistent with previous reports (34). Remarkably, the detection rates of all actionable mutations and *EGFR* in CSF were higher than those in plasma samples; moreover, *EGFR* in CSF was higher than that in tissue samples from treated patients, regardless of distant metastases other than CNS metastases (all $p < 0.0001$). Thus, for treated NSCLC patients with CNS metastases, CSF outperformed plasma in detecting actionable mutations. For concordance between paired CSF and plasma samples, the MSAF of the CSF was significantly higher than that of the matched plasma cfDNA ($p < 0.001$). From multiple comparisons, it was seen that CSF is better than plasma in detecting alterations, especially CNV and SV alterations.

This study investigated the performance of CSF in detecting resistance mutations among treated patients. CSF cfDNA for identifying mutations can show why the efficacy of EGFR-TKIs was limited in 56 patients (78.87%, 56/71). A recent study evaluated the role of CSF-NGS in osimertinib-treated *EGFR*-mutated NSCLC and found that the detection rate of *EGFR* mutations using CSF genotyping was 97.1%, compared to 95.5% in our study (35). It also showed that CSF might reveal resistance mechanisms such as C797S mutation, MET dysregulation, and cell cycle pathway alterations implicated in osimertinib failure, which our study also confirmed (35). A previous study confirmed

that identifying resistant ALK secondary mutations is essential in ALK fusion-positive NSCLC patients progressing after ALK-TKI therapy, as it can influence sensitivity to subsequent ALK-TKIs (36). In this study, patients with ALK-TKI resistance were identified with ALK secondary mutations using CSF cfDNA, and patients with ALK- or ROS1-TKI resistance were identified as having an ALK or ROS1 fusion and known ALK-TKI resistance mechanisms. We demonstrated that CSF cfDNA was more informative in identifying secondary mutations among drug-resistant patients and initial sensitive mutations than plasma, as previously reported. CSF cfDNA could be used for intracranial biopsy to test for acquired resistance in NSCLC patients with CNS metastases.

Precision medicine has led to improvements in the prognosis of patients with advanced NSCLC. Adoption of NGS is time-saving and cost-efficient (37). For NSCLC patients with CNS metastases, the selection of the appropriate sample type for NGS to save time and reduce the overall cost of testing is important. Real-world data demonstrates that analysis of cfDNA isolated from CSF may provide important genomic information for NSCLC patients with CNS metastases. Overall, more treated patients choose CSF for genetic testing than treatment-naive patients do. However, fewer patients choose CSF as a test sample than plasma or tissue in the real world, possibly because it is more traumatic and more difficult to perform a lumbar puncture than to obtain CSF from blood. Therefore, it is important to select NSCLC patients with CNS metastases who are clinically suitable for CSF testing. Meanwhile, a previous study reported that clinical factors such as the diameter of the largest intracranial lesion and the minimum lesion–ventricle distance for all intracranial lesions had significant influence on the detection of CSF (38). In clinical practice, specific clinical manifestations of patients can be combined to select suitable specimens. However, plasma is the preferred choice for molecular profiling in all groups in the real-world, especially in treated patients. Meanwhile,the detection rate was unsatisfactory, especially in patients who had distant metastases only from the CNS, and in the detection of CNV alteration. Moreover, the detection rate of genomic alterations has been reported to be lower in the plasma ctDNA of patients with isolated CNS disease, and complementary tests such as CSF cfDNA may be useful for these patients (39). Therefore, for patients with isolated CNS metastases and those who have retested CNV mutations, other samples such as CSF are recommended for real-world genetic testing.

This study has some limitations, which include its retrospective snapshot study design, without consideration of additional clinical factors and therapeutic efficacy. Hence, we could not precisely determine optimal specimens for patients in different clusters. The number of treatment-naive patients included in this study was small, and the role of CSF in this subset of patients is limited. Furthermore, the paired CSF and plasma samples were small, and no tissue samples were included. Therefore, we could not investigate concordance between tissue, plasma, and CSF at the same time point. The clinical implications of CSF genotyping on treatment outcomes were not analyzed in this study.

CONCLUSIONS

This large-scale, real-world study confirmed that liquid biopsy using CSF showed high potential for identifying actionable mutations and exploring the underlying resistance mechanisms in NSCLC patients with CNS metastases. CSF can be used as a liquid biopsy source to facilitate the broad exploration of potential resistance mechanisms in clinical practice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found in the GVM in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences, under accession number GVM000345, https://ngdc.cncb.ac.cn/gvm/getProjectDetail?project=GVM000345.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Shaanxi Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FS, JB, NL, WG, and ZF contributed to conception and design of the study. JB, JZ, WG, GD, MZ, and QH organized the database. KW and MY performed the statistical analysis. KW and RC wrote the first draft of the manuscript. KJ, XW, YM, and GD wrote sections of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest: KW, MY, and RC are current employees of Geneplus-Beijing.

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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Efficacy and safety of bevacizumab biosimilar compared with reference bevacizumab in locally advanced and advanced non-small cell lung cancer patients: A retrospective study

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Background: Bevacizumab has played an important role in the systemic treatment of patients with advanced non-small-cell lung cancer (NSCLC) without gene mutation. In recent years, bevacizumab biosimilar has received marketing approval based on the results of phase III clinical studies. However, more clinical data are needed to verify the efficacy and safety of bevacizumab biosimilar in clinical application.

Materials and methods: We identified 946 patients with locally advanced or metastatic NSCLC who were treated with bevacizumab biosimilar or bevacizumab from January 1, 2019 to November 30, 2021. Comparisons and statistical analyses of bevacizumab biosimilar and bevacizumab were made in terms of efficacy and safety. Efficacy evaluation was performed directly in accordance with RECIST v1.1. Adverse events were graded following the National Cancer Institute Common Terminology Criteria for Adverse Events v5.0.

Results: The objective response rates (ORRs) were 28.9% in the biosimilar group (n=551) and 30.9% in the reference group (n=395; unstratified ORR risk ratio: 0.934, 95% confidence interval [CI]: 0.677–1.138; unstratified ORR risk difference: −0.020, 95% CI: −0.118–0.035). The estimated median progression-free survival (mPFS) were 6.27 (95% CI: 5.53–7.01) and 4.93 (95% CI: 4.24–5.62) months in the biosimilar and reference groups, respectively (P=0.296). The number of treatment lines, combined treatment regimens and

with or without radiotherapy were significant factors affecting the PFS of both groups (P<0.001, P=0.001, P=0.039). Different genetic mutations and dose intensity were not the main factors affecting PFS (P=0.627, P=0.946). The incidences of treatment-emergent adverse events (TEAEs) were 76.41% in the biosimilar group and 71.65% in the reference group (P=0.098). The incidences of grade 3 or higher TEAEs were 22.14% and 19.49% in the biosimilar and reference groups, respectively (P=0.324).

Conclusions: Bevacizumab biosimilar is equivalent in efficacy to bevacizumab in patients with locally advanced and advanced NSCLC. It showed acceptable toxicity profile and no new adverse events. Patients who were excluded by clinical trials can also benefit from bevacizumab biosimilar.

KEYWORDS

antiangiogenic treatment, bevacizumab, biosimilar, non-small cell lung cancer, lung cancer

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide with an estimated 1.8 million deaths each year (1). Approximately 57% of patients have distant metastasis at the time of initial diagnosis and lose surgical indications (2). Therefore, systemic therapy plays an important role in lung cancer treatment. Vascular endothelial growth factor (VEGF) acts as a key regulator of tumor angiogenesis and is associated with increased risks of recurrence, metastasis, and death (3, 4). Bevacizumab is a recombinant humanized monoclonal antibody that specifically interrupts the interaction between human VEGF and endothelial cell surface receptors, inhibiting the biological activity of VEGF and limiting angiogenesis (5). Randomized controlled trials have demonstrated that bevacizumab in combination with chemotherapy improves overall survival (OS) and progression-free survival (PFS) relative to chemotherapy alone (6, 7). In addition, recent evidence points to novel combinations of bevacizumab with another targeted therapy or immunotherapy, as well as maintenance therapy after disease progression (8).

A biosimilar is a biological product that is highly similar to the reference product with no clinically remarkable differences in safety, purity, or potency (9). A biosimilar is an important avenue to reduce patient expenditure and the financial burden of national healthcare systems while maintaining therapeutic effect (10). However, a biosimilar is not an exact copy of its reference product and thus requires vigilance and concern in clinical application (11, 12).

Currently, prospective clinical trials have confirmed that bevacizumab biosimilar in combination with platinum-containing two-drug chemotherapy has similar efficacy and safety compared with bevacizumab in patients with untreated advanced non-squamous non-small-cell lung cancer (NSCLC) (13, 14). In 2019, China National Medical Products Administration approved the marketing of the bevacizumab biosimilar developed by Qilu Pharmaceutical Co., Ltd. (trade name: Encoda) with all indications approved for bevacizumab in China. However, no clinical data has verified the efficacy and safety of this biosimilar in clinical application. Existing trials (15–17) have excluded patients receiving previous treatment, patients receiving combination with targeted therapy or immunotherapy, patients with brain metastases, patients with rare genetic mutations (such as EML4-ALK rearrangement), and patients with Eastern Cooperative Oncology Group (ECOG) scores greater than 2. The efficacy and safety of bevacizumab biosimilar have no concensus in these populations. The purpose of the current investigation was to retrospectively analyze and compare the efficacy and safety of bevacizumab biosimilar and reference bevacizumab in patients with locally advanced and advanced NSCLC in clinical application and to provide reference for clinical decision-making.

Materials and methods

We studied the medical records of all patients with locally advanced and advanced non-squamous NSCLC treated at Jiangsu Cancer Hospital from January 1, 2019 to November 30, 2021. We screened patients who received bevacizumab biosimilar or bevacizumab treatment. All patients included in the study had at least one measurable disease. This study was approved by the Academic Ethics Committee of Jiangsu Cancer Hospital (reference No.036 (2022)). All patients gave informed consent and signed the consent form.

Data collection and response assessment

Medical records were examined and separated by clinical pathologic features and treatment histories. Radiographic examinations were performed to assess the efficacy at two cycles after initiation, and then the state of the tumor was assessed every two cycles or when symptoms of suspected disease progression occurred. Data and follow-up records were updated as of December 1, 2021. Efficacy evaluation was performed directly in accordance with the Response Evaluation Criteria in Solid Tumor 1.1. The best response to bevacizumab biosimilar or bevacizumab treatment was defined as a complete response (CR) or partial response (PR) and stable disease achieved at least once during therapy. The primary efficacy endpoint was the objective response rate (ORR) defined as the CR or PR rate. The secondary efficacy endpoint was the progression-free survival (PFS) defined as the time from treatment initiation to clinical or radiographic progression or death. Adverse events (AEs) were graded by the National Cancer Institute Common Terminology Criteria for Adverse Events v5.0.

Statistical analysis

Differences in baseline clinical features, efficacy, and safety between groups were measured by χ^2 test or independent T test. Survival data were estimated using the Kaplan–Meier method, including 95% confidence intervals (CIs). Significant differences between these curves were determined using log-rank test. Multivariate analysis of PFS was conducted by Cox proportional risk analysis. All statistical analyses were conducted using the SPSS (version 25.0) and R software (version 3.6.3). Statistical significance was set at $P<0.05$.

Results

Patient characteristics and treatment exposure

A total of 946 patients were included in this study, including 551 patients who received bevacizumab biosimilar (biosimilar group) and 395 patients who received bevacizumab (reference group). The baseline characteristics of the patients are summarized in Table 1. The subjects' demographics and baseline disease characteristics were well balanced between the treatment groups with no statistical differences. Overall, the median age of the 946 patients was 60.5 years, including 533 males (56.34%) and 413 females (43.66%). Adenocarcinoma was the most common pathological type (98.52%). The remaining 14 nonadenocarcinomas included 7 adenosquamous carcinoma, 3 sarcomatoid carcinoma, 2 large cell carcinoma, and 2 undifferentiated carcinoma. The proportion of patients without gene mutation was the highest (44.83%), followed by patients with EGFR L858R mutation (22.69%) and patients with EGFR exon 19 deletion (17.12%). Before initial treatment with bevacizumab or bevacizumab biosimilar, 209 patients (21.88%) had brain metastases, 136 patients (14.38%) had liver metastases, and 136 patients (14.38%) had clinically diagnosed hypertension.

Exposure to treatment agents was comparable between the groups. The mean durations of exposure were 6.98 (standard deviation [SD], 6.03) and 8.31 (9.74) months (P=0.121) and the mean number of doses were 7.5 (5.45) and 7.8 (6.02, P=0.364) in the biosimilar and reference groups, respectively. The mean dose intensities in the biosimilar and reference groups were 8.37 and 7.64 mg/kg per cycle, respectively (P=0.823).

Efficacy

No patient achieved CR. In the biosimilar group (n=551), 159 patients experienced PR and 334 patients experienced SD with an ORR of 28.9% (95% CI: 25.1%–32.7%) and a disease control rate (DCR) of 89.5% as shown in Figure 1A. In the reference group (n=395), 122 patients developed PR and 223 patients developed SD with an ORR of 30.9% (95% CI: 26.3%–35.5%) and a DCR of 87.3%. As is shown in Table 2, the unstratified ORR risk ratio was 0.934 with a 95% CI of 0.767–1.138 and a 90% CI of 0.792–1.103. The unstratified ORR risk difference was −0.020 with a 95% CI of −0.118–0.035 and a 90% CI of −0.105–0.023. The results fell within the range prescribed by the Food and Drug Administration (FDA), Japan's Pharmaceuticals and Medical Devices Agency (PMDA), and the European Medicines Agency (EMA). This result indicates that the bevacizumab biosimilar showed similar efficacy to bevacizumab. As shown in Table 3, the subgroup analyses were performed based on the following subgroups: sex, age, pathology, stage, number of treatment lines, radiotherapy, combined treatment regimens, combined chemotherapy regimens (if combined with chemotherapy), dose intensity, genetic mutations, brain metastasis, liver metastasis, and history of hypertension. In a subgroup analysis of combined treatment regimens, the ORR of the biosimilar group was lower than that of the reference group when combined with chemotherapy and immunotherapy (22.58% vs. 33.64%, P=0.048, Figure 1B). Further logistic regression analysis showed that the influencing factors of ORR in patients under the biosimilar combined with chemotherapy and immunotherapy were the number of treatment lines and combination with radiotherapy (P=0.021, 0.008). Product type (i.e., bevacizumab or bevacizumab biosimilar) was not a main factor (P=0.604). Overall, patients in the first-line treatment group revealed relatively higher ORR and DCR than those in the second- or later-line therapy (biosimilar group: ORR: 37.29% vs.

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TABLE 1 Clinical characteristics and treatment exposure of patients.

	Total (n=946)	biosimilar group (n=551)	reference group (n=395)	P
Sex				
Male	533 (56.34%)	305 (55.35%)	228 (57.72%)	0.469
Female	413 (43.66%)	246 (44.65%)	167 (42.48%)	
Age (years)				
≥60	524 (55.39%)	303 (54.99%)	221 (55.95%)	0.770
<60	422 (44.61%)	248 (45.01%)	174 (44.05%)	
Pathological type				
adenocarcinoma	932 (98.52%)	543 (98.55%)	389 (98.48%)	0.933
Nonadenocarcinoma	14 (1.48%)	8 (1.45%)	6 (1.52%)	
Stage of cancer				
IIIB	54 (5.71%)	32 (5.81%)	22 (5.57%)	0.876
IV	892 (94.29%)	519 (94.19%)	373 (94.43%)	
Gene mutation type				
No genetic mutation	448 (48.41%)	247 (44.83%)	201 (50.89%)	0.066
EGFR exon 18 point mutation	4 (0.42%)	2 (0.36%)	2 (0.51%)	0.738
EGFR exon 19 deletion	162 (17.12%)	101 (18.33%)	61 (15.44%)	0.245
EGFR exon 20 insertion	24 (2.54%)	15 (2.72%)	9 (2.28%)	0.669
EGFR the L858R point mutation	209 (21.04%)	125 (22.69%)	84 (21.27%)	0.604
EGFR double mutation	10 (1.06%)	4 (0.73%)	6 (1.52%)	0.335
ALK rearrangement	26 (2.75%)	14 (2.54%)	12 (3.04%)	0.645
ROS1 rearrangement	8 (0.85%)	6 (1.09%)	2 (0.51%)	0.480
RET rearrangement	7 (0.74%)	6 (1.09%)	1 (0.25%)	0.249
BRAF mutation	6 (0.63%)	4 (0.73%)	2 (0.51%)	1.000
HER2 mutation	9 (0.95%)	6 (1.09%)	3 (0.76%)	0.742
KRAS mutation	26 (2.75%)	17 (3.09%)	9 (2.28%)	0.454
MET aberration	7 (0.74%)	4 (0.73%)	3 (0.76%)	1.000
with brain metastases				
Yes	209 (21.88%)	120 (21.78%)	89 (22.03%)	0.783
No	737 (78.12%)	431 (78.22%)	306 (77.97%)	
with liver metastases				
Yes	136 (14.38%)	72 (13.07%)	64 (16.20%)	0.175
no	810 (85.62%)	479 (86.93%)	331 (83.80%)	
with a history of hypertension				
yes	136 (14.38%)	81 (14.70%)	55 (13.92%)	0.737
no	810 (85.62%)	470 (85.30%)	340 (86.08%)	
the mean duration of exposure (m)	7.54	6.98	8.31	0.121

(Continued)

TABLE 1 Continued

	Total (n=946)	biosimilar group (n=551)	reference group (n=395)	P
the mean number of doses	7.64	7.53	7.81	0.364
the mean dose intensity (mg/kg)	8.07	8.37	7.64	0.823

19.72% vs. 16.98%, *P*<0.001, Figure 1C; DCR: 93.07% vs. 85.92% vs. 83.96%, *P*=0.009; reference group: ORR: 35.96% vs. 26.00% vs. 25.00%, *P*=0.080; DCR: 93.10% vs. 82.00% vs. 80.43%, *P*=0.002). Patients with combined radiotherapy showed higher ORR than those without combined radiotherapy (biosimilar group: 34.55% vs. 28.23%, *P*=0.326; reference group: 47.92% vs. 28.53%, *P*=0.006; Figure 1D). Remarkable differences in ORR and DCR were observed in the different combination groups as shown in Figure 1E. Patients in the combination chemotherapy and another targeted therapy had the highest ORR (56.34%) and patients in the combination of another targeted therapy had the highest DCR (92.50%). In addition, patients without brain metastases showed higher DCR than patients with brain metastases (84.69% vs. 89.69%, *P*=0.045, Figure 1F).

A total of 385 (69.9%) patients progressed or died in the biosimilar group compared with 363 (91.9%) patients in the reference group. Based on the Kaplan–Meier analysis, the estimated median PFS (mPFS) values were 6.27 (95% CI: 5.53–7.01) months in the biosimilar group and 4.93 (95% CI: 4.24–5.62) months in the reference group as shown in Figure 2. Based on the Cox regression model, the estimated hazard ratio (HR) for bevacizumab biosimilar and bevacizumab comparison was 1.084 (95% CI: 0.932–1.260, *P*=0.296). The analyses showed that the long-term efficacies of the two treatment groups were similar. Overall, the mPFS was 5.53 months (95% CI: 4.98–6.09) for all patients. The number of treatment lines, radiotherapy, and combined treatment regimens were statistically significant for PFS as shown in Table 4. In

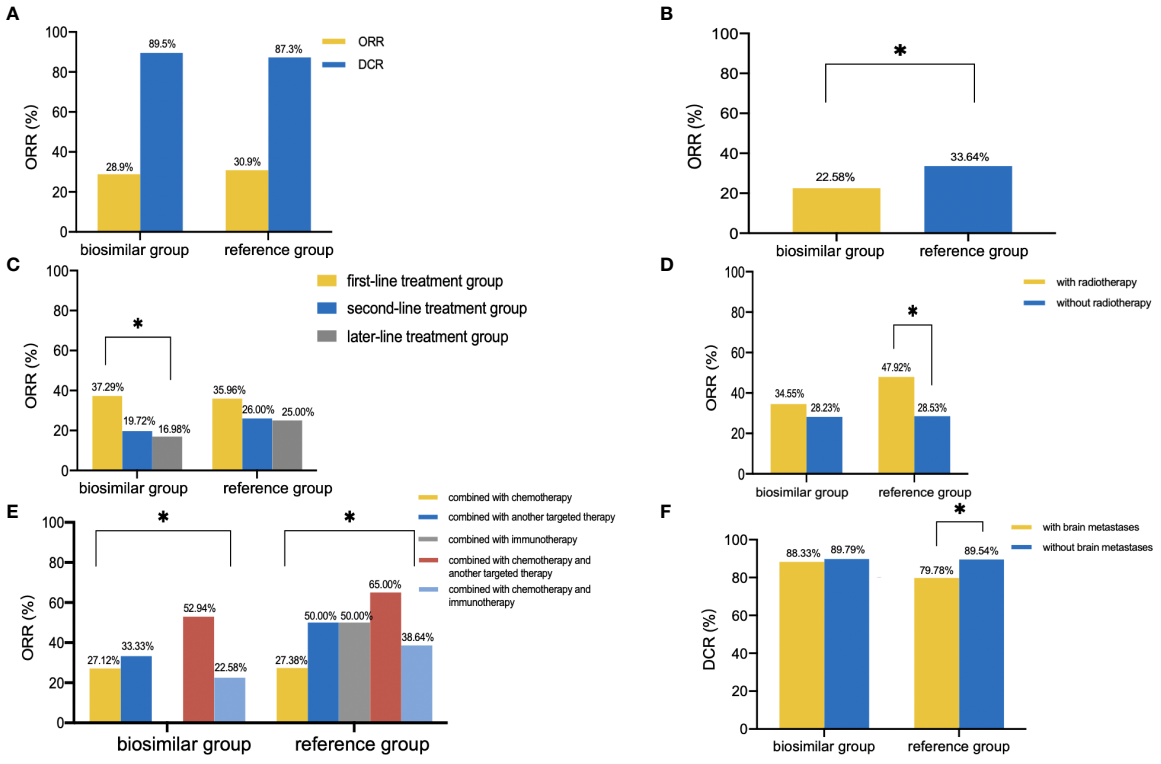

FIGURE 1
Clinical outcomes. **(A)**, ORR and DCR of the biosimilar and reference groups. **(B)**, ORR of patients treated with bevacizumab (or biosimilar) in combination with chemotherapy and immunotherapy. **(C)**, ORR of different number of treatment lines in the two groups. **(D)**, ORR of patients with or without radiotherapy in the two groups. **(E)**, ORR of different combined treatment regimens in the two groups. **(F)**, DCR of patients with or without brain metastases in the two groups. **P*<0.05.

TABLE 2 Comparison of overall response rate between biosimilar and reference groups.

	biosimilar group (n=551)	reference group (n=395)
Best overall response, n (%)		
PR	159 (28.9%)	122 (30.9%)
SD	334 (60.6%)	223 (56.4%)
PD	58 (10.5%)	50 (12.7%)
ORR	28.9%	30.9%
95% exact CI	25.1%-32.7%	26.3%-35.5%
Treatment comparison (vs referrence bevacizumab group)		
Unstratified ORR risk ratio	0.934	
95% CI of risk ratio	0.767-1.138	
90% CI of risk ratio	0.792-1.103	
Unstratified ORR risk difference	-0.020	
95% CI of difference	-0.118-0.035	
90% CI of difference	-0.105-0.023	

ORR, overall/objective response rate; PR, partial response; SD, stable disease; PD, progressive disease.

addition, based on the Cox regression analysis of the subgroups, no statistical difference was observed in the PFS of the bevacizumab biosimilar and bevacizumab groups except for the subgroup with a history of hypertension as shown in Figure 3. In a subgroup of patients with a history of hypertension, bevacizumab biosimilar obtained longer PFS than reference bevacizumab (8.85 vs. 6.63 months, *P*=0.047). The mean dose intensity of the biosimilar group was significantly higher than that of the reference group (8.28 vs. 7.37 mg/kg, *P*=0.007) in this subgroup. Based on multivariate analysis, the product type was not the main factor affecting PFS (*P*=0.595).

Safety

As shown in Table 5, the incidence of treatment-related grade AEs in all patients was 74.42% (704/946). Among which, 199 cases (21.04%) were more than grade 3 AEs. No fatal effects happened. Similar incidences of TEAEs at any grade were observed in the biosimilar and reference groups (421 subjects [76.41%] vs. 283 subjects [71.65%], *P*=0.098), and most of them were classified as grade 1 or 2 AEs. No statistically significant differences in the incidence of grade 3 and 4 TEAEs were observed between the two groups (22.14% vs. 19.49%, *P*=0.324). Among the treatment-related AEs in the biosimilar group, neutropenia had the highest incidence (22.87%), followed by anemia (16.15%), alopecia (14.70%), nausea (12.89%), fatigue (10.16%), thrombocytopenia (10.16%), hypertension (9.62%), and fever (8.71%). In the reference group, neutropenia had the highest incidence (24.05%), followed by anemia (16.46%), alopecia (13.67%), fatigue (11.90%), thrombocytopenia (10.89%), nausea (10.63%), loss of appetite (9.37%), and bleeding (7.34%). The incidence of hypertension at any grade in the biosimilar group was higher than that in the reference group (9.62% vs. 5.82%, *P*=0.033), but no difference in the incidence of hypertension was found at grade 3 or 4 (1.63% vs. 0.51%, *P*=0.093). We further analyzed the clinical data to understand the origin of the differences. Among patients over 70 years of age who received high-dose treatment, the incidence of hypertension caused by the biosimilar (n=48) was significantly higher than that of reference bevacizumab (n=32; 37.5% vs. 15.6%, *P*=0.034). Among patients over 70 years of age with a history of hypertension, the incidence of hypertension caused by the biosimilar (n=25) was higher than that of reference bevacizumab (n=17; 40% vs. 23.5%, *P*=0.266). Among these patients, we first hypothesized that differences in the incidence of hypertension might be attributed to some patients in the subgroup over 70 years of age. We then compared the incidence of hypertension in patients over 70 years of age after excluding patients with high-dose therapy and a history of hypertension. The results showed that the incidence of hypertension was similar between the two products (17.3% vs. 10.0%, *P*=0.511). After imbalanced factors were eliminated, no statistically significant difference in the incidence of hypertension of any grade was found between the biosimilar

TABLE 3 Subgroup analysis of ORR and DCR between and within biosimilar and reference groups.

	ORR(%)					DCR(%)				
Variable	biosimilar group	p^1	reference group	p^2	p^3	biosimilar group	p^1	reference group	p^2	p^3
Sex										
male (n=533)	29.18%	0.852	31.58%	0.728	0.551	88.52%	0.419	85.09%	0.115	0.244
female (n=413)	28.46%		29.94%		0.744	90.65%		90.42%		0.937
Age (years)										
≥60 (n=524)	27.72%	0.516	30.77%	0.955	0.448	88.78%	0.557	88.69%	0.365	0.974
<60 (n=422)	30.24%		31.03%		0.862	90.32%		85.63%		0.954
Pathological type										
adenocarcinoma (n=932)	28.55%	0.349	31.11%	0.753	0.399	89.32%	1.000	87.66%	0.169	0.432
nonadenocarcinoma (n=14)	50.00%		16.67%		0.301	100.00%		66.67%		0.165
Stage of cancer										
IIIB (n=54)	34.38%	0.478	36.36%	0.567	0.880	87.50%	0.938	90.91%	0.851	1.000
IV (n=892)	28.52%		30.56%		0.508	89.60%		87.13%		0.254
Number of treatment lines										
1 (n=506)	37.29%	**0.000***	35.96%	0.080	0.760	93.07%	**0.009***	93.10%	**0.002***	0.988
2 (n=242)	19.72%		26.00%		0.248	85.92%		82.00%		0.410
≥3 (n=198)	16.98%		25.00%		0.165	83.96%		80.43%		0.516
Combined with radiotherapy										
with (n=103)	34.55%	0.326	47.92%	**0.006***	0.168	90.91%	0.715	95.83%	0.059	0.445
without (n=843)	28.23%		28.53%		0.923	89.31%		86.17%		0.166
Combined treatment regimens										
combined with chemotherapy (n=690)	27.12%	**0.001***	27.38%	**0.003***	0.939	88.77%	0.068	87.38%	0.387	0.575
combined with another targeted therapy (n=40)	33.33%		50.00%		0.602	94.44%		75.00%		0.277
combined with immunotherapy (n=8)	0.00%		50.00%		0.250	50.00%		50.00%		1.000
combined with chemotherapy and another targeted therapy (n=71)	52.94%		65.00%		0.357	92.16%		90.00%		1.000
combined with chemotherapy and immunotherapy (n=137)	22.58%		38.64%		**0.048***	91.40%		88.64%		0.756
Combined chemotherapy regimens (if combined)										
combined with pemetrexed (n=26)	10.00%	0.358	12.50%	0.609	1.000	70.00%	0.180	81.25%	0.148	0.508
combined with taxane (n=46)	16.67%		25.00%		0.698	86.67%		68.75%		0.241
combined with gemcitabine (n=9)	25.00%		40.00%		1.000	75.00%		80.00%		1.000
combined with pemetrexed–platinum (n=474)	29.84%		27.78%		0.621	90.31%		89.35%		0.731
combined with taxane–platinum (n=95)	24.14%		32.43%		0.377	89.66%		89.19%		0.942

(Continued)

TABLE 3 Continued

Variable	ORR(%)					DCR(%)				
	biosimilar group	p^1	reference group	p^2	p^3	biosimilar group	p^1	reference group	p^2	p^3
Dose intensity										
low-dose (n ≤ 7.5mg/kg) (n=461)	33.19%	0.061	29.79%	0.567	0.432	92.48%	0.055	88.09%	0.590	0.112
high-dose (7.5mg/kg<n ≤ 15.0mg/kg) (n=485)	25.85%		32.50%		0.125	87.38%		86.25%		0.727
Gene mutation type										
No genetic mutation (n=448)	26.32%	0.196	32.84%	0.165	0.131	87.45%	0.169	90.55%	0.190	0.225
EGFR exon 18 point mutation (n=4)	100.00%		0.00%		0.333	100.00%		100.00%		NA
EGFR exon 19 deletion (n=162)	23.76%		24.59%		0.905	84.16%		88.52%		0.440
EGFR exon 20 insertion (n=24)	46.67%		22.22%		0.389	93.33%		66.67%		0.130
EGFR the L858R point mutation (n=209)	35.20%		29.76%		0.412	90.20%		79.38%		0.057
EGFR double mutation (n=10)	50.00%		0.00%		0.133	100.00%		83.33%		1.000
ALK rearrangement (n=26)	28.57%		41.67%		0.683	100.00%		91.67%		0.462
ROS1 rearrangement (n=8)	33.33%		100.00%		0.429	83.33%		100.00%		1.000
RET rearrangement (n=7)	33.33%		100.00%		0.429	100.00%		100.00%		NA
BRAF mutation (n=6)	0.00%		0.00%		NA	100.00%		100.00%		NA
HER2 mutation (n=9)	33.33%		66.67%		0.524	66.67%		100.00%		0.500
KRAS mutation (n=26)	29.41%		44.44%		0.667	94.12%		100.00%		1.000
MET aberration (n=7)	0.00%		0.00%		NA	100.00%		100.00%		NA
Brain metastases										
with (n=209)	26.67%	0.549	23.60%	0.091	0.614	88.33%	0.645	79.78%	**0.015***	0.089
without (n=737)	29.47%		33.01%		0.306	89.79%		89.54%		0.913
Liver metastases										
with (n=136)	23.61%	0.292	28.13%	0.601	0.548	86.11%	0.319	87.50%	0.967	0.926
without (n=810)	29.65%		31.42%		0.589	89.98%		87.31%		0.235
A history of hypertension										
with (n=136)	30.86%	0.666	40.00%	0.115	0.272	91.36%	0.550	90.91%	0.391	0.928
without (n=810)	28.51%		29.41%		0.780	89.15%		86.76%		0.300

*: p<0.05; ORR, overall/objective response rate; DCR, disease control rate;p1, hypothesis testing parameters within the biosimilar group;p2, hypothesis testing parameters within the referrence group;p3, hypothesis testing parameters between the biosimilar group and the reference group

group (n=541) and reference group (n=392; 7.9% vs. 5.1%, *P*=0.087).

Discussion

The results of this study demonstrated that bevacizumab biosimilar is equivalent in efficacy to bevacizumab in locally advanced and advanced NSCLC. Patients receiving previous treatment, patients receiving regimens other than in combination with chemotherapy, patients with rare genetic mutations, and patients with brain metastases also benefit clinically from both products. The AE spectra and incidence rates of the two products were similar.

Bevacizumab is a humanized monoclonal antibody targeting VEGF and is prepared by recombinant DNA technology. By

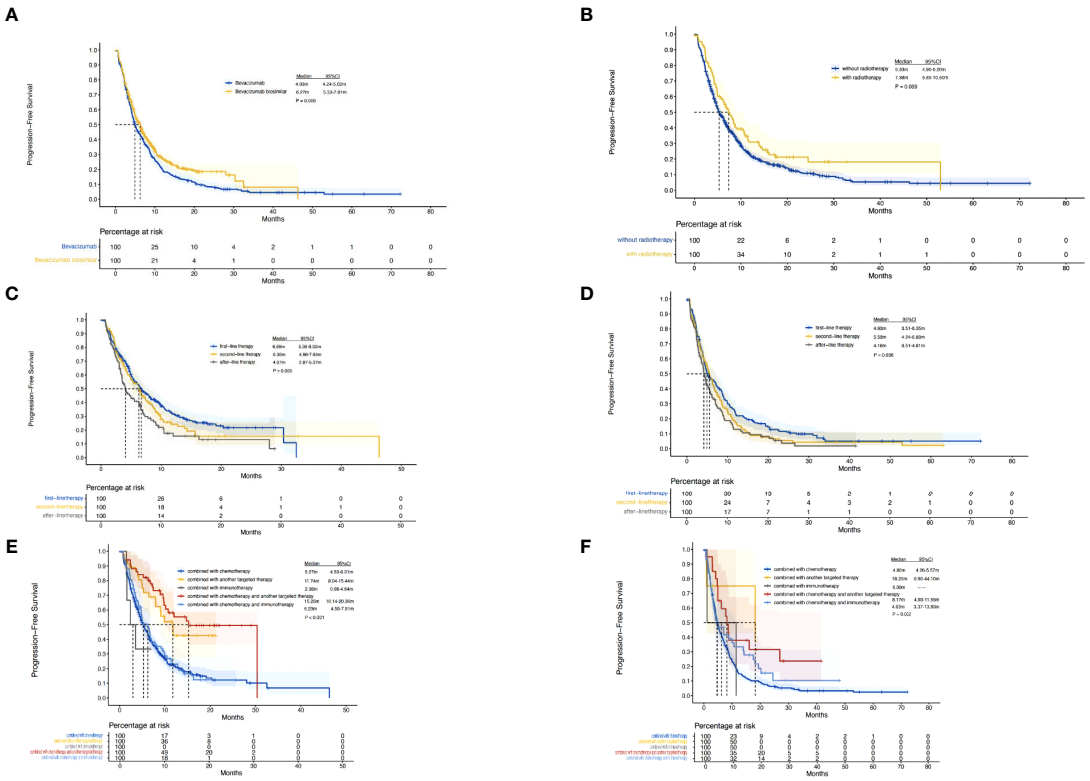

FIGURE 2
Kaplan−Meier curves. **(A)**, PFS of the biosimilar and reference groups. **(B)**, PFS of all the patients with or without radiotherapy. **(C)**, PFS of patients in the biosimilar group with different number of treatment lines. **(D)**, PFS of patients in the reference group with different number of treatment lines. **(E)**, PFS of patients in the biosimilar group with different combined treatment regimens. **(F)**, PFS of patients in the reference group with different combined treatment regimens.

combining with VEGF, it can inhibit the binding of VEGF to its receptor and block the signaling pathway of angiogenesis in tumor tissues. Bevacizumab has become an important component of systemic therapy for advanced NSCLC without genetic mutations. A biosimilar is an approved biological product that is highly similar to the original drug with no clinically remarkable difference in safety, purity, or potency. Biosimilar therapy is an important avenue to reduce patient expenditure and the financial burden of national healthcare systems while maintaining therapeutic effect (18). However, a biosimilar is not an exact copy of its reference product and requires vigilance and concern in clinical application.

Current prospective clinical trials have confirmed that bevacizumab biosimilar in combination with platinum-containing two-drug chemotherapy has similar efficacy and safety compared with bevacizumab in patients with untreated advanced non-squamous NSCLC. In 2019, China National Medical Products Administration approved the marketing of bevacizumab biosimilar developed by Qilu Pharmaceutical Co., Ltd. (trade name: Encoda) with all indications approved for bevacizumab in China. However, no clinical data has verified the efficacy and safety of the biosimilar in clinical application. Existing trials have excluded patients receiving previous treatment, patients receiving combination with targeted therapy or immunotherapy, patients with brain metastases, patients with rare genetic mutations (such as EML4–ALK rearrangement), and patients with ECOG scores greater than 2. No consensus has been reached on the efficacy and safety of bevacizumab biosimilar in these populations. Among the published retrospective studies comparing bevacizumab biosimilar with bevacizumab, most have focused on patient clinical characteristics, cost-effectiveness, and economic impact rather than efficacy and safety. Only one study mentioned the statistical analysis of treatment modalities for 18 patients with NSCLC who used bevacizumab (19). Our study is not comparable to this one because of the lack of other information and its limited patient population. Our study greatly expanded the sample size and provided detailed information. It is the first retrospective study to evaluate the efficacy and safety of bevacizumab biosimilar.

Subject demographics, baseline disease characteristics. And exposure to treatment were well balanced and comparable between the two treatment groups. In the biosimilar group, the ORR was 28.9% (95% CI: 25.1%–32.7%), and the DCR was

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TABLE 4 Univariate analysis and multivariate analysis of factors affecting the progression-free survival (PFS) of all patients.

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Sex				
male vs female	0.978 (0.846-1.130)	0.759		
Age				
≥60 years vs <60 years	1.013 (0.877-1.171)	0.862		
Pathological type				
adenocarcinoma vs nonadenocarcinoma	0.923 (0.494-1.723)	0.801		
Stage of cancer				
IIIB vs IV	0.777 (0.558-1.081)	0.135		
Product				
Bevacizumab biosimilar vs reference Bevacizumab	1.226 (1.061-1.417)	0.006	1.084 (0.932-1.260)	0.296
Dose intensity				
low-dose vs high-dose	0.995 (0.862-1.149)	0.946		
Combined with radiotherapy				
yes vs no	0.743 (0.587-0.940)	0.014	0.778 (0.613-0.987)	0.039*
Number of treatment lines				
1.000	1.000	0.000	1.000	0.001*
2.000	1.174 (0.988-1.394)		1.139 (0.957-1.355)	
≥3	1.485 (1.237-1.7782)		1.407 (1.168-1.695)	
Combined treatment regimens				
combined with chemotherapy	1.000	0.000	1.000	0.000*
combined with another targeted therapy	0.417 (0.617-3.089)		0.446 (0.284-0.700)	
combined with immunotherapy	1.381 (0.267-0.519)		1.260 (0.559-2.841)	
combined with chemotherapy and another targeted therapy	0.372 (0.267-0.519)		0.399 (0.295-0.560)	
combined with chemotherapy and immunotherapy	0.847 (0.686-1.046)		0.835 (0.673-1.036)	
Combined chemotherapy regimens (if combined)				
combined with pemetrexed	1.000	0.068		
combined with taxane	1.484 (0.876-2.514)			
combined with gemcitabine	1.497 (0.665-3.368)			
combined with pemetrexed–platinum	0.983 (0.639-1.512)			
combined with taxane–platinum	1.200 (0.749-1.920)			
Gene mutation type				
no genetic mutation	1.000	0.627		
EGFR exon 18 point mutation	0.607 (0.195-1.892)			
EGFR exon 19 deletion	1.089 (0.892-1.330)			
EGFR exon 20 insertion	0.985 (0.628-1.545)			

(Continued)

TABLE 4 Continued

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
EGFR the L858R point mutation	0.889 (0.735-1.074)			
EGFR double mutation	1.093 (0.517-2.309)			
ALK rearrangement	1.025 (0.660-1.592)			
ROS1 rearrangement	0.308 (0.099-0.961)			
RET rearrangement	0.723 (0.299-1.749)			
BRAF mutation	1.226 (0.457-3.287)			
HER2 mutation	0.662 (0.295-1.483)			
KRAS mutation	0.856 (0.551-1.329)			
MET aberration	0.855 (0.354-2.068)			
Brain metastases				
with vs without	1.137 (0.958-1.349)	0.142		
Liver metastases				
with vs without	1.309 (1.077-1.592)	0.007	1.207 (0.990-1.470)	0.062
History of hypertension				
with vs without	1.205 (0.927-1.751)	0.030		

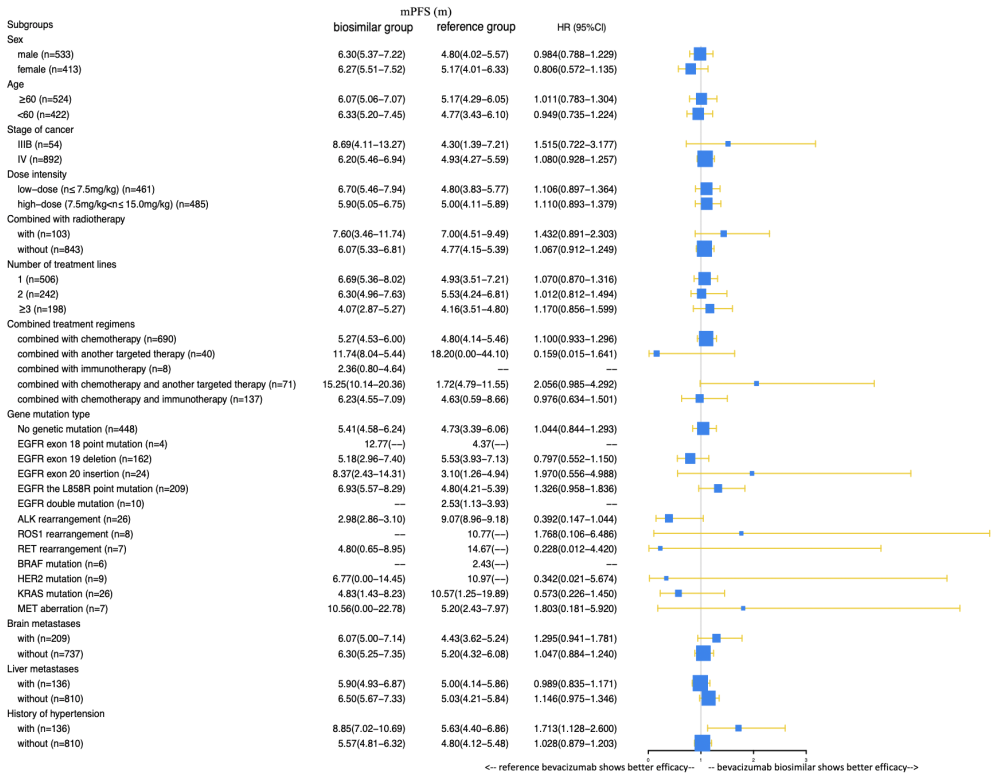
*p < 0.05.

89.5%. The ORR and DCR of the control group were 30.9% (95% CI: 26.3%–35.5%) and 87.3%, respectively. The unstratified ORR risk ratio was 0.934 with a 95% CI of 0.767–1.138 and a 90% CI of 0.792–1.103. The unstratified ORR risk difference was −0.020 with a 95% CI of −0.118–0.035 and a 90% CI of −0.105–0.023. The definition of equivalence by FDA, PMDA, and EMA corresponds to the 90% CI of ORR hazard ratio in the range of 0.73–1.37; the 95% CI of the ORR HR is 0.729–1.371, and the 95% CI of the ORR risk difference is −13%–13% (20, 21). In the present study, the CIs of ORR risk ratio and ORR risk difference were within these predefined equivalence margins. The estimated mPFS values were 6.27 (95% CI: 5.53–7.01) months in the biosimilar group and 4.93 (95% CI: 4.24–5.62) months in the reference group. The estimated HR for bevacizumab biosimilar and bevacizumab comparison was 1.084 (95% CI: 0.932–1.260, *P*=0.296). Therefore, bevacizumab biosimilar and reference bevacizumab are equivalent in efficacy.

In different historical clinical trial results for bevacizumab biosimilar, the ORR ranges from 41.5% to 53.1%, and the mPFS is about 7.5 months (22–24). Numerically, our study showed a lower ORR and a shorter PFS than other studies. In view of the differences between clinical application and clinical trials, such as the number of treatment lines, drug combinations, and dose intensity, the above results are not highly comparable. The subgroup analyses were performed based on the following subgroups: sex, age, pathology, stage, number of treatment lines, radiotherapy, combined treatment regimens, combined chemotherapy regimens (if combined with chemotherapy), dose intensity, genetic mutations, brain metastasis, liver metastasis, and history of hypertension. To avoid the increased risk of potential bias associated with a small sample size, subgroups with less than 3 cases were not included in the subgroup analysis.

In the subgroup analysis of combined treatment regimens, the ORR of the biosimilar group was lower than that of the reference group when combined with chemotherapy and immunotherapy (22.58% vs. 33.64%, *P*=0.048). Further logistic regression analysis showed that the influencing factors of ORR in patients combined with chemotherapy and immunotherapy were the number of treatment lines and combination with radiotherapy (*P*=0.021, 0.008). Product type (i.e., bevacizumab or bevacizumab biosimilar) was not a main factor (*P*=0.604). In a subgroup of patients with a history of hypertension, bevacizumab biosimilar obtained a longer PFS than reference bevacizumab (8.85 vs. 6.63 months, *P*=0.047). The mean dose intensity of the biosimilar group was significantly higher than that of the reference group (8.28 vs. 7.37 mg/kg, *P*=0.007) in this subgroup. Based on multivariate analysis, the product type was not the main factor affecting PFS (*P*=0.595). Among the remaining subgroups, the ORR and PFS analyses support the equivalence between the two groups.

The number of treatment lines, combined treatment regimens, and radiotherapy were the significant factors



affecting the PFS of both groups (P<0.001, P=0.001, P=0.039). Combined chemotherapy regimens (if combined with chemotherapy), different genetic mutations, dose intensity, brain metastases, liver metastases, and a history of hypertension were not the main factors affecting PFS (P=0.104, 0.627, 0.946, 0.142, 0.062, 0.030).

The treatments combined with chemotherapy and another targeted therapy (biosimilar group: ORR=52.94%, mPFS=15.25 months; reference group: ORR=65.00%, mPFS=8.17 months) and combined with another targeted therapy group (biosimilar group: ORR=33.33%, mPFS=11.74 months; reference group: ORR=50.00%, mPFS=18.20 months) showed better efficacy than other treatment regimens. Among these treatments, approximately 80% of other targeted therapies are EGFR–TKI. Preclinical studies have shown that VEGF and EGFR share a common downstream signaling pathway (25–28), but the clinical trial data of EGFR–TKI combined with bevacizumab are still immature and have many uncertainties (29). Our study confirmed the benefits of bevacizumab (or bevacizumab biosimilar) combined with EGFR–TKI in ORR and PFS but failed to obtain OS results for all patients because of the short follow-up period. Previous studies have shown that patients with exon 19 deletions have a better prognosis after EGFR–TKI treatment than those with 21 p.L858 mutations (30). However, our study revealed similar ORR and PFS benefits between the two mutation types in both groups. The synergistic antiproliferative effects of EGFR–TKI and antiangiogenic treatment might eliminate the prognostic differences caused by genetic mutations. More prospective studies or in-depth retrospective clinical data are expected to better explore the above conclusions.

In addition to EGFR mutations, studies on patients with other rare mutations receiving targeted therapy combined with bevacizumab are few, and all of which are exploratory studies with small samples. The American Society of Clinical Oncology reported a study from China in which 16 patients with EML4–ALK rearrangement receiving crizotinib and bevacizumab have a mPFS of 13.0 months. Our study included 89 patients that harbor other rare driving gene mutations, including ALK rearrangement, ROS1 rearrangement, RET rearrangement, BRAF mutation, HER2 mutation, KRAS mutation, and MET aberration. These populations have also been proven to benefit from bevacizumab or bevacizumab biosimilar.

In the subgroup analysis of combined with chemotherapy, multivariate analysis showed the combined chemotherapy regimen was not the main factor affecting PFS (P=0.068). But

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TABLE 5 Treatment-related adverse events.

	biosimilar group (n=551)		reference group (n=395)	
Adverse events	any grade	Grade 3-4	any grade	Grade 3-4
General disorders				
fatigue	56 (10.16%)	7 (1.27%)	47 (11.90%)	4 (1.01%)
fever	48 (8.71%)	18 (3.27%)	22 (5.57%)	9 (2.28%)
Infectious diseases				
pneumonia	7 (1.27%)	2 (0.36%)	3 (0.76%)	0 (0.00%)
Blood and lymphatic system disorders				
anemia	89 (16.15%)	2 (0.36%)	65 (16.46%)	2 (0.51%)
leucopenia	36 (6.53%)	3 (0.54%)	21 (5.32%)	4 (1.01%)
neutropenia	126 (22.87%)	67 (12.16%)	95 (24.05%)	42 (10.63%)
thrombocytopenia	56 (10.16%)	11 (2.00%)	43 (10.89%)	5 (1.27%)
Vascular disorders				
hypertension	53 (9.62%)*	9 (1.63%)	23 (5.82%)*	2 (0.51%)
bleeding	39 (7.08%)	5 (0.91%)	29 (7.34%)	4 (1.01%)
thromboembolism	3 (0.54%)	0 (0.00%)	2 (0.51 %)	0 (0.00%)
Urinary disorders				
creatinine increased	10 (1.81%)	2 (0.36%)	9 (2.28%)	6 (1.52%)
proteinuria	5 (0.91%)	3 (0.54%)	6 (1.52%)	2 (0.51%)
Respiratory disorders				
cough	2 (0.36%)	1 (0.18%)	0 (0.00%)	0 (0.00%)
Gastrointestinal disorders				
diarrhea	3 (0.54%)	0 (0.00%)	1 (0.25%)	1 (0.25%)
nausea	71 (12.89%)	6 (1.09%)	42 (10.63%)	3 (0.76%)
vomiting	34 (6.17%)	3 (0.54%)	17 (4.30%)	3 (0.76%)
intestinal obstruction	2 (0.36%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
elevated ALT or AST	14 (2.54%)	6 (1.09%)	7 (1.77%)	3 (0.76%)
Nervous system disorders				
headache	13 (2.36%)	1 (0.18%)	11 (2.78%)	1 (0.25%)
paresthesia	3 (0.54%)	0 (0.00%)	1 (0.25%)	0 (0.00%)
Skin and subcutaneous tissue disorders				
hair loss	81 (14.70%)	2 (0.36%)	54 (13.67%)	1 (0.25%)
rash	7 (1.27%)	0 (0.00%)	3 (0.76%)	2 (0.51%)
Musculoskeletal connective tissue disorders				
joint pain	2 (0.36%)	1 (0.18%)	1 (0.25%)	0 (0.00%)

(Continued)

TABLE 5 Continued

	biosimilar group (n=551)		reference group (n=395)	
Adverse events	any grade	Grade 3-4	any grade	Grade 3-4
Metabolism and nutrition disorders				
poor appetite	42 (7.62%)	1 (0.18%)	37 (9.37%)	2 (0.51%)
Total	421 (76.41%)	122 (22.14%)	283 (71.65%)	77 (19.49%)

Number of patients with an event (percent). *p < 0.05; ALT, alanine aminotransferase; AST, aspartate transaminase.

it is worth noting that, numerically, the treatment combined with gemcitabine showed the shortest PFS and the treatment combined with pemetrexed–platinum showed the longest PFS in both the biosimilar and reference groups (biosimilar group: pemetrexed vs taxane vs gemcitabine vs pemetrexed–platinum vs taxane–platinum: 3.17m vs 3.20m vs 1.15m vs 4.43m vs 3.41m, *P*=0.119; reference group:1.97m vs 2.27m vs 1.59m vs 4.23m vs 3.57m, *P*=0.055). Previous studies (14) have confirmed that gemcitabine is inferior to pemetrexed or paclitaxel in advanced NS-NSCLC patients. Our results suggest that this trend may remain in the context of bevacizumab in combination. Whether the efficacy of bevacizumab in combination with different platinum-based doublets is different is still controversial. PointBreak trial (31) confirmed that pemetrexed–platinum combined with bevacizumab regimen obtained significantly longer PFS than taxane–platinum combined with bevacizumab regimen, but no statistical difference was observed in PRONOUNCE trial (32) and ERACLE trial (33). In our study, no statistical difference was obtained on this question, probably due to the inherent influence of selection bias and missing data. Larger prospective studies are expected to investigate this issue.

In the previous phase III clinical trial studies, only the AVAiL and AVAPERL studies used bevacizumab at 7.5 mg/kg, and the other studies used 15 mg/kg. These studies lacked the ability to directly compare the two doses of bevacizumab. With 7.5 mg/kg as the boundary line, our study showed that no statistical differences in the ORR and PFS between the low- and high-dose groups (biosimilar group: 33.19% vs. 25.85%, 6.70 vs. 5.90 months; reference group: 29.79% vs. 32.50%, 4.80 vs. 5.00 months). In addition, the patients with the brain metastases (BMS) also benefit from bevacizumab or biosimilar (biosimilar group: ORR=26.67%, mPFS=6.07 months; reference group: ORR=23.60%, mPFS=4.43 months).

Similar incidences of TEAEs at any grade were observed in the biosimilar group and the reference group (421 [76.41%] vs. 283 subjects [71.65%], *P*=0.098), and most of them were classified as grade 1 or 2 events. No statistically significant difference in the incidence of grade 3 or 4 TEAEs was observed between the two groups (22.14% vs. 19.49%, *P*=0.324). No fatal effects happened. Among the treatment-related AEs in the biosimilar group, neutropenia had the highest incidence (22.87%), followed by anemia (16.15%), alopecia (14.70%), nausea (12.89%), fatigue (10.16%), thrombocytopenia (10.16%), hypertension (9.62%), and fever (8.71%). In the reference group, neutropenia had the highest incidence (24.05%), followed by anemia (16.46%), alopecia (13.67%), fatigue (11.90%), thrombocytopenia (10.89%), nausea (10.63%), loss of appetite (9.37%), and bleeding (7.34%). In general, the AE spectra and AE rates of bevacizumab biosimilar and reference bevacizumab were similar. The overall incidences of grade 3 and 4 TEAEs were low and similar, indicating that bevacizumab biosimilar and bevacizumab have favorable safety profiles.

The incidence of hypertension at any grade in the biosimilar group was higher than that in the reference group (9.62% vs. 5.82%, *P*=0.033), but no differences in the incidence of hypertension were observed at grades 3 and 4 (1.63% vs. 0.51%, *P*=0.093). We further analyzed the clinical data to understand the origin of the differences. Among patients over 70 years of age who received high-dose treatment, the incidence of hypertension caused by the biosimilar (n=48) was significantly higher than that of reference bevacizumab (n=32; (37.5% vs. 15.6%, *P*=0.034). Among patients over 70 years of age with a history of hypertension, the incidence of hypertension caused by biosimilar (n=25) was higher than that of reference bevacizumab (n=17; 40% vs. 23.5%, *P*=0.266). Among these patients, we first hypothesized that differences in the incidence of hypertension might be attributed to some patients in the subgroup over 70 years of age. We then compared the incidence of hypertension in patients over 70 years of age after excluding patients with high-dose therapy and a history of hypertension. The results showed that the incidence of hypertension was similar between the two products (17.3% vs. 10.0%, *P*=0.511). After imbalanced factors were eliminated, no statistically significant difference in the incidence of hypertension of any grade was found between the biosimilar group (n=541) and reference group (n=392; 7.9% vs. 5.1%, *P*=0.087).

In the subgroup analysis of combined with chemotherapy, similar incidences of TEAEs at any grade were observed (pemetrexed vs taxane vs gemcitabine vs pemetrexed–platinum vs taxane–platinum: 69.2% vs 78.3% vs 55.6% vs 71.9% vs 72.6%, *P*=0.695). Among these treatment-related AEs, neutropenia had the highest incidence (28.92%), followed by anemia (18.92%), thrombocytopenia (11.54%) and fatigue (10.77%). The incidence of a few adverse reactions varied with chemotherapy regiments, and these differences existed in both the biosimilar and reference groups. The incidence of anemia at any grade was significantly higher in the

treatment combined with pemetrexed–platinum than in the other chemotherapy regimens (biosimilar group:10.0% vs 13.3% 0.00% vs 26.8% vs 12.1%, *P*=0.042; reference group:12.5% vs 13.3% vs 0.00% vs 23.1% vs.10.8%, *P*=0.039). All 4 cases of sensory neuropathy were in the taxane–platinum combined with bevacizumab regimen (biosimilar group vs reference group: 5.2% vs 2.7%, *P*=0.654), but all were classified as grade 1 or grade 2 events. The above adverse reactions were consistent with previous studies on corresponding chemotherapy regimens. These were not attributed to the type of bevacizumab product. The toxicity of these adverse reactions is considered acceptable, but close monitoring of these patients is still required in clinical practice.

We acknowledge the limitations of the retrospective study design, which is inherently affected by selection bias and missing data. Moreover, reliance on electronic health records may mean that some events may be underestimated. Therefore, larger prospective studies are needed to confirm our findings.

Conclusions

The results of this study demonstrated that bevacizumab biosimilar is equivalent in efficacy to bevacizumab in patients with locally advanced and advanced NSCLC. Bevacizumab biosimilar showed acceptable toxicity profile and no new AEs. Patients receiving previous treatment, patients receiving regimens other than in combination with chemotherapy, patients with rare genetic mutations, and patients with brain metastases can also benefit clinically from both products. The number of treatment lines, radiotherapy, and combined treatment regimens were the substantial factors affecting the ORR and PFS of bevacizumab or biosimilar. Different genetic mutations and dose intensity were not the main factors affecting PFS.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Academic Ethics Committee of Jiangsu Cancer Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

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

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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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Clinical factors influencing long-term survival in a real-life cohort of early stage non-small-cell lung cancer patients in Spain

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Background: Current prognosis in oncology is reduced to the tumour stage and performance status, leaving out many other factors that may impact the patient´s management. Prognostic stratification of early stage non-small-cell lung cancer (NSCLC) patients with poor prognosis after surgery is of considerable clinical relevance. The objective of this study was to identify clinical factors associated with long-term overall survival in a real-life cohort of patients with stage I-II NSCLC and develop a prognostic model that identifies features associated with poor prognosis and stratifies patients by risk.

Methods: This is a cohort study including 505 patients, diagnosed with stage I-II NSCLC, who underwent curative surgical procedures at a tertiary hospital in Madrid, Spain.

Results: Median OS (in months) was 63.7 (95% CI, 58.7-68.7) for the whole cohort, 62.4 in patients submitted to surgery and 65 in patients submitted to surgery and adjuvant treatment. The univariate analysis estimated that a female diagnosed with NSCLC has a 0.967 (95% CI 0.936 - 0.999) probability of survival one year after diagnosis and a 0.784 (95% CI 0.712 - 0.863) five years after diagnosis. For males, these probabilities drop to 0.904 (95% CI 0.875 - 0.934) and 0.613 (95% CI 0.566 - 0.665), respectively. Multivariable analysis shows that sex, age at diagnosis, type of treatment, ECOG-PS, and stage are statistically significant variables ($p<0.10$). According to the Cox regression model, age over 50, ECOG-PS 1 or 2, and stage II are risk factors for survival (HR>1) while adjuvant chemotherapy is a good prognostic variable (HR<1). The prognostic model identified a high-risk profile defined by males over 71 years old, former smokers, treated with surgery, ECOG-PS 2.

Conclusions: The results of the present study found that, overall, adjuvant chemotherapy was associated with the best long-term OS in patients with resected NSCLC. Age, stage and ECOG-PS were also significant factors to take into account when making decisions regarding adjuvant therapy.

KEYWORDS

non-small cell lung cancer, risk stratification, prognostic model, early stage, long-term survival

Introduction

Lung cancer is the worldwide leading cause of cancer-related mortality, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all lung cancer patients. Owing to the tendency for late diagnosis and tumour recurrence (1, 2), the 5-year overall survival (OS) rate for NSCLC remains low at about 23% and significantly varies by stage, with 5-year OS rates being as high as 73% for patients with stage IA and as low as 2% for those with stage IV disease (3, 4). As a result, there has been considerable effort to identify patients with early-stage NSCLC who may benefit from additional treatment after surgery. Adjuvant radiotherapy is no longer recommended after surgery because it has been shown to have a deleterious effect on long-term survival, at least for stage I and II disease (5). Current European Society for Medical Oncology (ESMO) guidelines for early-stage NSCLC clearly indicate surgery for stages I and II, with adjuvant chemotherapy recommended for stage II and considered for stage IB. Radiotherapy is recommended as a nonsurgical option for stage I (6).

Current prognosis in oncology is reduced to the tumour stage and performance status of the patients, leaving out many other factors that may impact the patient´s management. Even if a few, more advanced stratification models for cancer patients have been proposed, these are usually focused on very specific typologies and require analyses not commonly available in the clinical practice (7) or have not been validated in multiple international cohorts (8–10). Smarter stratification models that leverage data about disease interactions, disease severity, and treatment pathways based on electronic health records (EHRs) can provide crucial information for making better clinical decisions about patients with cancer. Therefore, prognostic stratification of patients with poor prognosis after surgery, in order to assist physicians to make decisions on therapeutic strategies is of considerable clinical relevance.

We here report the results of a study aimed to identify clinical factors associated with long-term overall survival in a real-life cohort of patients with stage I-II NSCLC treated at a tertiary hospital in Madrid, Spain, and develop a prognostic model that identifies poor prognosis factors and stratifies patients by risk.

Methods

Data source

This cohort study used data obtained from a hospital-based lung cancer registry managed by the Department of Medical Oncology at Puerta de Hierro-Majadahonda University Hospital (HUPHM). It is a structured database registered in the RedCap platform, that collects de-identified clinical data from lung cancer patients at HUPHM. The study was approved by the Ethics Committee at HUPHM (No. PI 148/15) and was carried out in accordance with the Helsinki Declaration.

Study population

This is a hospital-based retrospective study that updates prospective follow-up data in the population of NSCLC patients diagnosed and treated at HUPHM from 2008, regardless of their treatment, sex, or age. The last follow-up or vital status information was updated in December 2021. All patients included underwent curative surgery as primary treatment and had pathological confirmation on surgical sample of NSCLC in early stages (I–II). The exclusion criteria were: performed neoadjuvant therapy; unavailable clinicopathological, vital status, or follow-up data; and age < 18 years old.

Clinical data from 2128 patients was extracted from the EHR and structured in a dataset (Figure 1). Of those, 1559 were excluded due to diagnosis of metastatic disease (stage III and IV). Additionally, 55 patients who received radiotherapy as primary treatment were excluded from the study.

Study variables

Patient, tumour, and treatment characteristics were collected from the EHR and structured in RedCap platform: demographic parameters, performance status (ECOG-PS, Eastern Cooperative Oncology Group-Performance status), tobacco habit, comorbidities, family history of cancer, histologic type, disease stage (patients were staged according to seventh edition of TNM classification by American Joint Committee on Cancer (AJCC7) and reclassification using AJCC eighth edition (AJCC8) was also performed), treatments (surgery, adjuvant chemotherapy), and relapse of the disease. Smoking status was defined as never smoker, former smoker, and current smoker. Smokers who claimed to have quit in the 8 weeks prior to diagnosis were classified as current smokers.

Statistical analysis

Statistical analysis was performed using R Software, version 4.0.5. Quantitative data were expressed as mean, median and standard

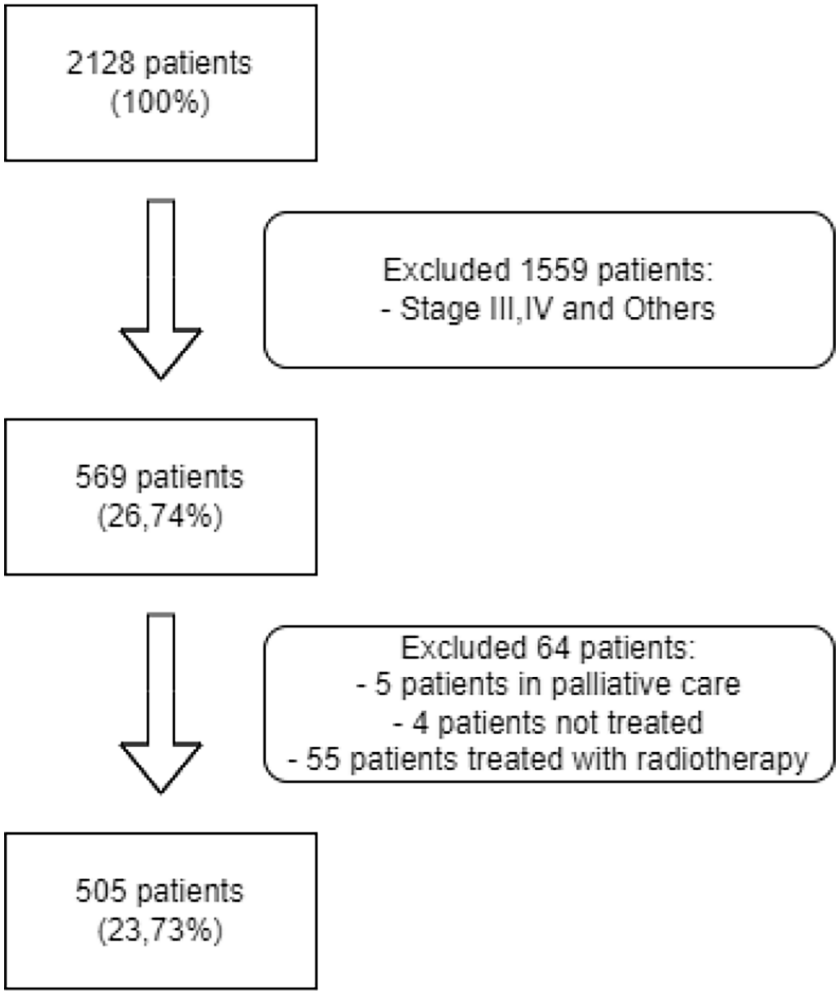

FIGURE 1
Flow Diagram for patient selection.

deviation (SD). The qualitative variables were expressed in the form of frequencies and percentages. Univariate and multivariate analyses were conducted to evaluate the primary patients' characteristics leading to better OS. Univariate survival analysis was performed using Kaplan-Meier curves and survival functions were compared using a log-rank test to check for differences. Statistical significance for the log-rank test was set at $p<0.05$. To investigate the contribution of each characteristic in the survival time, Cox Multivariate regression model was adjusted using a backward stepwise procedure. Significance level was set at $p<0.10$. The assumption of the proportionality of hazards was evaluated with Schoenfeld residuals. Survival Time for Stages I and II patients has a long right tail distribution, with outliers representing long survivors. Using univariate Kaplan Meier analysis, the probabilities of survival after 12 and 60 months were estimated (Estimate, SE, lower and upper 95% confidence interval). Disease free survival (DFS) was calculated from the date of surgery until the date of death or relapse. Using the Kaplan-Meier estimator, the OS curve and the DFS for both Stage I and II were estimated.

Results

Patient characteristics

A total of 505 patients with stage I and II NSCLC were included. The baseline characteristics of the patients are detailed in Table 1. Overall, there was a significantly greater number of men (76%) compared to women (24%). The median age at diagnosis was 60.6 years; 64% of the patients were aged between 51 and 70 years; 5.1% were under 50 and 31% over 71. Regarding smoking habits, 55.6% of the diagnosed patients were former smokers and 31.7% current smokers, with only 10.7% of never smokers. Most of the patients were diagnosed in stage I (62.8%) compared to stage II (37.2%); 96% of patients had an ECOG-PS of 0 or 1, 80% received surgery as primary treatment, while 20% received surgery plus adjuvant treatment, and 89% had comorbidities. Finally, in this patient cohort, 32.3% of patients relapsed during the follow-up period and received subsequent therapy.

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TABLE 1 Characteristics of the patients.

Characteristics	All Cohort						
	Total	%	Survival Mean			Deceased	
			Months	Median	SD		
Overall	505	100%	63,7	61,9	39,1	219	43%
Gender							
Female	121	24,0%	66,7	69,8	38,9	36	29,8%
Male	384	76,0%	60,3	59,5	38,8	183	47,7%
Age at Diagnosis [years}							
20-50	26	5,1%	73,3	88,8	65,9	7	26,9%
51-7O	323	64,0%	65,7	62.6	37,2	139	43,0%
71+	156	30,9%	59,9	56,1	35,0	73	46,8%
Smoking Habits							
Non Smoker	54	10,7%	70,8	78,1	38,7	14	25,9%
Former Smoker	281	55,6%	56,2	58,1	42,6	144	51,2%
Current Smoker	160	31,7%	65,6	63.0	31,2	58	36,3%
Unknown	10	2,0%	-	-	-	3	30,0%
Stage							
I	317	62,8%	67,7	64,6	33,1	120	37,9%
II	188	37,2%	45,1	57,5	47,2	99	52,7%
Comorbidities							
No	56	11,1%	63,9	67,8	45,3	22	39,3%
Yes	449	88.9%	63,2	61,2	38,2	197	43,9%
Patient with Previous Cancer							
No	343	67,9%	64,0	63,4	39,5	145	42,3%
Yes	149	29,5%	62,5	60,1	33,2	67	45,0%
Unknown	13	2,6%	-	-	-	7	53,3%
Treatment							
Surgery	402	79,6%	62,4	60,4	38,8	186	46,3%
Surgery + Adjuvant CHT	103	20,4%	65,0	67,9	39,5	33	32,0%
Performance status							
0	355	70,3%	66,8	63,2	32,7	117	33,0%
1	131	25,9%	44,4	61,5	53,0	90	68,7%
2	8	1,6%	16,0	28,4	25,6	7	87,5%
Unknown	11	2,2%	-	-	-	5	45,5%
Relapse							
No disease	226	44,75%	69,1	67,7	36,2	58	25,7%
Relapse/Progression	163	32,28%	42,7	54,7	43,4	114	69,9%
Unknown	116	22,97%	-	-	-	47	40,5%

CHT, chemotherapy; SD, standard deviation.

Survival analysis

Overall survival of the whole population

Median survival in our cohort was 63.7 months (95% CI, 56.7-64.4) (Figure 2A); 25% of the patients have a survival time less than 30.4 months, and the 3rd quartile indicates that the survival time of 25% of the patients is higher than 82 months. We can also observe that 14 patients are outliers, with the highest survival. In Figure 2B, the curve median estimator shows the influence of these patients, alive after more than 150 months since diagnosis. As illustrated by the CI amplitude, the statistical relevance above 120 months decreases due to the reduced number of patients. The observed survival time of long survivors has an impact on the Kaplan-Meier estimate for median time (Figure 2B) which differs significantly from the observed median of the dataset (Figure 2A).

Overall, 8% of the patients died within the first year since diagnosis, and 86% had a long-term OS (alive more than 2 years since diagnosis). Of note, 6% survived more than 10 years since diagnosis (Supplementary Material).

Disease-free survival

There was statistical difference (p=0.0085) in DFS between stage I and II, being the median DFS for stage I 98.87 months (8.23 years) and 81.07 months (6.76 years) for stage II. The median DFS for the whole cohort was 92.23 months (7.68 years). The 5-year DFS for the stage I cohort was 63% and for stage II group was 48% (Figure 3).

Results of the univariate analysis

The univariate analysis was performed based on survival to relate the different socio-demographic variables, as well as those related to the tumour and the type of treatment received. The analysis revealed statistically significant differences (Figure 4) according to sex (p<0.001), with a greater survival in women; age at diagnosis (p=0.015), with greater survival in the group of 20 to 50 years old; smoking habits (p<0.001) where survival drops dramatically in former and current smokers compared to never smokers; and stage (p=0.002), with greater survival in stage I compared to stage ll.

As for treatment received (Figure 5), survival is strongly improved by surgery with adjuvant chemotherapy compared to surgery (p=0.0085). Apart from the two pivotal prognostic factors, stage and treatment, ECOG-PS also stands as a statistically significant factor that impacts prognosis (p<0.001), especially ECOG-PS 1 and 2 (although the representation of this last group is very scarce), compared to ECOG-PS 0, along with relapse of the disease (p<0.0001), which lowers dramatically patient survival, compared to disease-free status.

Using Kaplan-Meier survival curves we are able to estimate the probability of a patient surviving a given time period, in a univariate approach. The univariate analysis has different sample dimensions for each characteristic because all the patients with *unknown* value are discarded. Estimates for the probability of survival at 1 and 5 years, for each of the considered covariates, as well as the corresponding 95% CI, are shown in Table 2. From a univariate point of view, we estimate that a female diagnosed with NSCLC has a 0.967 (95% CI 0.936 - 0.999) probability of survival one year after diagnosis and a 0.784 (95% CI 0.713 - 0.863) five years after diagnosis. For males, these probabilities drop to 0.903 (95% CI 0.875 - 0.933) and 0.613 (95% CI 0.566 - 0.665), respectively.

According to the p-value of the log-rank test, we can conclude that only comorbidities (p=0.1) and previous cancer (p=0.47) are not significant covariates, while all the other characteristics (p<0.05), reveal to be significant on survival, on a univariate approach. Of important note, survival probability estimates after 12 months do not vary much within each significant variable, while these estimates significantly differ after 60 months, such as relapse (0.423; 95% CI 0.352 - 0.510) vs no disease (0.809; 95% CI 0.759 - 0.863).

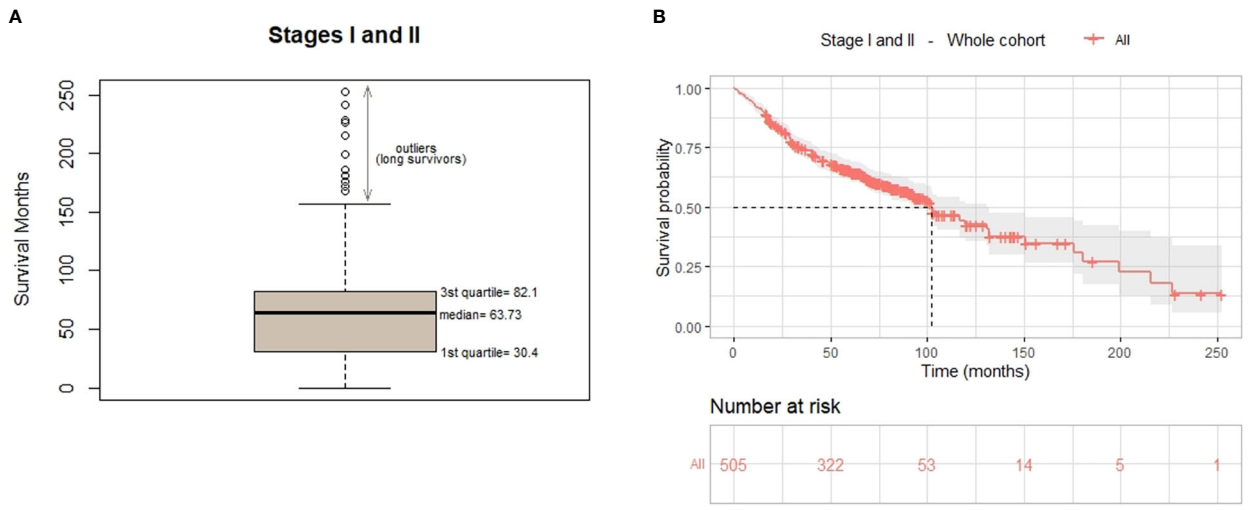

FIGURE 2
Overall survival of the whole cohort. **(A)** Box and whisker plot shows median AND quartiles survival (in months) and outliers. **(B)** OS curve for the 505 early stage patients estimated using the Kaplan-Meier estimator.

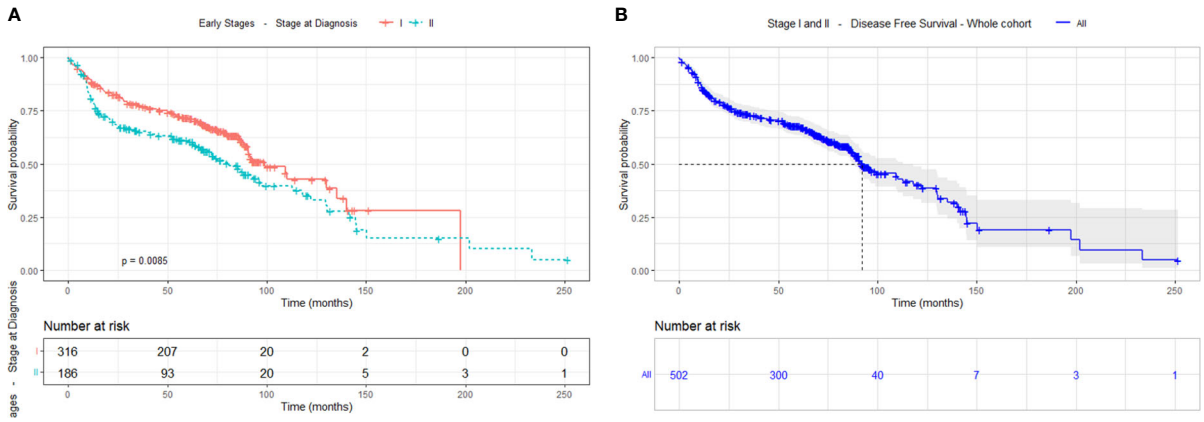

FIGURE 3
Disease-free survival, **(A)** by cancer stage (I-II) and **(B)** of the whole cohort.

Results of the multivariable analysis

Covariate inclusion of prognostic indicators was performed using a combination of two-sided Wald test and Likelihood ratio test ($p<0.05$) in addition to Akaike Information Criterion (AIC) and concordance (c-index) of the performed model. Multivariate Cox proportional hazards regression analysis showed that Sex, Age, Treatment, Stage and ECOG-PS were independent significant variables ($p<0.10$) associated with decreased OS, while sex, stage, comorbidities, and smoking habit were not ($p>0.10$). Nevertheless, despite not being significant probably due to the lack of data, being a non-smoker reduces 34% the risk of dying (HR=0.66; 95% CI 0.35-1.24). Model´s concordance (c-index) was 0.693.

The final Cox model indicated that, among the variables with statistical significance (Table 3), the one that revealed a protective or risk-lowering effect was surgery with adjuvant chemotherapy (HR=0.46; 95% CI 0.30-0.69), implying that, on average, this treatment reduces by 54% the risk of death when compared to patients who were treated with surgery alone. Of note, statistically significant variables that increase risk were age, especially in patients above 71 years old (HR=3.25; 95% CI 1.25-8.46), ECOG-PS 1 (HR=2.07; 95% CI 1.54-2.78) and 2 (HR=5.41; 95% CI 2.47-11.83), and stage ll (HR=1.46; 95% CI 1.08-1.98).

Accordingly, we identified and integrated significant variables in the patient cohort to build a prognostic model that explains the probability of survival (Table 4). For prognosis of patients with early-stage NSCLC, the Cox survival model included six discriminative features. The prognostic model, see Table 3, identified a high-risk profile defined by males over 70 years old, former smokers, stage II, ECOG-PS 2, treated with surgery alone. These features correspond to the highest positive estimates for each of the covariates. The identified features for the low-risk profile (corresponding to the lowest negative coefficients) were being a female between 20 and 50 years old, non-smoker, treated with surgery and adjuvant chemotherapy, with an ECOG-PS 0.

The predictions of survival probabilities according to the model are presented in Figure 6 for a high-risk profile (red line) and low-risk profile (blue line), compared to the reference category (green line). They reveal significant differences between high-risk and low-risk patients, while reference patients have a similar behavior to low-risk patients for the first 2 years since diagnosis.

Discussion

Changes in patient management and survival in patients with early-stage NSCLC may have brought about the majority of the lung cancer long survivors. Several studies have demonstrated that curative-intent surgery, when coupled with regional lymph node examination, is generally associated with the best long-term OS in patients with early-stage NSCLC (11).

The median survival in our cohort was 63.7 months (95% CI, 56.7-64.4). Significant differences were observed in survival in our patients. In our cohort, female gender is associated with greater survival, as previously reported in other studies (12, 13). In relation to smoking habit, approximately 88% of our patients diagnosed with stage I and II NSCLC were current or former smokers and survival was significantly lower in former or current smokers at diagnosis, compared to non-smokers. These findings support the idea that tobacco is the main cause of this type of tumour (14, 15). Of note, older age is usually associated with lower current smoking and higher former smoking prevalence (16), which could explain the lower odds for adverse outcome in current compared with former smokers. In any case, these results suggest that all levels of smoking exposure are likely to be associated with lasting and progressive lung damage (17), and therefore, anti-tobacco measures should be reinforced to reduce tobacco consumption, especially among young people (18, 19).

Very significant differences were also observed in survival among treatments. For stage I–II NSCLC patients medically fit for surgery, surgical resection remains the treatment of choice, yielding the best potential choice of cure for these patients. Lobectomy was performed in 88% of our patients, matching with the current standard procedure (20). Median age at diagnosis (60.6 years) and ECOG-PS 0 or 1 among the majority of our patients are consistent with general population candidates for surgery. In addition, hospital volume affects five-year survival. In an analysis of over 2000 patients from the Surveillance, Epidemiology, and End Results (SEER) Program database, five-year survival was better among individuals undergoing

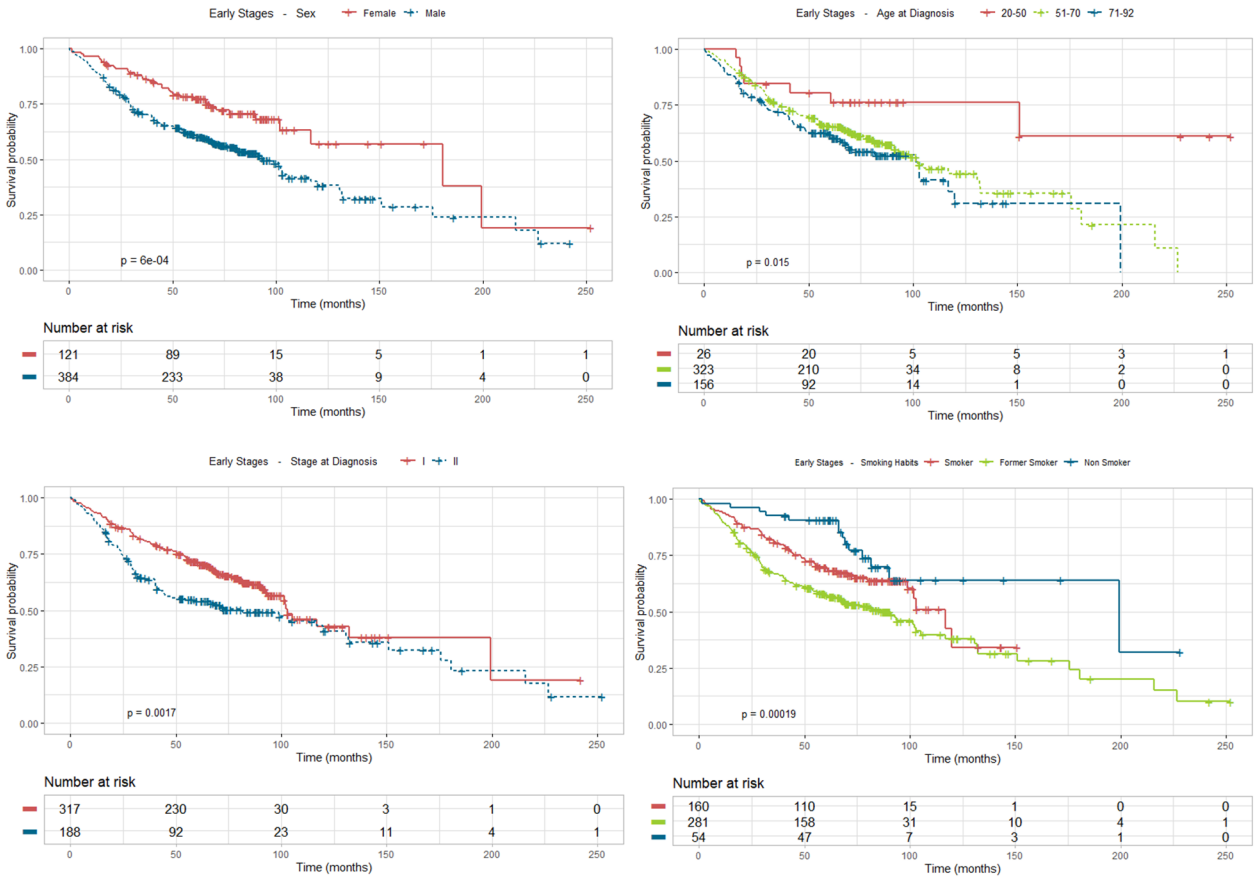

FIGURE 4
Survival analysis using Kaplan Meier estimates in stages I and II according to sex, age at diagnosis, stage and smoking habit.

resection at high-volume institutions (44 versus 33 percent at low-volume centers) (21). Our institution, being a tertiary hospital with a high volume of thoracic surgery procedures, may explain the significantly lower perioperative mortality rates, compared to those performed at lower-volume institutions (only 8% of our patients died within the first year since diagnosis).

After years of research evaluating the benefit of adding systemic therapy to surgery, two-phase III trials (8, 9) have shown an absolute survival benefit of 12 to 15% with the use of adjuvant chemotherapy in patients with stage I and II NSCLC (22). Results from The Lung Adjuvant Cisplatin Evaluation (LACE) meta-analysis demonstrated a 5.4% absolute survival benefit at 5 years [HR: 0.89 (95% CI: 0.82–0.96, p=0.005)] (4). Although in general the adjuvant studies in NSCLC have discordant results, recent data from recent studies demonstrate the clear benefit of adjuvant chemotherapy after surgery with an absolute increase in survival of 4% at five years (23). Adjuvant chemotherapy with a platinum doublet has become standard treatment for resected lung cancer patients. Of the 505 patients included in our study, 20.4% received adjuvant treatment after surgery which was slightly lower than in other similar cohorts (24–26), maybe due to the high proportion of patients with stage I versus stage II (62.8% vs 37.2%, respectively). Patients who received chemotherapy after surgery had a median survival of 65 months (IQR= 48.8) compared to 62.4 months (IQR= 52.5) for the patients who underwent surgery alone in our cohort.

Disease relapse also stands as a pivotal survival factor. Risk of local recurrence increases with the stage in lung cancer, but even stage I patients experience local recurrence up to 19% of the time (27). There was statistical difference (p=0.0085) in DFS between stage I and II in our cohort, being the median DFS for stage I 98.87 months (8.23 years) and 81.07 months (6.76 years) for stage II. The 5-year DFS for the stage I cohort was 63% and for stage II group was 48%. Of note, 32.3% (163) of our patients relapsed, 20% stage I and 28% stage II. Of those, 40 (24.5%) had received surgery plus adjuvant treatment and 123 (75.5%) surgery alone. The possibility of identifying patients with high-risk for recurrence following surgical resection can help with surveillance plans and potentially personalize adjuvant therapy for these patients (28–30).

Prediction models are usually developed to guide healthcare professionals in their decision-making about further treatment management and to inform patients about their risks of having (diagnosis) or developing (prognosis) a particular disease or outcome. The tumour, node, and metastasis (TNM) classification is currently considered gold standard for NSCLC prognostication despite standing as a poor predictor of overall survival, accounting

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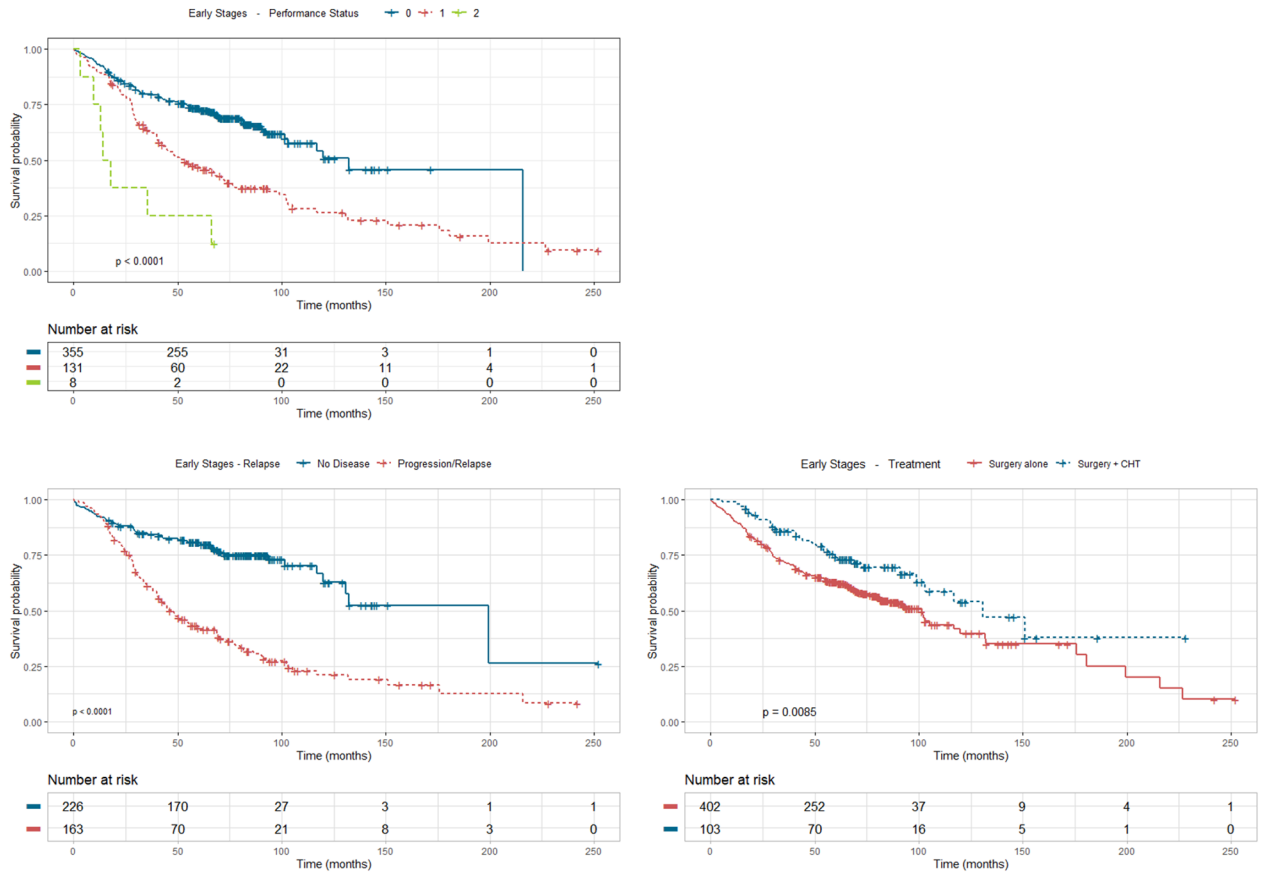

FIGURE 5
Survival analysis using Kaplan Meier estimates in stages I and II according to treatment, performance status and relapse.

TABLE 2 Univariate analysis of survival probability using Kaplan Meier estimates after 12 months and after 60 months.

Characteristics	Total	%	log-rank test		Survival Probability after 12 months				Survival Probability after 60 months		
			p-value	Estimate	St. Error	lower 95%	upper 95%	Estimate	St. Error	lower 95%	upper 95%
Overall	505	100%		0,919	0,0122	0,895	0,943	0,645	0,0216	0,613	0,697
Gender	506		<0.001								
Female	121	23,96%		0,9669	0,0163	0,9356	0,9993	0,7840	0,0384	0,7122	0,8629
Male	384	76,04%		0,9036	0,0151	0,8746	0,93336	0,6132	0,0253	0,5656	0,6648
Age at Diagnosis (years)	505		0,015								
20-50	26	5,15%		1	0	1	1	0,8060	0,0780	0,6670	0,9740
51-70	323	63,96%		0,9288	0,0143	0,9012	0,9573	0,6585	0,0269	0,6078	0,7135
71+	156	30,89%		0,8846	0,02560	0,8359	0,9362	0,6184	0,0396	0,5455	0,7011
Smoking Habits	495		<0.001								
Non Smoker	54	10,91%		0,9815	0,0183	0,9462	1	0,9066	0,0398	0,8319	0,9880
Former Smoker	281	56,77%		0,8932	0,0184	0,8579	0,9301	0,5770	0,0302	0,5207	0,6393
Current Smoker	160	32,32%		0,9375	0,0191	0,9007	0,9758	0,697	0,0371	0,628	0,7735

(Continued)

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TABLE 2 Continued

Characteristics	Total	%	log-rank test		Survival Probability after 12 months				Survival Probability after 60 months		
			p-value	Estimate	St. Error	lower 95%	upper 95%	Estimate	St. Error	lower 95%	upper 95%
Stage	505		0,0017								
I	317	62,77%		0,9340	0,0140	0,9070	0,9620	0,7195	0,0256	0,6711	0,7714
II	188	37,23%		0,8936	0,0225	0,8506	0,9388	0,5409	0,0375	0,4722	0,6195
Comorbidities	505		0,32								
No	56	11,09%		0,9643	0,0248	0,9169	1	0,6540	0,0644	0,5391	0,7933
Yes	449	88,91%		0,9131	0,0133	0,8875	0,9396	0,6539	0,0229	0,6106	0,7003
Patient with Previous Cancer	547		0,47								
No	375	65,56%		0,9643	0,0248	0,9169	1	0,6540	0,0644	0,5391	0,7933
Yes	172	31,44%		0,9131	0,0133	0,8875	0,9396	0,6539	0,0229	0,6105	0,7003
Treatment	505		0,008								
Surgery	402	79,60%		0,9005	0,0149	0,8717	0,9302	0,631	0,0243	0,5850	0,6805
Surgery +Adjuvant CHT	103	20,40%		0,9900	9,0097	0,9720	1,0000	0,7488	0,0455	0,6597	0,8387
Perform ante status	494		<0.001								
0	355	71,86%		0,9296	0,0136	0,9033	0,9566	0,7344	0,0238	0,6891	0,7826
1	131	26,52%		0,9008	0,0261	0,8510	0,9534	0,4680	0,0450	0,3380	0,5650
2	8	1,62%		0,7500	0,1530	0,5030	1,0000	0,250O	0,1531	0,0753	0,8302
Relapse	389		<0.001								
No disease	226	58,10%		0,9248	0,0175	0,891	0,9598	0,8093	0,0265	0,7589	0,8631
Relapse/Progression	163	41,90%		0,9325	0,0I96	0,8948	0,9718	0,4238	0,0401	0,352	0,5101

ECOG-PS, Eastern Cooperative Oncology Group-Performance status; CHT, chemotherapy.

TABLE 3 Multivariate analysis - Cox regression model.

Characteristics	Coefficient		Standard Error	Lower L95%	Upper U95%	p value
Gender						
Female	Reference Category					
Male	0,3667	1,44	0,2019	0,97	2,14	0,0693
Age at Diagnosis						
20-50	Reference Category					
51-70	0,9942	2,70	0,4743	1,07	6,85	0,0361
71+	1,1795	3,25	0,4876	1,25	8,46	0,0156
Smoking Habits						
Current Smoker	Reference Category					
Former Smoker	0,2480	1,28	0,1636	0,93	1,77	0,1294
Non Smoker	-0,4134	0,66	0,3208	0,35	1,24	0,1975

(Continued)

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TABLE 3 Continued

Characteristics	Coefficient		Standard Error	Lower L95%	Upper U95%	p value
Stage						
I	Reference Category					
II	0,3816	1,46	0,1545	1,08	1,98	0,0135
Treatment						
Surgery	Reference Category					
Surgery + Adjuvant CHT	-0,7782	0,46	0,2092	0,30	0,69	0,0002
Performance Status						
0	Reference Category					
1	0,7279	2,07	0,1505	1,54	2,73	<0.001
2	1,6883	5,41	0,3992	2,47	11,83	<0.001

ECOG-PS, Eastern Cooperative Oncology Group-Performance status; AJCC7, American Joint Committee on Cancer, seventh edition; CHT, chemotherapy.

TABLE 4 Prognostic model of survival including significant variables from the multivariate analysis.

Cox Survival Model		
Variables	Higher Risk Profile	Lower Risk Profile
Gender	Male	Female
Age at Diagnosis	71+	20-50
Smoking Habits	Former Smoker	Non Smoker
Stage	II	1
Treatment	Surgery	Surgery + Adjuvant CHT
Performance Status	2	0

CHT, chemotherapy.

for less than half of prognostic variance (31). NSCLC patients are inherently heterogeneous, and their prognosis relies on many different factors, which is why accurate survival beyond TNM stage should be obtained with the development of prediction models that can obtain specific patient profiles accounting for a range of predictive factors. While different models have been published in the last years, none have demonstrated superior performance, applicability or global utility yet (31–33).

The goal of our study was to develop a clinically useful prognostic model based on currently available risk factors. Our model identified two patient risk profiles based on six discriminative factors (sex, age at diagnosis, smoking habits, stage, treatment, and performance status): a high-risk that allows identifying those patients who may benefit from adjuvant treatment or immunotherapy if they are not fit for chemotherapy; and a low-risk, that endorse adjuvant treatment in post-surgical patients who are fit for chemotherapy. It also allows adapting surveillance plans for each risk profile and avoids unnecessary tests or visits.

Our study had some limitations. First, the sample size of our cohort may have limited the significance of the results. Our model may benefit from being developed and validated with a larger cohort of patients. Furthermore, information on genomic characteristics of the patients was not provided, which may improve this model, even in early stages.

With additional prospective and multisite validation, this prognostic model could potentially serve as a predictive decision

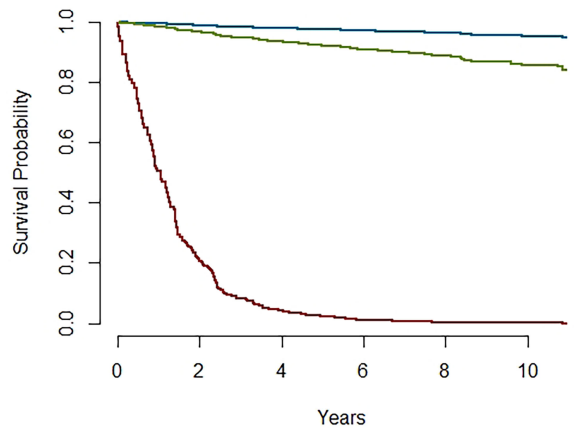

FIGURE 6
Survival probabilities for Higher (red) and Lower (blue) risk profiles and reference category (green).

support tool for deciding the use of adjuvant treatment in early stage lung cancer.

Conclusions

The results of the present study found that, overall, adjuvant chemotherapy was associated with the best long-term OS in patients with resected NSCLC. Age, stage and ECOG-PS were also significant factors to take into account when making decisions regarding adjuvant therapy. Continued work on individualized risk stratification, including prospective studies and research that incorporates this kind of prognostic models such as the one presented in this study as measures of risk, and is needed to better inform oncologists' decision-making regarding adjuvant therapy use after resection achieving a personalized care in practice standard.

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/s.

Ethics statement

The study was approved by the Ethics Committee at Hospital Universitario Puerta de Hierro-Majadahonda (No. PI 148/15) and was carried out in accordance with the Helsinki Declaration. Written informed consent was obtained from all patients' prior enrolment in the study.

Author contributions

MT, MP, and PS participated in the Conceptualization, Methodology, Investigation, Writing- and Original draft preparation; GG performed the formal analysis, Data curation, Validation, Writing- Reviewing and Editing; FF participated in the Conceptualization and Investigation; RH contributed to the Investigation, Writing- Reviewing and Editing; CP performed the Data curation and Validation; AS participated in the Data curation process, Validation, and Software; JC-C contributed to the Investigation, Writing- Reviewing and Editing; JP made critical revisions on the manuscripts and provided expert opinions on implications on the study findings. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1074337/full#supplementary-material

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CNS efficacy of afatinib as first-line treatment in advanced non-small cell lung cancer patients with EGFR mutations

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Background: Afatinib is a potent, irreversible second-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor which has demonstrated efficacy in advanced non-small cell lung cancer (NSCLC) patients harboring either common or uncommon EGFR mutations. However, data on its activity against brain metastases are limited. This study aimed to retrospectively evaluate the efficacy and safety of afatinib as first-line treatment for EGFR-mutant NSCLC patients with brain metastases.

Methods: Treatment-naive advanced NSCLC patients harboring EGFR mutations and brain metastases treated with afatinib were retrospectively reviewed to assess the central nervous system (CNS) efficacy and also the systematic benefits.

Results: Totally 43 patients with measurable or non-measurable brain metastases were enrolled in the CNS full analysis (cFAS) set. Among them, 23 patients with measurable brain metastases were included in the CNS evaluable for response (cEFR) set. The CNS ORR was 48.8% (95% CI, 33.3 - 64.5%) in the cFAS set and 82.6% (95% CI, 61.2 - 95.0%) in the cEFR set, respectively. CNS mDoR was 8.9 months (95% CI, 4.7 - 13.1 months) and CNS mPFS was 12.7 months (95% CI, 6.9 - 18.5 months) in the cFAS set. In the subgroup analysis stratified by EGFR mutation types, CNS ORR of cEFR set in the common mutation cohort was 100% (95% CI, 75.3 - 100%) and 60% (95% CI, 26.2 - 87.8%) in the uncommon mutation cohort (p = 0.024); CNS ORR of cFAS set was 57.7% (95% CI, 36.9 - 76.6%) and 35.3% (95% CI, 14.2 - 61.7%), respectively (p = 0.151). CNS mPFS was 14.4 months in patients with common mutations and 6.1 months in patients with uncommon mutations (hazard ratio, 0.47; 95% CI, 0.22 - 1.00; p = 0.045). Patients with common mutations showed a significantly lower cumulative incidence of CNS failure than uncommon mutation cohort (p = 0.0026). Most of patients experienced grade 1/2 treatment-related adverse events.

Conclusions: First-line afatinib demonstrated encouraging efficacy on brain metastases in NSCLC patients harboring either common or major uncommon

EGFR mutations in a real-world setting, with manageable toxicities. Patients with common mutations showed better CNS outcomes than those with uncommon mutations.

KEYWORDS

NSCLC, afatinib, brain metastases, CNS efficacy, uncommon EGFR mutations

1 Introduction

Brain metastases (BMs) occur in approximately 30% to 50% of non-small cell lung cancer (NSCLC) patients during the whole course of the disease, indicating poor prognosis and great challenges for treatment (1–3). Epidermal growth factor receptor (EGFR) mutation is one of the most pervasive oncogenic driver mutations in NSCLC, which is found in approximately 15% to 20% of Caucasian patients and 30% to 50% of Asian patients (4–6). The two most common EGFR mutations, exon 19 deletion (19 del) and exon 21 Leu858Arg (L858R) mutation account for approximately 80% to 90% of this oncogenic alteration, while uncommon EGFR mutations are estimated as approximately 10% to 20% (7–10). Patients with EGFR mutations are more prone to BMs than those with wild-type (11). Traditionally, the mainstream treatment options for NSCLC patients with BMs include surgical resection, stereotactic radiosurgery (SRS), and whole-brain radiotherapy (WBRT). However, these strategies may lead to radiation necrosis and significant compromises of loss of neurocognitive function (12–14). In the past two decades, the remarkable improvements by the molecular-targeted therapies have been seen in patients with NSCLC driven by oncogenic alterations, especially in EGFR tyrosine kinase inhibitors (TKIs). On the basis of the favorable results of prospective randomized trials, EGFR TKIs are now recommended as a standard first-line treatment replacing conventional platinum-based chemotherapy for patients with EGFR-mutated NSCLC (15–20). As a result of prolonging survival afforded by EGFR TKIs coupled with improvements of neuroimaging technology, patients seemed more inclined to develop BMs, with a 3-year cumulative risk of developing BMs increased to roughly 47% over the course of disease (21). Data on improved central nervous system (CNS) efficacy and manageable toxicities by some EGFR TKIs have also been reported previously (19, 20, 22–27). Given these, it's crucial to further explore the CNS efficacy of EGFR TKIs and optimize the first-line treatment and subsequent strategies for patients with BMs under the consideration of both overall survival benefit and patient quality of life.

Afatinib is a second-generation, irreversible ErbB family blocker that selectively blocks signals from ErbB family receptors (EGFR [ErbB1], HER2 [ErbB2], and ErbB4) and transphosphorylation of ErbB3, which cause a more sustained and wider-spectrum activity against EGFR mutations in contrast to reversible first-generation EGFR TKIs (erlotinib and gefitinib). Owing to its favorable efficacy in LUX-Lung series, afatinib was approved of the first-line treatment for NSCLC patients with EGFR mutations. In a combined analysis of LUX-lung 3 and 6 for common EGFR mutations and BMs (n = 48), afatinib demonstrated significant clinical activity against BMs with a CNS objective response rate (ORR) of 72.9% and median CNS progression-free survival (PFS) of 8.2 months (23). It also showed favorable CNS efficacy and survival outcomes in the real-world studies, irrespective of the EGFR mutation types (28, 29). And based on a series of reported findings mainly focus on common mutations, afatinib appeared to show a trend toward superiority over chemotherapy and first-generation EGFR TKIs in terms of CNS PFS, CNS ORR and cumulative incidence risk of CNS failure in patients with BMs (19, 22–25). Additionally, due to its significant clinical benefits in uncommon EGFR mutations such as G719X, S768I, L861Q, and some compound mutations (defined as ≥2 EGFR mutations and at least one uncommon EGFR mutation), afatinib is currently the only EGFR TKI approved for advanced NSCLC patients with G719X/L861Q/S768I (30). However, there were very few reports on the activity of afatinib for BMs in uncommon EGFR-mutant NSCLC patients.

There is still an unmet need to comprehensively assess the CNS efficacy of afatinib, especially in patients harboring uncommon mutations in the real-world setting. We conducted this study to explore its activity and tolerability in EGFR-TKI-naive patients with baseline BMs, expecting to help guide therapeutic selections of appropriate EGFR TKIs and thus to provide guidance for clinical practice.

2 Methods

2.1 Patients

EGFR-mutant NSCLC patients with BMs who received afatinib (30 mg or 40 mg, orally, once daily) as first-line treatment at Sun Yat-Sen University Cancer Center between March 2018 and January 2022 were retrospectively reviewed in this study. Patients received contrast computed tomography (CT) scans and contrast magnetic resonance imaging (MRI) at baseline and reviewed every 8 weeks from the start of afatinib until treatment discontinuation. Clinical and imaging data of eligible patients were extracted from the electronic medical records for response evaluation. This retrospective study was approved by the Institutional Review Board of Sun Yat-Sen University Cancer Center and conducted in accordance with the Declaration of Helsinki.

2.1.1 Inclusion and exclusion criteria

Patients who met the inclusion and exclusion criteria were eligible for evaluation in this retrospective study. The inclusion criteria details

were as follows (1): at least 18 years of age (2), pathologically confirmed NSCLC, (3) contrast MRI-detected BMs at baseline, (4) BMs without prior radiotherapy including asymptomatic BMs or BMs with focal neurological symptoms but no need for steroids, (5) laboratory-confirmed EGFR mutations detected by real-time PCR, Sanger sequencing, amplification-refractory mutation system (ARMS)-polymerase chain reaction (PCR) or next-generation sequencing, (6) at least one measurable extracranial lesion, defined as ≥10 mm, (7) an Eastern Cooperative Oncology Group (ECOG) performance status of 0 - 2, (8) no previous treatment with antineoplastic agents after initial diagnosis. The exclusion criteria were: (1) *de novo* EGFR T790M mutation and EGFR exon 20 insertion, (2) accompanied by other malignant tumors, (3) a combination with other anti-tumor agents.

2.2 Assessment

Treatment response was assessed by Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1) for both intracranial lesions and extracranial lesions. Measurable lesions were defined as target lesions (TLs)and non-measurable lesions as nontarget lesions (NTLs). Patients with measurable and/or non-measurable brain lesions at baseline were included in the CNS full analysis (cFAS) set. Patients with at least one measurable brain lesion at baseline were included in the CNS evaluable for response (cEFR) set. Besides that, subgroup analysis was made according to EGFR mutation subtypes. Severity of adverse events were recorded on the basis of Common Terminology Criteria for Adverse Events, version 5.0 (CTCAE 5.0).

2.3 Statistical analysis

CNS ORR, CNS disease control rate (DCR), CNS duration of response (DoR), CNS PFS, CNS time to response (TTR), cumulative incidence of CNS failure and best percentage change from baseline in TL size were recorded to evaluate the CNS response. CNS ORR was defined as the percentage of patients who achieved a best CNS response of complete response (CR) or partial response (PR). CNS DCR was defined as the proportion of patients with a CR or PR or stable disease (SD) in brain lesions. CNS DoR was defined as the time from first documentation of intracranial CR or PR until the time of progression (including intracranial progressive disease [PD] or extracranial PD) or death of any reason, whichever came first. CNS PFS was defined as the time from the first dose of afatinib until the time of progression (including intracranial PD or extracranial PD) or death of any reason, whichever came first. And CNS TTR was defined as the time from the first dose of afatinib to the time when the intracranial CR or PR to afatinib was first evaluated. The ORR and DCR were calculated with exact Clopper-Pearson 95% confidence intervals (CIs) based on the exact binomial distribution, and compared by chi-square test or Fisher's exact test. CNS DoR, PFS, and TTR were estimated by the Kaplan-Meier method with corresponding 95% CIs, and compared by log-rank test. Besides, a Cox proportional hazards model was applied to estimate HRs and 95% CIs with significance set at p <0.05 level. A competing risk analysis estimating the cumulative incidence for the event of interest (CNS progression) in the presence of competing risk event (non-CNS progression) was performed using a semiparametric Fine–Gray regression model. All the p values reported in the analysis were two-sided, and a p <0.05 level was considered statistically significant in the tests. And all statistical analyses were calculated using SPSS (version 26.0) except for the competing risks analysis, which were calculated with R software (version 4.1.2), and plots were executed using R software (version 4.1.2).

3 Results

3.1 Patient characteristics

By the data cut-off date as January 20, 2022, a total of 43 EGFR-mutant NSCLC patients with BMs at first diagnosis were enrolled in this retrospective analysis. The detailed baseline demographics and clinical characteristics of patients are presented in Table 1. Among these patients, 26 (60.5%) were male and 17 (39.5%) were female. The median age was 57 years (range, 37 - 79 years). All of them were Asians (Chinese), and most of them were adenocarcinoma (42 of 43, 97.7%) and nonsmokers (27/43, 62.8%). All patients were diagnosed with brain parenchymal metastases, none had leptomeningeal metastases. 4 (9.3%) patients had mild baseline CNS symptoms associated with brain metastases, including headache in 3 (7.0%) patients and dizziness in 1 (2.3%) patient. EGFR mutation status was confirmed by molecular pathology, with tumor biopsy tissue samples used in 35 (81.4%) patients, blood samples in 6 (14.0%) patients and pleural effusions samples in 2 (4.7%) patients. 26 (60.5%) patients were reported to have common EGFR mutations (16 [37.2%] were exon 19 deletions, 10 [23.3%] were exon 21 Leu858Arg), and 17 (39.5%) were reported to have uncommon mutations (3 [7.0%] were G719X, 2 [4.7%] were L861Q, 1 [2.3%] were S768I, and 11[25.6%] were compound mutations).

3.2 Treatment

Afatinib starting dose of 30 mg once daily was given to 26 patients and 40 mg once daily given to 17 patients as oncologist's option based on the integrative consideration of individual risk-benefit profile according to individual conditions such as age, weight and comorbidities, etc. Generally, older patients (≥70 years) and those with lower body weight (<50 kg) would more trend to start at 30mg once daily. All patients had never received prior EGFR TKIs or cytotoxic drugs for anti-cancer treatment.

3.3 Efficacy

3.3.1 CNS efficacy

Totally, 43 patients were eligible for CNS response evaluation as the cFAS set, of which 23 were included in the cEFR set.

In the cEFR set, the CNS ORR was 82.6% (95% CI, 61.2 - 95.0%) and the CNS DCR was 100% (95% CI, 85.2 - 100%), with 2 CR (8.7%), 17 PR (73.9%), and 4 SD (17.4%) (Table 2 and Figure 1). The CNS mPFS was 12.7 months (95% CI, 8.7 - 16.7 months). The CNS mDoR

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TABLE 1 Baseline demographics and clinical characteristics of patients.

Characteristic	Patients,n (%) (n = 43)
Gender	
Male	26 (60.5)
Female	17 (39.5)
Age,years	
Median age, years (range)	57 (37-79)
< 65	31 (72.1)
≥ 65	12 (27.9)
Race	
Asians	43 (100.0)
Smoking status	
Never	27 (62.8)
Current or former	16 (37.2)
ECOG PS	
0-1	40 (93.0)
2	3 (7.0)
Histologic type	
Adenocarcinoma	42 (97.7)
Adenosquamous carcinoma	1 (2.3)
EGFR mutation type	
Exon 19 del	16 (37.2)
L858R	10 (23.3)
Uncommon mutation	17 (39.5)
G719X	3 (7.0)
L861Q	2 (4.7)
S768I	1 (2.3)
Compound mutation	11 (25.6)
G719X+Exon 19 del	1 (2.3)
G719X+L861Q	2 (4.7)
G719X+E709X	2 (4.7)
G719X+S768I	2 (4.7)
G719X+V769M	1 (2.3)
S768I+L858R	1 (2.3)
E709X+L858R	1 (2.3)
L861Q+L833W	1 (2.3)
Patients with measurable brain lesions	
Yes	23 (53.5)
No	20 (46.5)
Number of brain lesions, n (%)	
1	8 (18.6)
2-5	12 (27.9)
>5	23 (53.5)
Site of distant metastasis	
Contralateral lung	24 (55.8)
Liver	12 (27.9)
Pleura	17 (39.5)
Pancreas	2 (4.7)
Bone	32 (74.4)
Adrenal gland	11 (25.6)
Abdominal/Pelvic cavity	5 (11.6)
Starting dose	
30mg	26 (60.5)
40mg	17 (39.5)

ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, epidermal growth factor receptor.

was 8.9 months (95% CI, 5.0 - 12.8 months). The median best percentage change from baseline in the sum of CNS TL size was -53.7% (range, -100.0% to -9.1%) (Figure 2).

In the cFAS set, the CNS ORR was 48.8% (95% CI, 33.3 - 64.5%) and the CNS DCR was 100% (95% CI, 91.8 - 100%), with 4 CR (9.3%), 17 PR (39.5%), and 22 SD (51.2%) (Table 2). The CNS mPFS was 12.7 months (95% CI, 6.9 - 18.5 months), with a 6-month CNS PFS rate of 74.7% (95% CI, 62.2 - 89.6%) and a 1-year CNS PFS rate of 51.2% (95% CI, 37.1 - 70.7%). The CNS mDoR was 8.9 months (95% CI, 4.7 - 13.1 months), with the estimated proportion of patients remaining in CNS response at 3, 6, and 9 months of 85.2%, 62.5% and 48.2%, respectively (Figures 3A, B). The CNS mTTR was 1.6 months (95% CI, 1.3 - 2.0 months), which were the same as the cEFR set. The baseline neurological symptoms in 4 patients were obviously improved after starting afatinib.

In the subgroup analysis stratified by EGFR mutation subtypes, as shown in Table 3, patients with common mutations (n = 13) achieved a significantly higher CNS ORR than those with uncommon mutations (n = 10) in the cEFR set (13 of 13 [100.0%] vs 6 of 10 [60.0%]; p = 0.024), as well as numerically higher CNS ORR in the cFAS set, though without statistical significance (15 of 26 [57.7%] vs 6 of 17 [35.3%]; p = 0.151). CNS mPFS was significantly longer in the common mutation group than the uncommon mutation group (14.4 months [95% CI, 12.0 - 16.8 months] vs 6.1 months [95% CI, 4.3 - 8.0 months]; HR 0.47; 95% CI, 0.22 - 1.00; p = 0.045) (Figures 4A, B). There were no significant differences in mDoR (12.0 months [95% CI, 4.1 - 19.9 months] vs 6.5 months [95% CI, 1.1 - 12.0 months]; HR, 0.57; 95% CI, 0.19 - 1.71; p = 0.310) and mTTR (2.0 months [95% CI, 0.6 - 3.3 months] vs 1.0 months [95% CI, 0.2 - 1.8 months]; HR, 0.43; 95% CI, 0.16 - 1.20; p = 0.097) in both groups. In the competing risk analysis for cumulative incidence of CNS failure, patients with common mutations showed a significantly lower cumulative incidence of CNS failure compared with those with uncommon

TABLE 2 CNS activity of afatinib in patients with brain metastases.

Analysis Set/Response	cEFR (n = 23)	cFAS (n=43)
CNS Best overall response, n (%)		
CR	2 (8.7)	4 (9.3)
PR	17 (73.9)	17 (39.5)
SD or non-CR/non-PD*	4 (17.4)	22 (51.2)
PD	0 (0.0)	0 (0.0)
CNS ORR,% (95% CI)	82.6 (61.2-95.0)	48.8 (33.3-64.5)
CNS DCR, % (95% CI)	100.0 (85.2-100.0)	100.0 (91.8-100.0)
CNS DoR		
Median, months (95% CI)	8.9 (5.0-12.8)	8.9 (4.7-13.1)
CNS PFS		
Median, months (95% CI)	12.7 (8.7-16.7)	12.7 (6.9-18.5)
Follow-up time		
Median, months (95% CI)	23.0 (5.6-40.4)	16.7 (10.9-22.5)
CNS TTR,month		
Median, months (95% CI)	1.6 (1.3-2.0)	1.6 (1.3-2.0)
Estimated % remaining in response (95% CI)		
At 3 months	83.5 (68.0-100.0)	85.2 (70.9-100.0)
At 6 months	64.2 (44.8-92.2)	62.5 (43.8-89.1)
At 9 months	47.6 (27.2-83.2)	48.2 (28.9-80.3)
CNS PFS, % (95% CI)		
Progression free at 6 months	85.5 (71.6-100.0)	74.7 (62.2-89.6)
Progression free at 12 months	55.3 (35.9-85.1)	51.2 (37.1-70.7)

CNS, central nervous system; cEFR, CNS evaluable for response; cFAS, CNS full analysis; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; CI, confidence interval; DoR, duration of response; PFS, progression-free survival; TTR, time to response.
*CNS response in patients with nontarget lesions only was classified as CR, non-CR, progressive disease (PD), or non-PD, but neither PR nor SD. Stable disease includes non-CR, non-PD in patients with nontarget lesions.

mutations (p = 0.0026), with the estimated probability of CNS progression at 12 months of 9.7% and 68.6%, respectively (Figure 4C). Briefly, the efficacy outcome in the common mutation group was generally better than the uncommon group.

3.3.2 Systemic efficacy

Forty-three people with measurable TLs were eligible for systemic response evaluation. ORR was 79.1% (95% CI, 64.0 - 90.0%) and DCR was 100% (95% CI 91.8 - 100.0%), with 34 PR (79.1%) and 9 SD (20.9%) (Table 4). The median best percentage change from baseline in systemic TL size was -47.7% (range, -83.0% to -6.3%). In subgroup analysis, ORR was 92.3% (95% CI, 74.9 - 99.1%) in patients with common mutations and 58.8% (95% CI, 32.9 - 81.6%) in patients with uncommon mutations (p = 0.018). DCRs of both subgroups were 100%.

3.4 Safety

Table 5 summarizes the most common treatment-related adverse events (TRAEs). The most common TRAEs (any grade) were skin rash or acne (37 of 43,86.0%), diarrhea (35 of 43, 81.4%), stomatitis or mucositis (30 of 43, 69.8%), and paronychia (25 of 43, 58.1%). Forty (93.0%) patients experienced at least one grade 1 - 2 TRAEs. Grade 3 TRAEs were reported in four (9.3%) patients, including one (2.3%) with rash or acne, two (4.7%) with diarrhea, one (2.3%) with thrombocytopenia. Grade 4 TRAEs or treatment related death were not seen. Among all 43 patients, only one patient permanently discontinued afatinib treatment due to Grade 3 rash. Four patients experienced temporary afatinib discontinuation for approximately one week due to intolerable TRAEs, after which the afatinib dose was reduced from 40mg to 30mg once daily to continue treatment. Five patients were tolerated well thus had afatinib dose escalation from 30mg to 40mg once daily for better clinical benefits. Overall, no unexpected TRAEs of afatinib were observed and most AEs were manageable and tolerable.

3.5 Follow-up

At data cut-off, 28 patients experienced disease progressions, with 13 intracranial PD only, 10 extracranial PD only, 5 both intracranial

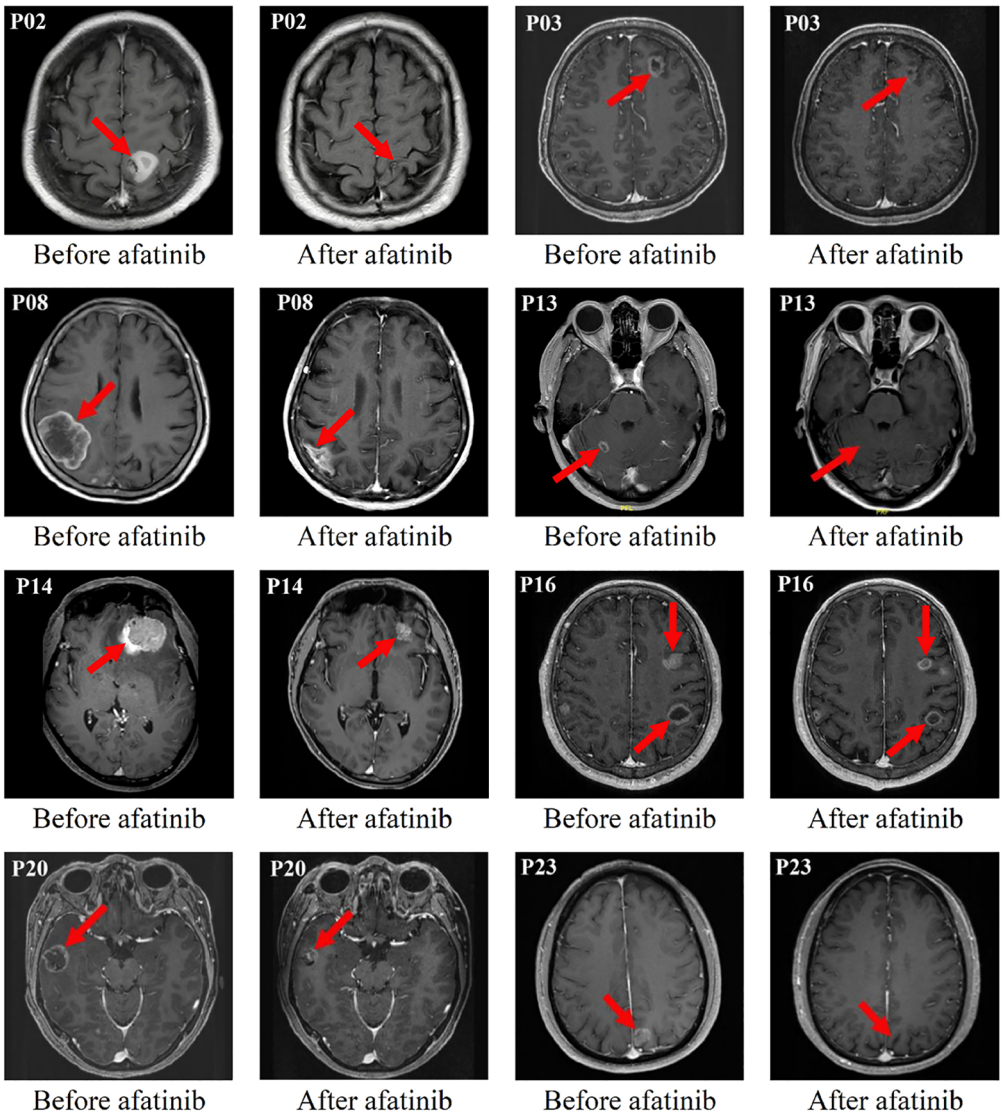

FIGURE 1
Eight typical examples of brain contrast MRI radiological changes in patients with measurable brain lesions (i.e. The red arrow points).

and extracranial PD. All patients with disease progressions discontinued afatinib treatment. Among the thirteen patients with intracranial progressions only, two patients were lost to follow-up, eight had genetic reassessment, of which acquired EGFR T790M-positive status was confirmed by blood sample in one patient and the same EGFR mutations remained detectable in the other seven patients, with blood samples used in five patients, cerebrospinal fluid sample in one patient and pleural effusion sample in one patient. Additionally, the remaining three patients without genetic reassessment were subsequently treated with radiotherapy for brain metastatic lesions, one had WBRT, one had stereotactic body radiotherapy (SBRT) and one had three-dimensional conformal radiotherapy (3D-CRT). The patient with EGFR T790M mutation switched to osimertinib. Other patients were switched to chemotherapy (with/without bevacizumab).

4 Discussion

To the best of our knowledge, afatinib is an irreversible second-generation EGFR TKI that has been approved for the first-line treatment for patients with EGFR-mutated NSCLC. Currently, evidence of its efficacy for initial treatment of BMs is rarely reported, especially in those harboring uncommon mutations. This retrospective study provided encouraging CNS ORR, CNS mPFS and other survival data to support that first-line afatinib was also favorable to control BMs in EGFR-positive NSCLC patients, with an acceptable safety profile, even in those with certain uncommon EGFR mutations.

Our data further strengthened the clinical benefits of afatinib to BMs. The efficacy of afatinib on BMs in the cEFR set as demonstrated by both CNS ORR (82.6%) and CNS mPFS (12.7 months) was relatively consistent with the previous findings (22–25, 28, 29).

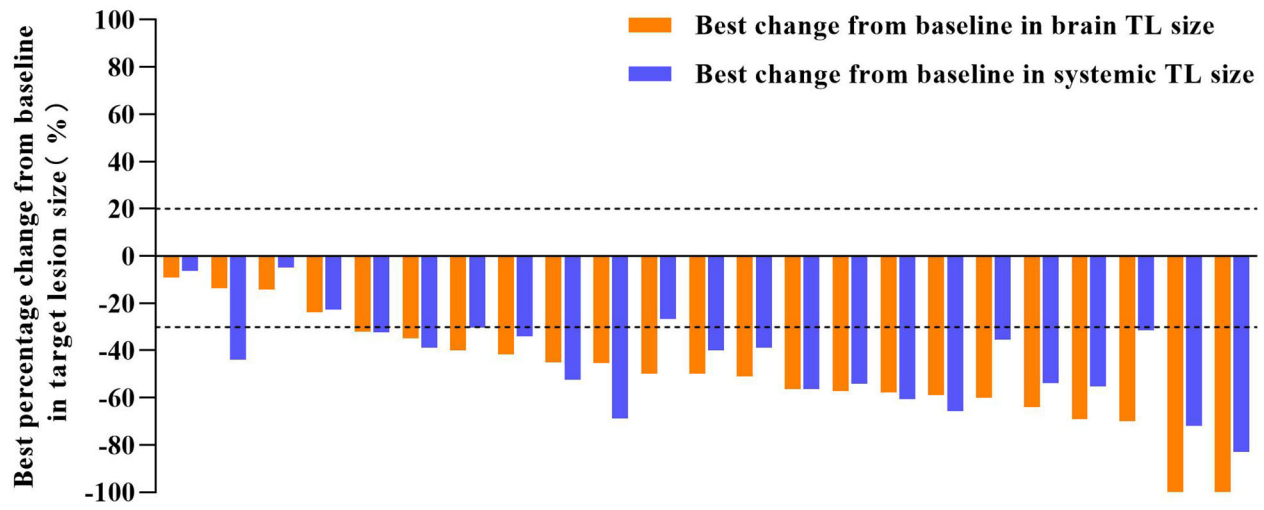

FIGURE 2
Tumor shrinkage in target lesion (TL) size of cEFR set. The median best percentage change from baseline in the sum of brain TL size was -53.7% (range, -100.0% to -9.1%). The median best percentage change from baseline in the sum of systemic TL size was -47.7% (range, -83.0% to -6.3%).

More specifically, in both of the cEFR and cFAS sets, CNS mPFS was longer than that reported in the combined analysis of LUX-lung 3 and 6 as well as LUX-lung 7, which is 8.2 months and 7.2 months in common EGFR-mutant patients, respectively (19, 23). A probable explanation for this could be the inherent limitation of this single-center retrospective analysis, in which selection bias was inevitable. Long-term maintenance of afatinib and more effective management of TRAEs may contribute in part to longer PFS in our analysis. Besides, we noticed that CNS ORR of the cFAS set was 48.8%, which appeared to be lower than that in the cEFR set. This was mainly due to the high proportion of included patients with non-measurable brain lesions. That's to say, many cases in the cFAS set cannot be calculated into the ORR, unless the response of patients was assessed as a CR.

The median mTTR was 1.6 months in both cEFR and cFAS sets, indicating a rapid CNS response to afatinib. This comparison supported that afatinib may rapidly shrink the brain metastasis, regardless the tumor size and location, without worrying about radiation necrosis and neurocognitive dysfunctions which may led by brain radiation.

There are few clinical data reporting the CNS activity of afatinib in patients carrying uncommon EGFR mutations. BMs seemed to exert a detrimental influence on the survival of advanced NSCLC patients with G719X/L861Q/S768I (31). Based on a combined analysis of LUX-Lung 3 and 6, CNS ORR was 33.3% (3 of 9) in patients with uncommon EGFR mutations and BMs (23). Outcomes presented by Yang et al. also indicated that afatinib might have encouraging CNS activity against tumors harboring uncommon EGFR mutations (56% in major uncommon mutations, 25% in exon 20 insertions, 9% in T790M and 10% in others) with median CNS TTF of 8.2 months in a subgroup of patients with BMs (32). Our study represents a more comprehensive analysis exploring CNS response to afatinib in patients with BMs harboring uncommon EGFR mutations, as well as comparing the differences of CNS efficacy between the two EGFR mutation cohorts. In our subgroup analysis, the uncommon EGFR mutation cohort consisted of 64.7% (11 of 17) patients with compound mutations and 35.3% (6 of 17) patients with single major uncommon EGFR mutation. We mainly used two statistical methods for the time-to-event analysis to

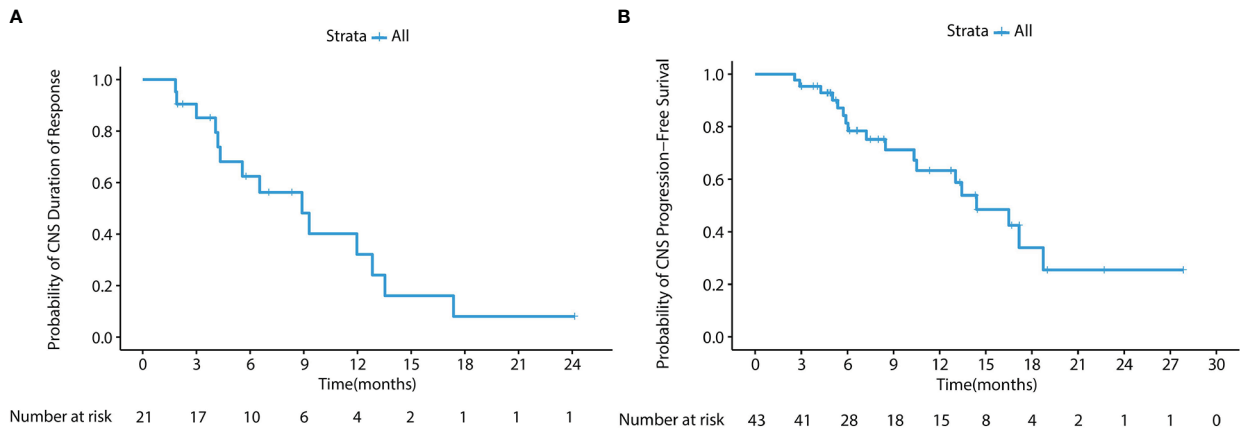

FIGURE 3
(A) Kaplan-Meier survival curve of CNS DoR in the cFAS set. The CNS mDoR was 8.9 months (95% CI, 4.7 - 13.1 months). **(B)** Kaplan-Meier survival curve of CNS PFS in the cFAS set. The CNS mPFS was 12.7 months (95% CI, 6.9 - 18.5 months).

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TABLE 3 CNS activity of afatinib in patients harboring common mutations or uncommon mutations.

Analysis Set/Response	cEFR		cFAS	
	Uncommon mutation (n =10)	Common mutation (n =13)	Uncommon mutation (n=17)	Common mutation (n=26)
CNS Best overall response, n (%)				
CR	1 (10.0)	1 (7.7)	1 (5.9)	3 (11.5)
PR	5 (50.0)	12 (92.3)	5 (29.4)	12 (46.2)
SD or non-CR/non-PD	4 (40.0)	0 (0.0)	11 (64.7)	11 (42.3)
PD	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CNS ORR, % (95% CI)	60.0 (26.2-87.8)	100.0 (75.3-100.0)	35.3 (14.2-61.7)	57.7 (36.9-76.6)
CNS DCR, % (95% CI)	100.0 (69.2-100.0)	100.0 (75.3-100.0)	100.0 (80.5-100.0)	100.0 (86.8-100.0)
CNS DoR				
Median, months (95% CI)			6.5 (1.1-12.0)	12.0 (4.1-19.9)
CNS PFS				
Median, months (95% CI)			6.1 (4.3-8.0)	14.4 (12.0-16.8)
TTR,month				
Median, months (95% CI)			1.0 (0.2-1.8)	2.0 (0.6-3.3)
Estimated % remaining in response (95% CI)				
At 3 months			83.3 (58.3-100.0)	86.7 (71.1-100.0)
At 6 months			66.7 (37.9-100.0)	60.7 (38.6-95.3)
At 9 months			33.3 (10.8-100.0)	45.5 (22.1-94.0)
PFS, % (95% CI)				
Progression free at 6 months			54.6 (34.5-86.5)	87.6 (75.4-100.0)
Progression free at 12 months			24.6 (9.6-62.9)	68.7 (51.7-91.2)
12-month cumulative incidence rate of CNS failure			68.6%	9.7%

sufficiently evaluate CNS efficacy in both common and uncommon mutation cohorts: CNS PFS and cumulative incidence of CNS failure. We found that afatinib demonstrated pronounced CNS activity in the common EGFR mutation cohort with a significantly superior CNS ORR (cEFR), CNS mPFS and a significantly lower cumulative incidence of CNS failure versus the uncommon EGFR mutation cohort. Nonetheless, the uncommon EGFR mutation cohort also showed favorable outcomes with a CNS ORR of 60% (cEFR) and CNS mPFS of 6.1months. The subgroup analysis provided a preliminary exploration on the activity of afatinib in the uncommon EGFR-mutant NSCLC patients with BMs, and the results showed that afatinib also had encouraging CNS efficacy in patients with uncommon EGFR mutations although inferior to that of common EGFR mutations.

Currently, the first-, second- and third-generation EGFR TKIs are available for EGFR-mutant NSCLC patients with BMs. This inevitably leads to the question of tailing different lines of EGFR TKI treatment to deploy the best whole-course strategy for patients. It's known that second- and third-generation EGFR TKIs confer superior efficacy over first-generation TKIs in patients with BMs based on a series of clinical trials and retrospective analyses (19, 20, 24–27). However, to date, only limited retrospective analyses have demonstrated clinical efficacy of dacomitinib on BMs since patients with BMs are excluded in Phase III ARCHER 1050 trial. Further prospective studies and real-world analyses are warranted to validate the intracranial efficacy of dacomitinib. Afatinib, another irreversible second-generation EGFR TKI, demonstrates superior survival benefits to first-generation TKIs in EGFR-mutant NSCLC patients with BMs. Data presented in our research also lend support to the use of afatinib as a treatment option for BMs in NSCLC patients with either common EGFR mutations or uncommon EGFR mutations. Due to a stronger ability to cross the BBB and penetrate the CNS, osimertinib, an irreversible third-generation EGFR TKI, has superior CNS activity to first- and second-generation TKIs (33). In FLAURA, osimertinib demonstrated pronounced CNS efficacy with an CNS ORR of 91% in the cEFR set and 66% in the cFAS set, which is superior to first-generation EGFR TKIs, representing a clinically significant treatment option for patients with EGFR mutations and BMs (20). However, there's a lack of head-to-head clinical trial comparing the CNS efficacy of osimertinib with second-generation EGFR TKIs. The optimal management of targeted therapy for BMs is still unclear. Based on the above, it seems that second- or third-generation TKI is supposed to serve as a prior treatment selection expecting to maximize the efficacy to control brain lesions. Opinions

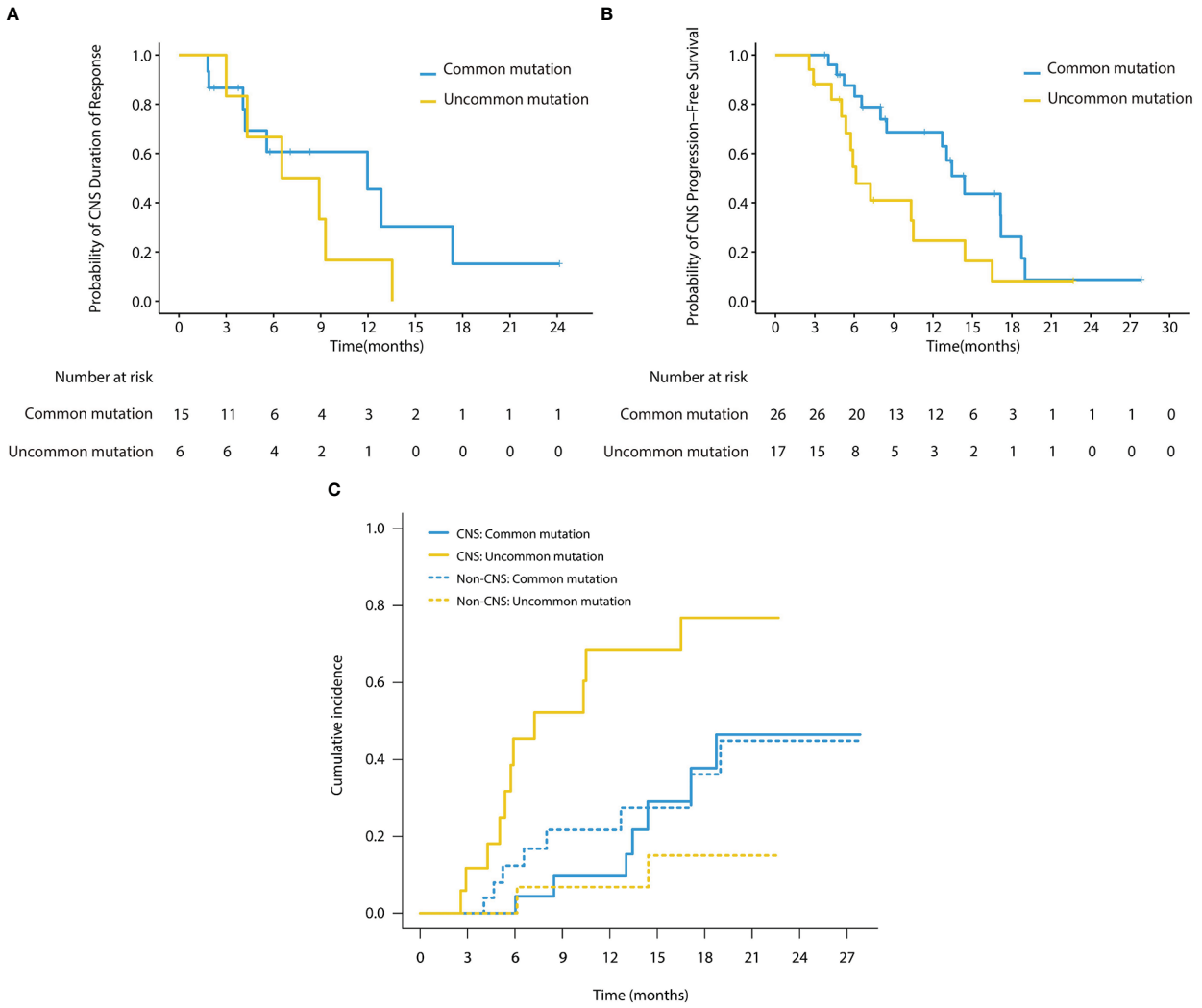

FIGURE 4
(A) Kaplan-Meier survival curves of CNS DoR in subgroup analysis (cFAS). The CNS mDoR was 12.0 months (95% CI, 4.1 - 19.9 months) in patients with common mutations and 6.5 months (95% CI, 1.1 - 12.0 months) in patients with uncommon mutations (HR, 0.57; 95% CI, 0.19 - 1.71; p = 0.31). **(B)** Kaplan-Meier survival curves of CNS PFS in subgroup analysis (cFAS). The CNS mPFS was 14.4 months (95% CI, 12.0 - 16.8 months) in patients with common mutations and 6.1 months (95% CI, 4.3 - 8.0 months) in patients with uncommon mutations (HR, 0.47; 95% CI, 0.22 - 1.00; p = 0.045). **(C)** Cumulative incidence of CNS failure in patients with baseline brain metastases. Patients with common mutations showed a significantly lower cumulative incidence of CNS failure compared with those with uncommon mutations (p = 0.0026), with the estimated probability of CNS progression at 12 months of 9.7% and 68.6%, respectively.

TABLE 4 Systemic activity of afatinib in patients with brain metastases.

Analysis Set/Response	Uncommon mutation (n = 17)	Common mutation (n = 26)	All patients (n = 43)
Best overall response, n (%)			
CR	0 (0.0)	0 (0.0)	0 (0.0)
PR	10 (58.8)	24 (92.3)	34 (79.1)
SD	7 (41.2)	2 (7.7)	9 (20.9)
PD	0 (0.0)	0 (0.0)	0 (0.0)
ORR, % (95% CI)	58.8 (32.9-81.6)	92.3 (74.9-99.1)	79.1 (64.0-90.0)
DCR, % (95% CI)	100.0 (80.5-100.0)	100.0 (86.8-100.0)	100 (91.8-100.0)

TABLE 5 TRAEs of afatinib in patients with brain metastases.

TRAEs (n = 43)	All grades, n (%)	Grades 3–4, n (%)
Any TRAE	40 (93.0)	4 (9.3)
Rash or acne	37 (86.0)	1 (2.3)
Diarrhea	35 (81.4)	2 (4.7)
Stomatitis or mucositis	30 (69.8)	0 (0.0)
Paronychia	25 (58.1)	0 (0.0)
Pruritus	4 (9.3)	0 (0.0)
Decreased appetite	6 (14.0)	0 (0.0)
Vomiting	0 (0.0)	0 (0.0)
Nausea	2 (4.7)	0 (0.0)
Constipation	2 (4.7)	0 (0.0)
Fatigue	3 (7.0)	0 (0.0)
Alopecia	0 (0.0)	0 (0.0)
Increased ALT/AST	6 (14.0)	0 (0.0)
Anaemia	0 (0.0)	0 (0.0)
Leukopenia	2 (4.7)	0 (0.0)
Neutropenia	0 (0.0)	0 (0.0)
Thrombocytopenia	0 (0.0)	1 (2.3)

TRAEs, treatment-related adverse events.

differ from each other when it comes to the selection of second- or third-generation TKIs as first-line treatment. There are pros and cons to both treatment options. It's reported that T790M accounts for more than half of all cases of acquired resistance to first or second-generation TKIs, but the resistance mechanism of osimertinib remains obscure (34, 35). Based on the subgroup analysis of AURA 3, osimertinib also shows promising CNS efficacy with an CNS ORR of 70% in the cEFR set for patients with BMs and metastatic T790M-positive NSCLC (36). Given the high incidence of acquired T790M-positive status in patients with disease progression following the first- or second-generation TKIs and favorable CNS activity of osimertinib given as a subsequent treatment, sequential use of second-generation TKIs and osimertinib may be potentially a feasible first-choice therapeutic strategy for patients with BMs. In terms of the immature CNS efficacy of dacomitinib, sequential afatinib followed by osimertinib may be a priority, especially in those harboring uncommon mutations. More prospective clinical trials including head-to-head trials are needed to address the question of the optimal management of BMs.

Our study had certain limitations. First, this is a single-center retrospective study that potential for selection bias is inevitable and adverse events data may be under-reported, which may result in slightly inconsistent data compared with other studies. Second, due to the relatively small cohort size of the study, there are limitations to draw firm conclusions on the clinical benefits across different subgroups. A further limitation is that the efficacy of afatinib on leptomeningeal metastases remained unclear as all patients observed in this study had parenchymal but no leptomeningeal metastases. In the next stage, we could conduct a multi-center study as well as expand the sample size to further validate our results and supplement the deficiencies.

Briefly, in this study, afatinib first-line treatment was found to have encouraging efficacy in brain metastases in advanced NSCLC patients harboring either common or major uncommon EGFR mutations in a real-world setting, with manageable toxicities. Patients with common mutations showed better CNS outcomes than those with uncommon mutations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Sun Yat-Sen University Cancer Center and conducted in accordance with the Declaration of Helsinki.

Author contributions

Conception and design: YBL, YL, LK. Provision of study materials or patients: YBL, YL, WL. Data collection: LK, JM, CH, QZ. Analysis and interpretation of data: LK, JM, WL. Drafting and revision of the manuscript: All authors. Study supervision: YBL, YL. 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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Adjuvant chemotherapy compared with observation in patients with T2aN0 stage IB lung adenocarcinoma

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Introduction: For patients with T2aN0 stage IB lung adenocarcinoma, benefits of adjuvant chemotherapy remain controversial. Here, we aimed to evaluate such benefits.

Methods: This retrospective cohort study was conducted on the database of the National Taiwan Cancer Registry. We analyzed patients with T2aN0 stage IB lung adenocarcinoma (re-classified by AJCC 8th edition) diagnosed during the period from January 2011 to December 2017. They were divided into two groups: (1) group 1: tumor <=3 cm with visceral pleural invasion (VPI); (2) group 2: tumor >3 cm, but <=4 cm. Overall survival (OS) and cancer specific survival (CSS) were evaluated. Risk factors for survival were determined.

Results: A total of 2,100 patients with T2aN0 stage IB lung adenocarcinoma (1,265 in group 1 and 835 in group 2) were enrolled for study. The proportions of patients receiving adjuvant chemotherapy in group 1 and 2 were 39.1% and 68.6%, respectively. Amongst group 1 patients, adjuvant chemotherapy was not an independent risk factor for OS and CSS. Amongst group 2 patients, high-grade histologic findings and receiving sublobar resection were two risk factors for poorer survival. Adjuvant chemotherapy was also associated with an OS (adjusted hazard ratio (aHR), 0.52; 95% confidence interval (CI), 0.38-0.72; P<0.001) and CSS (aHR, 0.54; 95% CI, 0.37-0.78; p=0.001) benefit regardless of the presence or absence of risk factors.

Conclusion: For patients with T2aN0 stage IB lung adenocarcinoma, adjuvant chemotherapy improved OS and CSS in those with tumors >3 cm, but <=4 cm.For patients with tumors <=3 cm with VPI, adjuvant chemotherapy had no survival benefit.

KEYWORDS

lung adenocarcinoma, adjuvant chemotherapy, T2aN0, stage IB, early lung cancer

Introduction

Lung cancer is by far the leading cause of cancer-related death (1). Complete surgical resection of the tumor provides a hope for a cure for those patients with resectable disease (2). However, post-operative recurrence poses a main problem of the treatment (3). Therefore, identifying populations who may benefit from additional treatment after surgery may improve the clinical outcomes in those patients with resectable lung cancer.

Several randomized clinical trials reported the efficacy of adjuvant chemotherapy following surgery in patients with resectable lung cancer (4–8). The pooled analysis of 5 trials of cisplatin-based adjuvant chemotherapy revealed benefit of adjuvant chemotherapy in completely resected lung cancer patients at an overall hazard ratio (HR) of 0.89 (95% CI, 0.82-0.96; p=0.005). In further subgroup analysis, the benefit is restricted to patients with stage II or IIIA disease. There was no significant improvement of survival in patients with stage IB or IA lung cancer (9). Another study, Cancer and Leukemia Group B (CALGB) 9633, a randomized controlled trial, was designed to solve the unmet need. Patients enrolled had pathologically confirmed T2N0 (according to the International System for Staging Lung Cancer edition in 1997) (10) non-small-cell lung carcinoma (NSCLC) undergoing complete surgical resection. The study showed a significant survival benefit of adjuvant chemotherapy for patients with tumors 4 cm or larger in diameter (HR, 0.69; 95% CI, 0.48- 0.99; p=0.043) (11).

Tumors larger than 4 cm, but 5 cm or less in size without lymph node metastasis are now classified as T2bN0 stage IIA lung cancer, according to AJCC staging system 8th edition (12). Their benefits of adjuvant chemotherapy are mentioned above (11). On the other hand, for patients with T2aN0 stage IB lung cancer, benefits of adjuvant chemotherapy remain unclear. Though several studies advocated the benefit of adjuvant chemotherapy for patients with stage IB lung cancer (8, 11, 13–16). the cancer staging was based on the 5th, 6th, or 7th international staging criteria (10, 17). Furthermore, prior randomized controlled trials enrolled NSCLC patients and did not subdivide them according to histology types. Nevertheless, there is increasing evidence that different histology types (lung adenocarcinoma vs. non-adenocarcinoma) presented with different clinical outcomes (18, 19). A meta-analysis partially answered the above-mentioned questions. The author pooled the studies regarding the impact of adjuvant chemotherapy in stage IB NSCLC in the context of the 8th TNM staging system. Subgroup analysis by histology indicated that adjuvant chemotherapy conferred more favorable survival to both squamous cell carcinoma and adenocarcinoma. However, the eligible studies were retrospective and with population heterogeneity, and subgroup analysis according to tumor size (e.g., tumor <=3 cm vs. tumor >3 but <=4 cm) was not performed (20). Apart from tumor size, other high-risk histopathologic features (e.g., tumor differentiation, vascular invasion, visceral pleural involvement) and surgical factors (e.g., sublobar resection, unknown lymph node status) are presumably indications for adjuvant chemotherapy (21). Little evidence is available to support these indications. Here, we conducted a retrospective cohort study on a nationwide population database in Taiwan, aiming to determine benefits of adjuvant chemotherapy for patients with completely resected T2aN0 stage IB lung adenocarcinoma.

Materials and methods

Data source

This retrospective cohort study used data from the National Taiwan Cancer Registry. The database was established by the Ministry of Health and Welfare in 1979, and it kept standardized records of patients' characteristics and clinical information on all newly diagnosed malignant cancer cases in Taiwan (22–24). Detailed information on the smoking status for lung cancer patients has been recorded in the database starting since 2011. We analyzed newly diagnosed lung cancer patients from January 2011 to December 2017. The main outcome parameter was overall survival and cancer-specific survival. This study was approved by the Research Ethics Committee of the National Taiwan University (NTU-REC No.202101HM030), with waiver of informed consent owing to the lack of personal information and use of secondary data in the study. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for observational studies was used in the revision of this article.

Data records and definition

Clinical data used for analysis included the following: age at diagnosis, sex, Eastern Cooperative Oncology Group (ECOG)

performance status, histologic types, tumor size, tumor stage, smoking status, histologic grade, visceral pleural invasion (VPI), extent of resection, adjuvant treatment, status of N2 stations dissection, and types of health care institution. Sublobar resection refers to wedge resection and segmentectomy. Histologic grade was grouped into low grade (well or moderately differentiated) and high grade (undifferentiated or poorly differentiated). The staging system of lung cancer before 2018 was conducted according to the AJCC staging system 7th edition (17).

Study population

We re-classified the enrolled patients according to the AJCC staging system 8th edition (12). Patients who met the criteria of pathological T2aN0 stage IB were analyzed. In other words, we excluded patients with tumors larger than 4 cm. As mentioned above, patients with different histology types experienced different prognosis (18, 19), we focused on adenocarcinoma in the present study. We also excluded those who had unknown tumor size, unknown VPI, unknown histological grading, and unknown smoking status. Patients with incomplete resection of the tumor and those who received adjuvant targeted therapy or other treatments were not included. Patients younger than 20 years old, greater or equal to 75 years old, with ECOG performance status of 2 or greater were not included. The selection algorithm of participants is illustrated in Figure 1.

According to AJCC staging system 8th edition, T2aN0 stage IB lung cancer includes tumors larger than 3 cm, but 4 cm or less in size, with involvement of main bronchus without carina, with visceral pleural invasion, or atelectasis or post obstructive pneumonitis. We categorized patients with tumors 3 cm or less into group 1. We focused on those with VPI because these populations accounted for most group 1 patients. Patients with tumors larger than 3 cm, but 4 cm or less in size were categorized into group 2.

Statistical analyses

To compare inter-group differences for categorical and continuous variables, Pearson's chi-square test, and t test were used respectively. Overall survival (OS) is the length of time from the date of cancer diagnosis to the date of death due to any cause, or to the date of last follow-up. Cancer-specific survival (CSS) is the length of time from the date of cancer diagnosis to the date of death from the disease. Disease-free survival (DFS) is the length of time from primary treatment for the cancer to the date of disease recurrence or death. Survival status was determined based on the national death certificate database from the Department of Statistics, Ministry of Health and Welfare, Taiwan, and the status was updated until December 31, 2020. OS and CSS of patients were estimated using the Kaplan–Meier method, whereas the inter-group differences were assessed using the stratified log-rank test. Associations between clinicopathologic variables and outcomes were assessed using Cox proportional hazards regression model. The strength of association was presented as the Hazard ratio (HR) and 95% confidence interval (CI). In this study, we used the two-tailed test, and the significant level was set at $P < 0.05$. All analyses were performed using SAS, version 9.4 statistical software (SAS Institute Inc).

Results

Study population

We analyzed 2,100 patients with T2aN0 stage IB lung adenocarcinoma. There were 1,265 (60.2%) patients having tumors 3 cm or less, and 835 (39.8%) patients having tumors larger than 3 cm, but 4 cm or less in size. Amongst patients with tumors 3 cm or less, 495 (39.1%) patients received adjuvant chemotherapy, whereas patients with tumors larger than 3 cm, but 4 cm or less in size, 573 (68.6%) received adjuvant

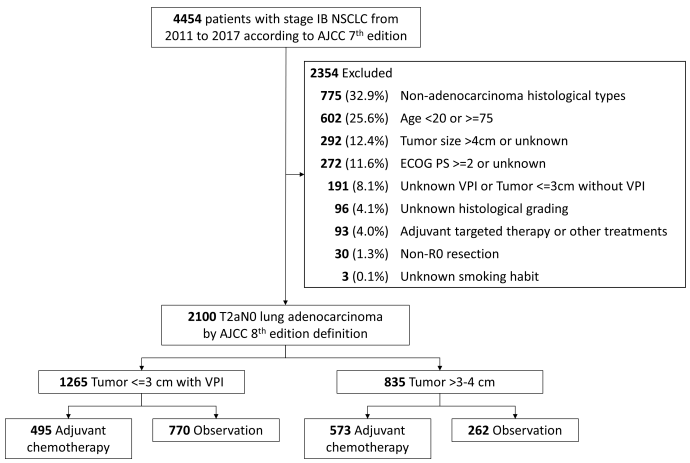

FIGURE 1
Algorithm for inclusion of study participants. Abbreviations: NSCLC, non-small-cell lung carcinoma; ECOG PS, Eastern Cooperative Oncology Group Performance Status; AJCC, American Joint Committee on Cancer; VPI, visceral pleura invasion.

chemotherapy. The details of the patient characteristics were shown in Supplementary Table 1. OS and CSS were significantly different between patients with different tumor sizes (Supplementary Figure 1A, B). For patients in group 1 (tumors <=3 cm with VPI) and groups 2 (tumor >3 cm, but <=4 cm), the 5-year OS were 90.6% vs 84.4%, while the 5-year CSS were 93.0% vs 88.2%.

Patient characteristics

Group 1: tumor <=3 cm with VPI

The characteristics of patients with tumors 3 cm or less is shown in Table 1A. Clinicopathological parameters were compared between the observation group and adjuvant chemotherapy group. More patients in the adjuvant chemotherapy group had high-grade histologic findings (26.7% vs. 20.9%, p=0.002), received lobectomy (86.1% vs. 76.6%, p<0.001), had larger tumors (>2 cm, but <=3 cm in size; 63.2% vs. 50.8%, p<0.001), and were treated in regional hospitals (24.8% vs. 16.5%, p<0.001). The recurrence rates between patients with and without chemotherapy were not significantly different (19.4% vs. 17.7%, p=0.418).

Group 2: tumor >3 cm, but <=4 cm

The characteristics of patients with tumors larger than 3, but 4 cm or less in size are shown in Table 1B. Patients in the adjuvant chemotherapy group had more visceral pleural invasion (43.6% vs. 34.7%, p=0.02), more received lobectomy (95.1% vs. 90.8%, p=0.02), more with ECOG performance status of 0 (76.8% vs. 61.8%, p<0.001), and more treated in medical centers (82.4% vs. 63.0%, p<0.001). The recurrence rates between patients with and without chemotherapy were not significantly different (26.0% vs. 26.1%, p=0.517).

Prognostic factors for survivals

Group 1: tumor <=3 cm with VPI

In the multivariable Cox proportional hazard model, age >65 to 74 years old, tumor size larger than 2 cm, but <=3 cm, and being treated in regional hospitals were identified as independent prognostic factors for OS (Table 2A). Regarding CSS, tumor size larger than 2 cm, but <=3 cm, and being treated in regional hospitals were identified as independent prognostic factors (Table 2A).

Group 2: tumor >3 cm, but <=4 cm

In the multivariable Cox proportional hazard model, age >65 to 74 years old, high grade histologic findings, smoking habit, and received adjuvant chemotherapy were identified as independent prognostic factors for OS. Receiving sublobar resection was identified as a prognostic factor in the univariate analysis (Table 2B). As regard to CSS, high grade histologic findings, receiving sublobar resection, and receiving adjuvant chemotherapy were identified as independent prognostic factors (Table 2B). VPI had no influence on OSS or CSS. Accordingly, we defined having either high-grade histologic findings or receiving sublobar resection as having risk factors.

Association between adjuvant chemotherapy and OS or CSS

Group 1: tumor <=3 cm with VPI

As shown in Figure 2A, amongst patients with tumors 3 cm or less with VPI, adjuvant chemotherapy was not associated with improved OS (adjusted HR [aHR], 0.98; 95% CI, 0.70-1.35; p=0.892). As mentioned previously, tumor size larger than 2 cm, but <=3 cm was identified as a prognostic factor for survival. Therefore, we sub-divided group 1 patients according to their tumor sizes (cut-off size at 2 cm), and again found no benefit of adjuvant chemotherapy on OS irrespective of tumor size. Regarding CSS, results were similar (Figure 2B).

In group 1, OS and CSS between patients with and without adjuvant chemotherapy were similar (Figure 3A, D). For patients with and without adjuvant chemotherapy, their 5-year OS were 89.9% vs 91.1%, while the 5-year CSS were 91.7% vs 93.8%. In subgroup analysis according to tumor size (cut-off size at 2 cm), no survival difference was found between those with and without adjuvant chemotherapy (Figures 3B, C, E, F).

Group 2: tumor >3 cm, but <=4 cm

As shown in Figure 2A, in all patients with tumors larger than 3 cm, but 4 cm or less in size, adjuvant chemotherapy was associated with improved OS (aHR, 0.52; 95% CI, 0.38-0.72; p<0.001). As stated above, having either high-grade histologic findings or receiving sublobar resection were defined as risk factors in group 2. We sub-divided group 2 patients according to the presence of any risk factors, and the benefit of adjuvant chemotherapy on OS was again found even in the absence of any risk factors. Regarding CSS, results were similar (Figure 2B).

In group 2, OS and CSS were significantly different between patients with and without adjuvant chemotherapy (Figures 4A, D). For patients with and without adjuvant chemotherapy, the 5-year OS were 87.4% vs 77.9%, while the 5-year CSS were 90.6% vs 82.8%. In subgroup analysis, the survival benefits of adjuvant chemotherapy persisted in patients with or without risk factors (Figures 4B, C, E, F).

Association between adjuvant chemotherapy and DFS

In group 1, adjuvant chemotherapy did not provide DFS benefit (aHR, 0.97; 95% CI, 0.74-1.27; p=0.835). Amongst patients with tumors 2 cm or less, adjuvant chemotherapy was not associated with improved DFS (aHR, 1.00; 95% CI, 0.63-1.59; p=0.984). Amongst patients with tumors larger than 2 cm, but <=3 cm, we again found no benefit of adjuvant chemotherapy on DFS (aHR, 0.95; 95% CI, 0.68-1.33; p=0.772).

With regard to group 2, adjuvant chemotherapy was not associated with improved DFS (aHR, 0.89; 95% CI, 0.66-1.21; p=0.44). Amongst patients with risk factor, adjuvant chemotherapy was not associated with improved DFS (aHR, 0.86; 95% CI, 0.54-1.42; p=0.542). Amongst patients without risk factor, we found no benefit of adjuvant chemotherapy on DFS (aHR, 0.92; 95% CI, 0.63-1.37; p=0.675).

TABLE 1A Patient characteristics in patients with (A) Group 1: Tumor <=3 cm with VPI, (B) Group 2: tumor >3 cm, but <=4 cm.

Table 1A. Group 1: tumor <=3 cm with VPI			
Patients, No. (%)			
Characteristic	Observation N=770	Adjuvant chemotherapy N=495	P value
Age, years			0.29
20-64	499 (64.8%)	335 (67.7%)	
65-74	271 (35.2%)	160 (32.3%)	
Sex			0.24
Male	321 (41.7%)	190 (38.4%)	
Female	449 (58.3%)	305 (61.6%)	
Histologic grade			0.02
Low	609 (79.1%)	363 (73.3%)	
High	161 (20.9%)	132 (26.7%)	
Surgery			<0.001
Sublobar resection	180 (23.4%)	69 (13.9%)	
Lobectomy	590 (76.6%)	426 (86.1%)	
N2 dissection, LN station			0.09
<3	182 (23.6%)	138 (27.9%)	
>=3	588 (76.4%)	357 (72.1%)	
Smoking habit			0.27
Ever	192 (24.9%)	110 (22.2%)	
Never	578 (75.1%)	385 (77.8%)	
ECOG			0.08
PS 0	607 (78.8%)	369 (74.5%)	
PS 1	163 (21.2%)	126 (25.5%)	
Tumor size			<0.001
<=2 cm	379 (49.2%)	182 (36.8%)	
>2-3.0 cm	391 (50.8%)	313 (63.2%)	
Hospital			<0.001
Medical left	643 (83.5%)	372 (75.2%)	
Regional hospital	127 (16.5%)	123 (24.8%)	
Tumor recurrence[#]			0.42
No	625 (82.3%)	395 (80.6%)	
Locoregional recurrence	41 (5.4%)	23 (4.7%)	
Distant recurrence	93 (12.3%)	72 (14.7%)	

PS, performance status.
[#]Patients with unknown tumor recurrence type were excluded.

Discussion

In this study, we found that the benefit of adjuvant chemotherapy was associated with tumor size amongst patients with T2aN0 stage IB lung adenocarcinoma. Adjuvant chemotherapy improved survival for those with tumors larger than 3 cm, but 4 cm or less in size. For patients with tumors 3 cm or less with VPI, adjuvant chemotherapy had no survival benefit.

TABLE 1B Group 2: tumor >3 cm, but <= 4cm

Patients, No. (%)			
Characteristic	Observation N=262	Adjuvant chemotherapy N=573	*P* value
Age, years			0.11
20-64	143 (54.6%)	346 (60.4%)	
65-74	119 (45.4%)	227 (39.6%)	
Sex			0.50
Male	106 (40.5%)	246 (42.9%)	
Female	156 (59.5%)	327 (57.1%)	
Histologic grade			0.24
Low	206 (78.6%)	429 (74.9%)	
High	56 (21.4%)	144 (25.1%)	
VPI			0.02
Absent	171 (65.3%)	323 (56.4%)	
Present	91 (34.7%)	250 (43.6%)	
Surgery			0.02
Sublobar resection	24 (9.2%)	28 (4.9%)	
Lobectomy	238 (90.8%)	545 (95.1%)	
N2 dissection, LN station			0.18
<3	59 (22.5%)	106 (18.5%)	
>=3	203 (77.5%)	467 (81.5%)	
Smoking habit			0.41
Ever	79 (30.2%)	157 (27.4%)	
Never	183 (69.8%)	416 (72.6%)	
ECOG			<0.001
PS 0	162 (61.8%)	440 (76.8%)	
PS 1	100 (38.2%)	133 (23.2%)	
Hospital			<0.001
Medical left	165 (63.0%)	472 (82.4%)	
Regional hospital	97 (37.0%)	101 (17.6%)	
Tumor recurrence[#]			0.52
No	187 (73.9%)	422 (74.0%)	
Locoregional recurrence	13 (5.1%)	40 (7.0%)	
Distant recurrence	53 (20.9%)	108 (18.9%)	

PS, performance status; VPI, visceral pleural invasion.

Tumor size is a topic of research in predicting the benefit for resectable lung cancer patients treated with adjuvant chemotherapy. For tumors larger than 4 cm, a survival advantage has been reported in association with adjuvant chemotherapy (11, 19, 25). Therefore, we focused our study on tumors 4 cm or less without nodal involvement and found the benefit of adjuvant chemotherapy was dependent on tumor size only.

For tumors 3 cm or less with VPI, we found no survival benefit with adjuvant chemotherapy. In a previous study using the Surveillance, Epidemiology and End Results (SEER) database, adjuvant chemotherapy does not improve survival in patients with tumors 4 cm or less with VPI. However, that study did not perform exploratory analysis focusing on tumors 3 cm or less (19). Pathak et al. conducted a cohort study using data from the

TABLE 2A Univariate and multivariable analysis for (A) overall survival (OS) and cancer-specific survival (CSS) of Group 1: tumor <=3 cm with VPI; and for (B) OS and CSS of Group 2: tumor >3-4 cm.

Table 2A. Group 1: tumor <=3 cm with VPI				
Variables	Univariate analysis		Multivariable analysis[$]	
	HR (95% CI)	*P* value	aHR* (95% CI)	*P* value
Overall survival:				
Age 65-74	1.54 (1.11-2.13)	0.008	1.50 (1.08-2.08)	0.016
Male	1.41 (1.03-1.94)	0.034		
High grade	1.36 (0.94-1.94)	0.097		
Sublobar resection	0.84 (0.52-1.29)	0.438		
N2 dissection, LN station <3	0.98 (0.68-1.39)	0.925		
Ever smoker	1.50 (1.05-2.11)	0.023		
ECOG PS 1	1.31 (0.91-1.85)	0.138		
Tumor size >2-3.0 cm	1.90 (1.35-2.72)	<0.001	1.83 (1.28-2.64)	0.001
Regional hospital	2.05 (1.45-2.87)	<0.001	2.13 (1.49-3.00)	<0.001
Adjuvant chemotherapy	1.10 (0.79-1.51)	0.570		
Cancer-specific survival:				
Age 65-74	1.22 (0.83-1.78)	0.290		
Male	1.24 (0.86-1.79)	0.243		
High grade	1.43 (0.94-2.13)	0.084		
Sublobar resection	0.67 (0.37-1.14)	0.166		
N2 dissection, LN station <3	0.87 (0.57-1.31)	0.526		
Ever smoker	1.28 (0.83-1.90)	0.242		
ECOG PS 1	1.12 (0.72-1.68)	0.598		
Tumor size >2-3.0 cm	2.05 (1.39-3.11)	<0.001	1.98 (1.33-3.04)	0.001
Regional hospital	2.14 (1.44-3.12)	<0.001	2.35 (1.57-3.48)	<0.001
Adjuvant chemotherapy	1.11 (0.77-1.60)	0.573		

HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval; PS, performance status. [$]In multivariable analysis, only factors reaching statistical significance were listed on the table. *Hazard ratio was adjusted by age, sex, histologic grade, extent of resection, smoking status, performance status, tumor size, and the types of health care institutions.

TABLE 2B Group 2: tumor >3 cm, but <=4 cm.

Variables	Univariate analysis		Multivariable analysis[$]	
	HR (95% CI)	*P* value	aHR* (95% CI)	*P* value
Overall survival:				
Age 65-74	1.45 (1.07-1.98)	0.018	1.46 (1.06-2.01)	0.019
Male	1.34 (0.98-1.83)	0.062		
High grade	1.67 (1.19-2.31)	0.003	1.62 (1.14-2.28)	0.006
VPI present	1.01 (0.74-1.38)	0.947		
Sublobar resection	1.91 (1.10-3.11)	0.014		
N2 dissection, LN station <3	1.11 (0.76-1.59)	0.573		
Ever smoker	1.68 (1.22-2.30)	0.001	1.55 (1.01-2.41)	0.049

(Continued)

TABLE 2B Continued

Variables	Univariate analysis		Multivariable analysis[$]	
	HR (95% CI)	*P* value	aHR* (95% CI)	*P* value
ECOG PS 1	1.33 (0.95-1.83)	0.088		
Regional hospital	1.23 (0.86-1.73)	0.242		
Adjuvant chemotherapy	0.49 (0.36-0.67)	<0.001	0.52 (0.38-0.72)	<0.001
>=1 Risk factors*	1.84 (1.34-2.52)	<0.001	1.73 (1.25-2.39)	<0.001
Cancer-specific survival:				
Age 65-74	1.38 (0.96-1.97)	0.079		
Male	1.32 (0.92-1.89)	0.131		
High grade	1.68 (1.13-2.45)	0.009	1.64 (1.09-2.44)	0.016
VPI present	0.93 (0.64-1.34)	0.715		
Sublobar resection	2.31 (1.26-3.89)	0.003	1.82 (0.98-3.15)	0.043
N2 dissection, LN station <3	1.26 (0.82-1.88)	0.279		
Ever smoker	1.70 (1.17-2.45)	0.004		
ECOG PS 1	1.33 (0.90-1.93)	0.142		
Regional hospital	1.24 (0.81-1.83)	0.303		
Adjuvant chemotherapy	0.50 (0.35-0.71)	<0.001	0.54 (0.37-0.78)	0.001
>=1 Risk factors*	1.95 (1.34-2.80)	<0.001	1.84 (1.26-2.67)	0.001

HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval; PS, performance status; VPI, visceral pleural invasion. * The risk factor refers to having either high-grade histologic findings or receiving sublobar resection. [$]In multivariable analysis, only factors reaching statistical significance were listed on the table. *Hazard ratio was adjusted by age, sex, histologic grade, visceral pleural invasion (VPI), extent of resection, smoking status, performance status, and the types of health care institutions.

National Cancer Database (NCDB) of the United States to assess the association between adjuvant chemotherapy and survival in patients with node-negative early-stage NSCLC. In subgroup analysis in 2,813 patients with tumors 3 cm or less with VPI, only 297 (10.6%) had received adjuvant chemotherapy. Adjuvant chemotherapy is not associated with a survival benefit in the population (HR, 0.90; 95% CI, 0.72-1.14; p=0.38) (25). Another study using NCDB evaluated the role of adjuvant chemotherapy in patients with tumors 4 cm or less with VPI. In subgroup analysis, 6,785 patients with tumors 3 cm or less with VPI and 608 (9.0%) of them received adjuvant chemotherapy. Adjuvant chemotherapy does not provide overall survival benefit (26). Our findings are consistent with prior studies that adjuvant chemotherapy is not associated with survival benefit for tumors 3 cm or less with VPI. Compared with prior research on patients with a low adjuvant chemotherapy rate, our present study had the highest proportion (39.1%) receiving adjuvant chemotherapy for patients with tumors 3 cm or less with VPI.

For patients with tumors larger than 3 cm, but 4 cm or less in size, we found that adjuvant chemotherapy had improved their OS and CSS even in the absence of risk factors. In a cohort study based on NCDB, patients with tumors larger than 3 to 7 cm were analyzed to evaluate the role of adjuvant chemotherapy. In subgroup analysis, there were 10,587 patients with tumors >3 cm, but <=4 cm and 1,608 (15.2%) of whom had received adjuvant chemotherapy. Adjuvant chemotherapy is associated with improved OS in the population with a hazard ratio of 0.75 (95% CI, 0.70-0.86; P <0.0001) (27). In aforementioned Pathak's study, 7,501 patients with tumors >3 cm, but <=4 cm were analyzed, and 896 (11.95%) of them had received adjuvant chemotherapy. In that population, adjuvant chemotherapy is not associated with an increase in OS (HR, 0.90; 95% CI, 0.78-1.03; p=0.21). On the other hand, adjuvant chemotherapy provides benefit only amongst patients who had received sublobar resection (HR, 0.72; 95% CI, 0.56-0.93; p=0.004) (25). In the present study, adjuvant chemotherapy was administered to 68.8% of patients with tumors >3 cm, but <=4 cm. Survival advantages in both OS and CSS were found in these patients regardless of the presence of risk factors. The difference in results across these studies may be related to differences in the studied population, chemotherapy regimen, and proportion of patients receiving adjuvant chemotherapy. Furthermore, performance status and smoking habits were not captured in the NCDB. The decision to offer adjuvant chemotherapy and survivals may be influenced by these factors. In contrast, these factors above were comprehensively recorded in the National Taiwan Cancer Registry database. Besides, CSS was unable to be evaluated in the NCDB, whereas the survival information was available in our database. With regard to the time to initiate adjuvant chemotherapy, we recommended starting adjuvant chemotherapy within 8 weeks following surgery according to prior randomized controlled trials (5, 7, 11).

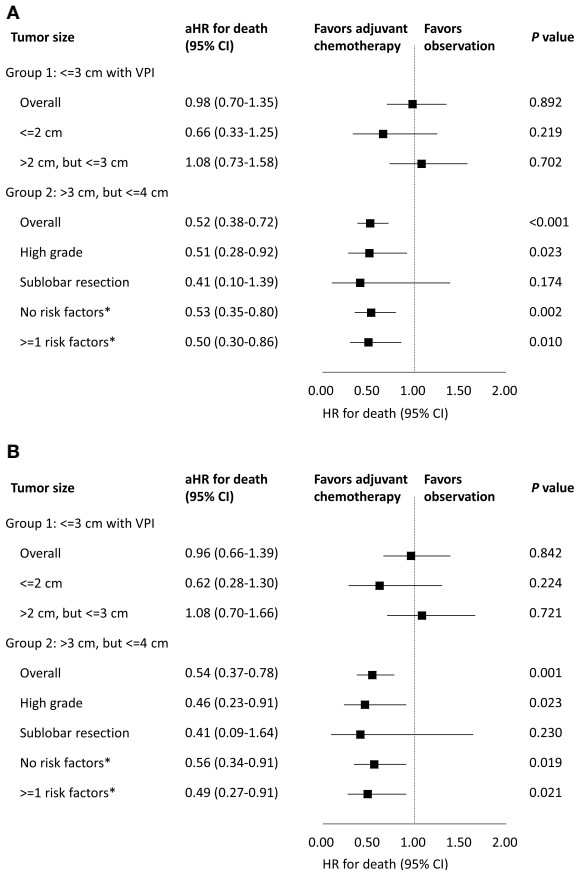

FIGURE 2
Association of **(A)** overall survival and **(B)** cancer-specific survival with adjuvant chemotherapy stratified by tumor size and risk factors. aHR, adjusted hazard ratio; CI, confidence interval; VPI, visceral pleura invasion. *The risk factor in group 2 refers to having either high-grade histologic findings or receiving sublobar resection.

The demographic characteristics of early lung cancer in Taiwan differ from that in non-Asian countries (28, 29). In our cohort, there were more female and non-smoking patients. Besides, the prevalence of *EGFR* mutation in lung cancer patients in Taiwan is higher than that in the western population (30). For patients with *EGFR*-mutant lung cancer experienced better response to EGFR-TKI or chemotherapy as compared to those with *EGFR* wild-type one if the patients suffered from disease recurrence into advanced stage (31–34), this may partly explain the discrepancy in results between our research and prior studies. Worldwide, the 5-year survival in pathologic stage IB is 73% (35), however they were 91.1% and 77.9% even in group 1 and group 2 stage IB without adjuvant chemotherapy in our study. Furthermore, according to the results from ADAURA trial, osimertinib is now standard-of-care therapy for stage IB *EGFR*-mutant lung cancer (36). This will make major improvement in survivals for stage IB *EGFR*-mutant lung cancer patients in the near future. As lung cancer is a highly heterogeneous disease, the treatment should be personalized and genetic testing could be encouraged for patients with stage IB lung adenocarcinoma. Further studies to clarify the role of driver gene mutations, immune status, and other novel treatments in adjuvant therapy following surgical resection for stage IB lung adenocarcinoma may be warranted.

There are some limitations of our study. First, it was a retrospective study. Second, the status of lymphovascular invasion, proposed as a high-risk histopathologic feature, was not recorded in the National Taiwan Cancer Registry. Third, the detailed information on adjuvant chemotherapy regimens was not collected in our cancer registry database. The regimen type, dose, and duration may influence treatment outcomes. Fourth, the treatment strategies could differ across health care institutions. Amongst patients treated in regional hospitals, the chemotherapy rates were similar between those with tumors >3 cm, but <=4 cm and those with tumors 3 cm or less (51.0% vs. 49.2%). On the other hand, in medical centers, patients with tumors >3 cm, but <=4 cm more likely to have received adjuvant chemotherapy as compared with those having tumors 3 cm or less (74.1% vs 36.7%). The inconsistency of treatment strategies across health care institutions may have introduced selection bias for adjuvant chemotherapy.

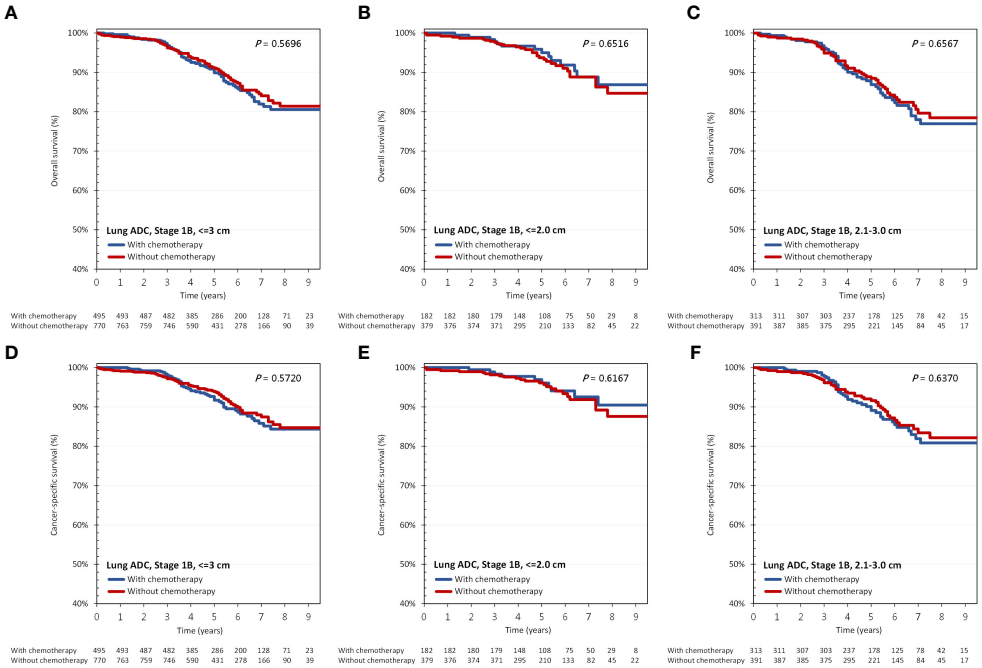

FIGURE 3
(A–C) overall survival and **(D–F)** cancer-specific survival according to the administration of adjuvant chemotherapy and tumor size in group 1: tumor <=3 cm with VPI. ADC, adenocarcinoma.

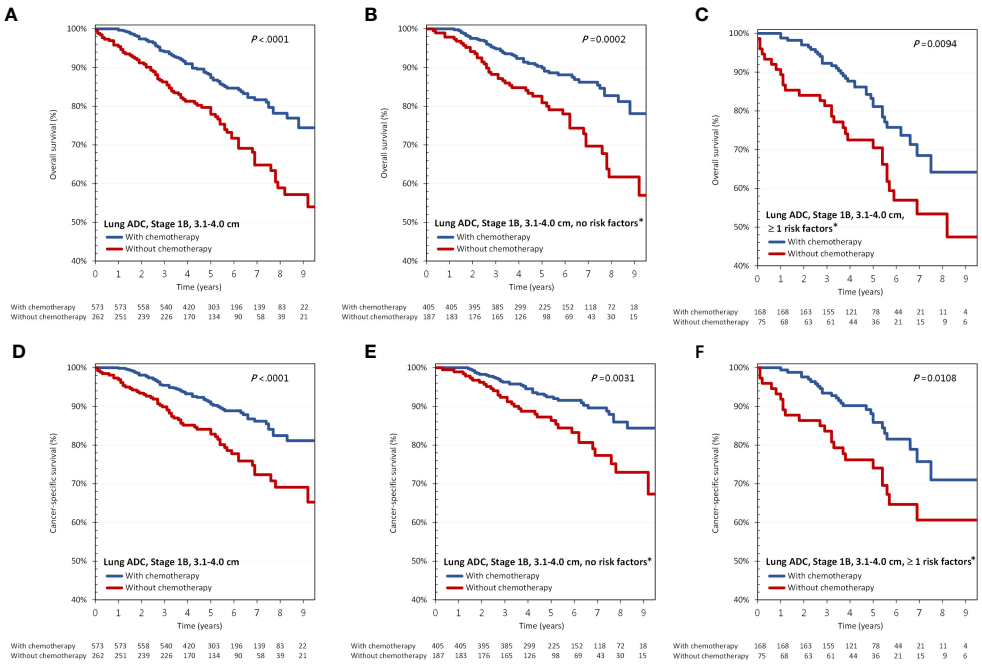

FIGURE 4
(A–C) overall survival and **(D–F)** cancer-specific survival according to the administration of adjuvant chemotherapy and presence of risk factors in group 2: tumor >3 cm, but <=4 cm. ADC, adenocarcinoma. *The risk factor in group 2 refers to having either high-grade histologic findings or receiving sublobar resection.

In conclusion, for patients with T2aN0 stage IB lung adenocarcinoma, the benefit of adjuvant chemotherapy depended on tumor size. Adjuvant chemotherapy within 8 weeks following surgery improved survival in those with tumors larger than 3 cm, but 4 cm or less in size. For patients with tumors 3 cm or less with VPI, adjuvant chemotherapy had no survival benefit.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Research Ethics Committee of the National Taiwan University (NTU-REC No.202101HM030), with waiver of informed consent owing to the lack of personal information and use of secondary data in the study. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for observational studies was used in the revision of this article. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Study concepts: P-HL, G-CC; Study design: C-JC, G-CC, Y-HH, J-ST; Data acquisition: C-JC, Y-HH, K-CC, K-HH; Quality control of data and algorithms: P-HL; Data analysis and interpretation: C-JC, P-HL, Z-RZ, C-HC; Statistical analysis: C-JC, W-CL; Manuscript preparation: P-HL; Manuscript editing: J-ST, T-WL; Manuscript review: G-CC, T-YY, J-YH. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1096683/full#supplementary-material

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Survival outcomes and prognostic factors of lung cancer patients with the *MET* exon 14 skipping mutation: A single-center real-world study

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Introduction: The *MET* exon 14 skipping *(MET*ex14) mutation is an important oncogenic driver in lung cancer. We performed a retrospective analysis of clinical data from lung cancer patients with the *MET*ex14 mutation to analyze their survival outcomes and associated prognostic factors.

Methods: A one-step reverse transcription-polymerase chain reaction to examine the presence of the *MET*ex14 mutation was performed using RNA samples from 1374 lung cancer patients with no detected *EGFR* and *ALK* mutations. Pathological features and immunohistochemistry (IHC) results for c-MET were analyzed in patients with *MET*ex14-positive tumors.

Results: *MET*ex14 was identified in 69 patients with lung cancer, including 53 adenocarcinoma (ADC) and 16 non-ADC patients. In comparison with patients without the *MET*ex14 mutation, lung cancer patients harboring the *MET*ex14 mutation were generally elderly individuals, never-smokers, and had poor performance scores. A higher frequency of *MET*ex14 mutations was detected in pulmonary sarcomatoid carcinoma (PSC) patients (24.3%, n = 9/37). However, stage IV PSC patients with or without the *MET*ex14 mutations showed similarly poor overall survival (OS) (p = 0.429). For all 36 *MET*ex14-positive lung ADCs, multivariate analysis showed several poor prognostic factors, including strong c-MET IHC staining (p = 0.006), initial brain metastasis (p = 0.005), and administration of only supportive care (p < 0.001). After excluding seven patients who received only supportive care, we further analyzed 29 stage IV lung ADC patients with *MET*ex14 mutations who received anti-cancer treatment. Multivariate analysis showed that pemetrexed treatment (p = 0.003), lung radiotherapy (p = 0.020), initial brain metastasis (p = 0.005), and strong c-MET IHC staining (p = 0.012) were independent prognostic factors for OS in these patients.

Conclusions: A higher frequency of *MET*ex14 mutations was detected in PSC patients. Stage IV PSC patients with or without the *METex14* mutations had similarly poor overall survival. Pemetrexed-based chemotherapy, strong c-MET ICH staining, initial brain metastasis, and lung radiotherapy, may help predict survival outcomes in patients with advanced lung ADCs harboring the *MET*ex14 mutation.

KEYWORDS

adenocarcinoma, immunohistochemistry, *MET* exon 14 skipping, pulmonary sarcomatoid carcinoma, overall survival

1 Introduction

Acquired gene alterations in lung tumors serve as driver mutations that initiate tumorigenic and invasive abilities. Some of these mutations can be targeted by specific small-molecule inhibitors or monoclonal antibodies (1). The c-mesenchymal-epithelial transition protooncogene (*MET*) is an important gene that encodes the MET protein, which functions as a transmembrane receptor tyrosine kinase and may trigger tumor growth under aberrant activation (2). *MET* exon 14 skipping (*MET*ex14) is one of the most common gene alterations of *MET*, and it acts as an important oncogenic driver in lung cancer (3). The *MET*ex14 mutation results in the loss of the juxtamembrane domain of the MET protein, which regulates and prevents MET over-signaling (4). Consequently, the E3 ubiquitin ligase c-cbl fails to bind to the MET protein, reducing receptor degradation and causing overactivation of MET-mediated signaling, thereby driving oncogenesis (5).

Among patients with lung cancer, the *MET*ex14 mutation occurs in 2%-4% of those with adenocarcinomas (ADC), 1%-2% of those with squamous cell carcinoma, and 7% to 31% of the patients with pulmonary sarcomatoid carcinoma (PSC) (6–8). Several small molecules targeting and inhibiting MET tyrosine kinase have been evaluated for their efficacy in the treatment of *MET*ex14-positive non-small cell lung cancer (NSCLC). Clinical studies have demonstrated that crizotinib, a multikinase inhibitor of receptor tyrosine kinases (RTKs), reduces the tumor size in advanced NSCLC patients carrying the *MET*ex14 mutation (9). However, the phase II METROS study reported limited benefits in terms of objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) (10). Capmatinib, an oral adenosine triphosphate (ATP)-competitive MET inhibitor, demonstrated anti-cancer efficacy with an ORR of 68% and a median PFS of 9.69 months in treatment-naïve patients in the phase II GEOMETRY mono-1 trial (11). Another ATP-competitive MET inhibitor, tepotinib, showed a favorable overall response rate and rapid as well as durable response in the phase II VISION study (12). Thus, both capmatinib and tepotinib are recommended as first-line treatments of choice for advanced NSCLC with *MET*ex14-positive tumors (13). Other MET-specific tyrosine kinase inhibitors (TKIs), multikinase inhibitors, and anti-MET antibodies are currently in ongoing clinical trials for the treatment of this patient population (14).

NSCLC patients carrying *MET*ex14 mutations receive conventional treatments without specific anti-MET therapy and have a poor prognosis and short OS (8, 15). Their OS is comparable to that of patients with undetected major driver mutations (16). Although *MET*ex14-positive NSCLC patients treated with selective MET TKIs reported longer OS, up to 30%-40% of these patients were reported to be non-responders (11, 17, 18). The factors associated with a poor prognosis in these patients remain unclear. Our previous study demonstrated that stage IV patients with *MET*ex14 mutations had diverse survival outcomes; some patients showed very poor survival, while others had a relatively long survival period (16). Therefore, identification of the potential factors that predict OS in these patients is important. In the present study, we performed a retrospective evaluation of clinical data from lung cancer patients with the *MET*ex14 mutation to analyze their survival outcomes and associated prognostic factors.

2 Patients and methods

2.1 Ethics statement

This study was approved by the institutional review board of National Taiwan University Hospital (NTUH), Taipei, Taiwan. Written informed consent was obtained from all patients before tumor specimen collection for clinical data acquisition and molecular analyses.

2.2 Patients

We retrospectively included patients diagnosed with lung cancer at the National Taiwan University Hospital between January 2006 and August 2020. Tumor specimens were consecutively and prospectively collected from either the primary lung tumors or distant metastatic sites by surgery, core needle biopsy, bronchial washing, endobronchial biopsy, and cell blocks of malignant pleural effusion. Only patients with lung cancer with no detected *EGFR* and *ALK* mutations were included

in this study. Tumors were confirmed by mutational analysis to exclude co-major driver mutations.

2.3 Mutational studies

EGFR mutation tests were performed using a one-step reverse transcription-polymerase chain reaction (RT-PCR) with RNA samples. *ALK* mutations were detected by either RT-PCR or immunohistochemistry (IHC) staining using the Ventana ALK (D5F3) antibody. Patients with lung cancer with no detected *EGFR* and *ALK* mutations were examined for the *MET*ex14 mutation. The presence of other major driver mutations, including *KRAS*, *HER2*, *BRAF* V600E, *ROS-1*, and *RET*, was also analyzed. Tumor specimen preparation, RNA extraction, primer selection, RT-PCR conditions, and sequencing methods for all driver mutations were performed using methods described previously (16, 19). Some of the patients with *ROS1* fusion and *RET* fusion underwent fluorescence *in situ* hybridization with a previously described standard protocol (19).

2.4 Acquisition of clinical and pathologic data

Demographic characteristics and clinical features of all enrolled patients were obtained from medical records. Patients who smoked less than 100 cigarettes in their lifetime were defined as nonsmokers. The Eastern Cooperative Oncology Group (ECOG) performance score (PS) was used to rank performance status (20). Distant metastases were evaluated and the number of different metastatic sites was recorded. Treatment modalities, including therapeutic surgery, chemotherapy, immunotherapy, MET TKI treatment, and local radiotherapy (RT) at the primary or metastatic sites were recorded. The endpoint of clinical analyses was OS, defined as the time from the initial diagnosis of lung cancer to death or the date of censoring at the last follow-up or loss of contact on April 30, 2022.

2.5 c-MET immunohistochemistry staining

MET protein expression was evaluated by performing c-MET IHC staining on formalin-fixed paraffin-embedded (FFPE) tissue sections of *MET*ex14-positive tumors. As described previously, 4-µm-thick FFPE sections were dewaxed, rehydrated, and reacted with a 1:50 dilution of anti-human c-MET antibody clone SP44 (Abcam, Cambridge, UK) (16). Staining was performed using an automated stainer (Ventana Benchmark; Roche Ventana, Tucson, AZ, USA) in accordance with the manufacturer's instructions. The intensity of MET expression was scored and classified as strong (score 3+), moderate (score 2+), weak (score 1+), or absent (score 0), as described previously (16). Staining distribution patterns were recorded as diffuse, focal, or negative. Other IHC stains, including pancytokeratin (CK), thyroid transcription factor-1 (TTF-1), and vimentin, were assessed as described previously (16). A portion of the IHC data was retrieved from the medical records.

2.6 Statistical analysis

Categorical variables were compared using the chi-squared test or Fisher's exact test when the expected number was less than 5. Continuous variables were expressed as median values with upper and lower values. OS and univariate analyses were estimated using the Kaplan–Meier method and the log-rank test to measure all differences in survival curves. We used a Cox proportional hazard regression model for multivariate analysis of OS with the backward-stepwise method. All tests were two-sided, and differences were considered significant when $p < 0.05$. Analyses were performed using the IBM SPSS software for Windows (version 26.0, IBM Corp., Armonk, NY, USA).

3 Results

3.1 Clinical features of lung cancer patients with and without the *MET*ex14 mutation

This cohort study enrolled 1374 lung cancer patients with no detected *EGFR* and *ALK* mutations (Figure 1). Among these patients, 170 had other driver mutations were excluded, including 71 with *KRAS* mutations, 39 with *HER2* mutations, 29 with *ROS-1* fusions, 19 with *RET* fusions, and 12 with *BRAF* V600E mutations (Figure 1). The *MET*ex14 mutation was identified in 69 patients, including 53 patients with ADC and 16 patients with non-ADC. Some patients with the *MET*ex14 mutation have been described in our previous report (16). In total, 1135 patients who did not show any driver mutations (*MET*ex14, *EGFR*, *ALK*, *KRAS*, *HER2*, *BRAF* V600E, *ROS-1*, or *RET*) were categorized into the non-*MET*ex14 group. Among the 69 patients with *MET*ex14-positive lung cancer, the median age was 74.2 years at initial diagnosis, 44 patients were male (64%), and more than half were never-smokers (41/69; 59%). The majority of the patients (48/69, 69%) had good ECOG PS scores (0-1), while 15 patients (22%) had a PS score of 2 and 6 patients had poor PS scores (3 and 4). A vast majority of the patients had stage

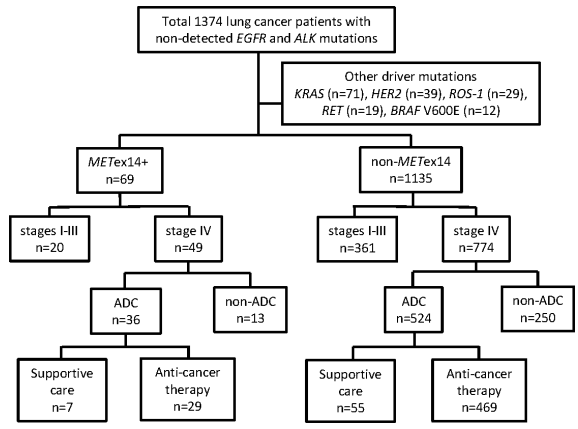

FIGURE 1
Overview of patient selection and patient groups. ADC, adenocarcinoma.

IV disease (49/69, 71%). In comparison with the non-*MET*ex14 group, lung cancer patients harboring the *MET*ex14 mutation were generally elderly individuals (≥70 years old, $p = 0.009$), never-smokers ($p = 0.020$), had poor ECOG PS ($p = 0.026$), and showed different subtypes of non-ADC ($p < 0.001$; Table 1). The highest frequency of *MET*ex14 mutations was observed in PSCs (9/37, 24.3%), followed by ADCs (53/803, 6.6%), pleomorphic carcinomas (1/19, 5%), squamous cell carcinoma (4/107, 3.7%), NSCLC-not otherwise specified (NOS) (1/80, 1.3%), and small cell lung cancer (1/159, 0.6%; Supplementary Table 1).

3.2 Univariate and multivariate analyses of prognostic factors for overall survival in all stage IV Adenocarcinoma patients harboring the *MET*ex14 mutation

We further focused on patients with lung cancer who were initially diagnosed with stage IV ADC, which included 36 patients harboring the *MET*ex14 mutation and 524 patients without major driver mutations (i.e., the non-*MET*ex14 group consisting of patients without detected *MET*ex14, *EGFR*, *ALK*, *KRAS*, *HER2*,

TABLE 1 Clinical characteristics of lung cancer patients harboring tumors with ($n = 69$) and without ($n = 1135$) the *MET*ex14 mutation.

Clinical characteristic	*MET*ex14+	No *MET*ex14	*p*-value[#]
Patients, n	69	1135	
Age, years			
Median (range)	74.2 (36-95)	67.2 (18-99)	0.013[§]
≥65, *n* (%)	54 (78)	679 (60)	0.002
≥70, n (%)	43 (62)	525 (46)	0.009
Sex, *n* (%)			0.938
M	44 (64)	729 (64)	
F	25 (36)	406 (36)	
Smoking status, *n* (%)			0.020
Current/Ever	28 (41)	624 (55)	
Never	41 (59)	511 (45)	
ECOG PS, *n* (%)			0.026
0–1	48 (69)	929 (83)	
2	15 (22)	130 (11)	
3–4	6 (9)	76 (6)	
Stage, *n* (%)			0.225
I	11 (16)	119 (10)	
II	3 (4)	53 (5)	
III	6 (9)	189 (17)	
IV	49 (71)	774 (68)	
Histology			0.066
Adenocarcinoma	53 (77)	750 (66)	
Non-adenocarcinoma	16 (23)	385 (34)	
Subtype of non-adenocarcinoma			< 0.001
Squamous cell carcinoma	4 (25)	103 (27)	
Sarcomatoid	9 (57)	28 (7)	
Small cell	1 (6)	158 (41)	
Pleomorphic carcinoma	1 (6)	18 (5)	
NSCLC-NOS	1 (6)	79 (20)	

ECOG PS, Eastern Cooperative Oncology Group performance status; F, female; M, male; n, number; NOS, not otherwise specified; NSCLC, non-small cell lung cancer.
#p-values were calculated using the chi-squared test or Fisher's exact test when the expected number was less than 5.
§Using Kruskal–Wallis test.

BRAF V600E, *ROS-1*, and *RET mutations*) (Figure 1). We examined the prognostic role of various factors for OS, including age, sex, smoking status, pathologic features of c-MET IHC, ECOG PS, presence and number of distant metastatic sites, and provision of anti-cancer therapy or only supportive care. Univariate analyses of OS was performed using Kaplan–Meier survival analysis in 36 stage IV ADC patients, of which c-MET IHC analysis data were available for 33 patients (Table 2).

Demographic factors, such as age (<70 vs. ≥70 years), sex, and smoking status, did not show statistically significant differences in relation to median OS (mOS). Pancytokeratin (CK) staining was not associated with differences in survival rates. Among the 33 tumors available for c-MET IHC, all *MET*ex14-positive tumors showed c-MET-positive expression and were categorized on the basis of staining scores (1+ ~ 2+ vs. 3+, Figure 2A). All c-MET patterns were either focal or diffuse (Figure 2C). We observed that patients with tumor samples showing strong (score 3+) c-MET IHC staining had a shorter mOS than those with weak or moderate (score 1+ ~ 2+) c-MET IHC staining (5.7 vs. 24.8 months, $p = 0.013$; Figure 2B). Similar findings were observed for the c-MET IHC distribution patterns; patients with tumor samples showing a diffuse pattern had shorter mOS than those with samples showing a focal pattern (3.8 vs. 27.6 months, $p = 0.036$; Figure 2D). Next, we evaluated the characteristics of the metastatic status for OS analysis. Patients with multiple initial metastatic sites (≥2) showed poorer survival outcomes than those with only one metastatic site (2.8 vs. 18.4 months, $p = 0.037$). A shorter OS was also observed in patients with metastatic brain tumors at the initial presentation (2.8 vs. 18.4 months, $p = 0.036$). The presence of malignant pleural effusion was not associated with survival outcomes. Among stage IV ADC patients with the *MET*ex14 mutation, seven patients received only supportive care without anti-cancer therapy and had a shorter mOS (1.4 months, 95% confidence interval [CI], 0.7-1.3) than those who received anti-cancer treatment (n = 29; mOS, 20.1 months; $p < 0.001$). Finally, multivariate analysis for OS revealed that a strong c-MET IHC staining score of 3+ (hazard ratio [HR]: 2.05, 95% CI: 1.23–3.43; $p = 0.006$), initial brain metastasis (HR: 3.86, 95% CI: 1.52–9.82; $p = 0.005$), and treatment with supportive care without anti-cancer therapy (HR: 11.78, 95% CI: 3.40–40.86; $p < 0.001$) were associated with poor survival outcomes (Table 2).

3.3 Univariate and multivariate analyses of prognostic factors for overall survival in stage IV adenocarcinoma patients harboring the *MET*ex14 mutation who received anti-cancer therapy

We next aimed to determine whether patient characteristics and differences in treatment modalities would affect the OS in the 29 lung ADC patients with the *MET*ex14 mutation who received at least one anti-cancer therapy (Table 3). All the patients had an ECOG PS score of 0-2. In univariate analysis, strong c-MET ICH staining (score 3+) was consistently associated with shorter mOS than weak-to-moderate staining (score 1+ to 2+; mOS, 7.3 and 27.1 months, respectively; $p = 0.015$). Although the c-MET IHC distribution pattern ($p = 0.068$) and initial brain metastasis ($p = 0.061$) showed a trend, the findings did not reach statistical significance. Other characteristics, such as the number of metastatic sites and malignant pleural effusion, were not associated with survival outcomes. Nevertheless, longer survival periods were observed in some subgroups. Consistently better mOS was observed in patients who received lung radiation therapy than patients who did not receive this treatment (27.6 vs. 12.1 months, $p = 0.002$). Patients who received immunotherapy showed a favorable mOS (n = 5; mOS, 44.9 months) than those who did not (n = 24; mOS, 13.6 months; $p = 0.032$). Similarly, patients treated with pemetrexed (n = 19; mOS, 20 months) showed a more favorable mOS than those who were not (n = 10; mOS, 5.7 months; $p = 0.011$). Finally, patients treated with MET TKIs (n = 6; mOS, 19.2 months) showed a trend of prolonged OS in comparison with those who did not receive MET TKI (n = 23; mOS, 13.6 months; $p = 0.065$). Other therapeutic modalities, including lung surgery, brain RT, bone or spine RT, and chemotherapy with cisplatin doublet, gemcitabine, or taxanes, did not significantly predict OS.

Multivariate analyses for 29 stage IV ADC patients carrying the *MET*ex14 mutation were performed, and the variables with *p*-values less than 0.1 in the univariate analysis were included (Table 3). After adjusting for clinicopathological factors, a significantly longer OS was observed in patients who received pemetrexed (HR: 0.20; 95% CI: 0.07–0.56; $p = 0.003$) and those who were treated with lung radiotherapy (HR, 0.26; 95% CI: 0.09–0.81; $p = 0.020$). Similar to the findings for all stage IV ADC patients harboring the *MET*ex14 mutation, anti-cancer therapy with initial brain metastasis (HR: 5.24, 95% CI: 1.65–16.60; $p = 0.005$) and strong c-MET IHC staining (HR: 2.06, 95% CI: 1.17–3.62; $p = 0.012$) consistently predicted poor survival outcomes in these 29 patients.

3.4 Survival outcomes of stage IV *MET*ex14-positive lung cancer patients in comparison with those without the *MET*ex14 mutation

For the stage IV PSC cases in our cohort, the estimated mOS was 4.8 months in the seven *MET*ex14-positive patients and 3.8 months in the 23 patients without *MET*ex14, indicating a similar mOS and poor survival in both groups ($p = 0.429$; Figure 3A). Among the *MET*ex14-positive patients, five of the seven PSC patients were poor chemotherapy responders. Most of these patients received less than four courses of first and/or second-line cisplatin doublet-based chemotherapy with rapid progression. One patient with an ECOG PS of 4 died within 2 weeks of diagnosis who received best supportive care. Another patient received a course of pembrolizumab and was lost to follow-up. After excluding patients who received only supportive care, similar mOS was observed between *MET*ex14-positive patients (n=6; mOS, 4.8 months) and non-*MET*ex14 patients (n=18; mOS, 5.4 months, $p=0.388$; Supplementary Figure 1A).

We next evaluated and compared the survival outcomes of patients with stage IV lung ADC with and without the *MET*ex14 mutation. The mOS was 7.3 months in *MET*ex14-positive patients

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TABLE 2 Univariate and multivariate analyses of prognostic factors for overall survival in all patients with *MET*ex14-positive stage IV adenocarcinoma (n = 36).

Factor	Patient, n	Univariate analysis[#]		Multivariate analysis[$]	
		Median OS (months)	*p*-value	HR (95% CI)	*p*-value
Age, years					
<70	13	7.8	0.559		
≥70	23	2.5			
Sex					
M	21	3.8	0.298		
F	15	13.6			
Smoking status					
Non-smoker	23	13.6	0.244		
Smoker/ex-smoker	13	3.8			
ECOG PS					
0-2	33	13.6	< 0.001	1	0.243
3-4	3	1.0		4.30 (0.37-49.51)	
CK					
Positive	7	18.7	0.678		
Non-positive	29	6.6			
MET IHC score (n = 33)					
1-2	10	24.8	0.013	1	0.006
3	23	5.7		2.05 (1.23-3.43)	
MET IHC distribution (n = 33)					
Focal	5	27.6	0.036	1	0.312
Diffuse	28	3.8		1.75 (0.59-5.15)	
Metastatic sites (numbers)					
1	19	18.4	0.037	1	0.378
≥2	17	2.8		1.43 (0.64-3.19)	
Initial brain metastasis					
No	25	18.4	0.036	1	0.005
Yes	11	2.8		3.86 (1.52-9.82)	
Malignant pleural effusion					
No	18	5.7	0.637		
Yes	18	12.1			
Supportive care only*					
No	29	20.1	<0.001	1	<0.001
Yes	7	1.4		11.78 (3.40-40.86)	

CI, confidence interval; CK, pancytokeratin; ECOG PS, Eastern Cooperative Oncology Group performance status; F, female; IHC, immunohistochemistry; M, male; n, number; OS, overall survival.

*Patients received only supportive care without anti-cancer therapy.

#Univariate analysis was performed using the Kaplan–Meier method and log-rank test.

[$]Multivariate analysis was performed using the backward-stepwise method for the Cox regression model.

(n = 36; 95% CI: 0-18.9) and 12.9 months in the patients without the *MET*ex14 mutation (n = 524; 95% CI: 10.8-15.0; Figure 3B). Although the OS was shorter in patients with the *MET*ex14 mutation, this trend did not show statistical significance (p = 0.061). After excluding patients who received only supportive care, a comparable survival outcome was observed between *MET*ex14-positive patients (n=29; mOS, 18.4 months; 95% CI: 9.4-27.4) and non-*MET*ex14 patients (n=469; mOS, 15.9 months; 95% CI: 12.3-19.5 p=0.236; Supplementary Figure 1B).

We further evaluated 29 patients who had been treated with pemetrexed and/or MET TKI. The detailed duration of whole treatment regimens for patients with *MET*ex14 were shown in Supplementary Figure 2. Pairwise comparisons of the 524 patients without the *MET*ex14 mutation (non-*MET*ex14), 19 *MET*ex14-positive patients receiving pemetrexed (*MET*ex14+, PEM+), and 10 *MET*ex14-positive patients treated with chemotherapeutic agents other than pemetrexed (*MET*ex14+, PEM-) were performed (Figure 3C). As mentioned in the univariate analysis, *MET*ex14+, PEM+ patients showed better mOS than *MET*ex14+, PEM- patients (p = 0.011). No significant difference in mOS was observed between *MET*ex14+, PEM+ patients (20.0 months) and non-*MET*ex14 patients (p = 0.615). However, *MET*ex14+, PEM- ADC patients had a worse mOS (5.7 months) than the non-*MET*ex14 patients (p = 0.019; Figure 3C). Six patients received one or two lines of MET TKIs (Supplementary Table 2). Four patients treated with sequential pemetrexed with MET TKIs (at different time periods) had an mOS of NR (not reached), which was longer than that of the 15 patients who received pemetrexed

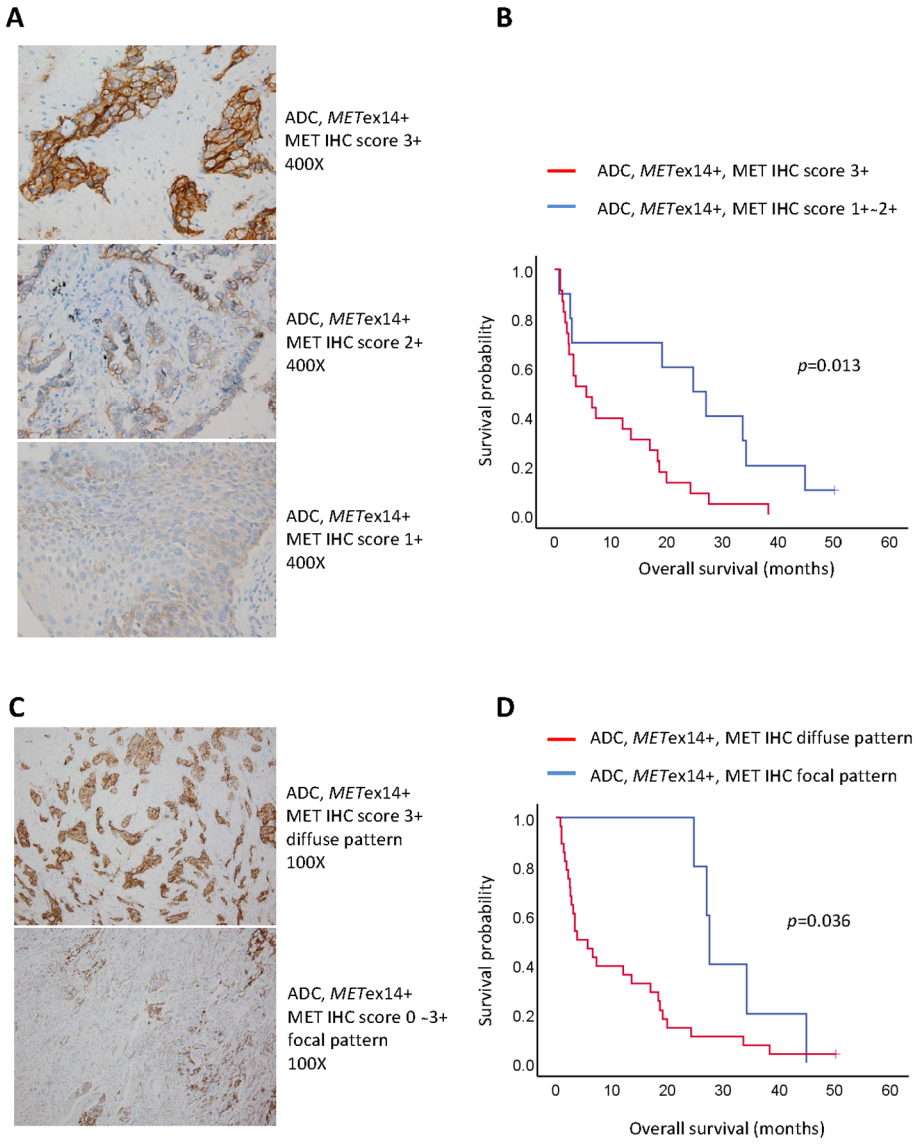

FIGURE 2
Pathological factors associated with poor prognosis in stage IV lung adenocarcinoma harboring *MET*ex14 (*MET*ex14+). **(A)** Representative figures of c-MET immunohistochemistry staining score 3+ (upper panel; original magnification: 400x), score 2+ (middle panel; original magnification: 400x), and score 1+ (lower panel; original magnification: 400x); **(B)** Kaplan-Meier curves of overall survival for score 1+ ~ 2+ and score 3+; **(C)** Representative figures of c-MET immunohistochemistry distribution patterns —— diffuse pattern (upper panel; original magnification: 100x) and focal pattern (lower panel; original magnification: 100x); **(D)** Kaplan-Meier curves of overall survival for diffuse and focal patterns.

TABLE 3 Univariate and multivariate analyses of prognostic factors for overall survival in patients with stage IV *MET*ex14-positive adenocarcinomas who received anti-cancer treatments (n = 29).

Factor	Patients n	Univariate analysis[#]		Multivariate analysis[$]	
		Median OS (month)	*p*-value	HR (95% CI)	*p*-value
Age, years					
<70	12	18.4	0.983		
≥70	17	17.0			
Sex					
M	16	17.0	0.408		
F	13	20.0			
Smoking status					
Non-smoker	20	17.0	0.460		
Smoker/ex-smoker	9	18.4			
MET IHC score (n = 27)					
1-2	9	27.1	0.015	1	0.012
3	18	7.3		2.06 (1.17-3.62)	
MET IHC distribution (n = 27)					
Focal	5	27.6	0.068	1	0.179
Diffuse	22	7.3		2.24 (0.69-7.27)	
Metastatic sites (numbers)					
1	17	18.7	0.130		
≥2	12	6.6			
Initial brain metastasis					
No	21	19.2	0.061	1	0.005
Yes	8	3.1		5.24 (1.65-16.60)	
Malignant pleural effusion					
No	14	18.4	0.508		
Yes	15	17.0			
Lung surgical treatment*					
No	27	17.0	0.176		
Yes	2	28.4			
Lung radiotherapy					
No	19	12.1	0.002	1	0.020
Yes	10	27.6		0.26 (0.09-0.81)	
Brain radiotherapy					
No	22	18.4	0.484		
Yes	7	5.7			
Bone or spine radiotherapy					
No	21	18.4	0.605		
Yes	8	13.6			
MET inhibitor					

(Continued)

TABLE 3 Continued

Factor	Patients n	Univariate analysis[#]		Multivariate analysis[$]	
		Median OS (month)	*p*-value	HR (95% CI)	*p*-value
No	23	13.6	0.065	1	0.723
Yes	6	19.2		0.74 (0.14-4.00)	
Immunotherapy					
No	24	13.6	0.032	1	0.699
Yes	5	44.9		0.76 (0.18-3.12)	
Cisplatin doublet					
No	9	6.6	0.443		
Yes	20	18.7			
Pemetrexed					
No	10	5.7	0.011	1	0.003
Yes	19	20.0		0.20 (0.07-0.56)	
Gemcitabine					
No	19	7.3	0.309		
Yes	10	24.3			
Taxanes					
No	16	6.6	0.284		
Yes	13	24.3			

CI, confidence interval; F, female; IHC immunohistochemistry; M, male; n, number; OS, overall survival; RT, radiotherapy.
* One patient received wedge resection for surgical biopsy. Another patient initially received right upper lobectomy but concurrent malignant pleural effusion was found during operation.
# Univariate analysis was performed using the Kaplan–Meier method and log-rank test.
[$]A multivariate analysis was performed using the backward-stepwise method for the Cox regression model.

without MET TKIs (mOS, 18.4 months; $p = 0.040$; Figure 3D), and was much better than that of the 10 patients who did not receive pemetrexed (including two patients who received MET TKIs but no pemetrexed), whose mOS was 5.7 months ($p = 0.011$; Figure 3D).

4 Discussion

Several clinical studies and trials have reported that NSCLC patients harboring *MET*ex14-positive tumors benefit from MET TKIs (9, 11, 13, 17, 21). However, not all patients showed clinical efficacy, and the response duration was limited. In the real world, some patients do not receive a specific MEK-TKI. In this study, we performed a multi-faceted evaluation of several prognostic factors associated with survival outcomes in a cohort of patients with lung cancer. We first successfully performed RNA-based PCR analysis and identified higher frequencies of *MET*ex14 in PSC, followed by ADC, and smaller frequencies in other lung cancer subtypes. For patients with stage IV lung ADC, we comprehensively analyzed the potential variables influencing survival outcomes. We showed that initial brain metastases and strong MET IHC staining may help predict OS. These results provide important information and shed light on the survival characteristics of lung cancer patients with *MET*ex14-positive tumors.

Previous studies reported that the overall incidence of the *MET*ex14 mutation was approximately 20%-30% in PSC and 3%-4% in ADC (6, 22). Our study reported a similar frequency of the *MET*ext14 mutation in PSC. Although pleomorphic carcinoma is categorized as a subtype of PSC in the 2015 World Health Organization (WHO) classification of lung tumors (23), we classified it as an independent subtype of lung cancer because the frequency of the *MET*ex14 mutation in pleomorphic carcinoma (5%) was quite different from that in PSC (24%). Moreover, the *MET*ex14 mutation was detected in 6.6% of lung ADC patients without *EGFR* and *ALK* mutations. Other characteristics of *MET*ex14-positive lung cancer, such as a predominance in female patients and an association with smoking, have been reported in previous studies (22, 24) but were not shown in our cohort and other studies (25, 26). The advanced age of patients with the *MET*ex14 mutation has been reported in the current and previous studies (21, 22, 25). Finally, these patients were more fragile and generally had a poorer ECOG PS than those without the *MET*ex14 mutation. As reported in previous studies, these demographic characteristics were associated with a poor OS, which may contribute to a highly aggressive subtype and short survival outcome for lung cancer patients carrying *MET*ex14-positive tumors (8, 27).

PSC is considered an aggressive subtype of lung cancer. Patients with PSCs generally show rapid progression, early metastasis, and dismal prognosis (28). The mOS of stage IV PSC patients was only 5.4 months in results from the National Cancer Database (29) and 2

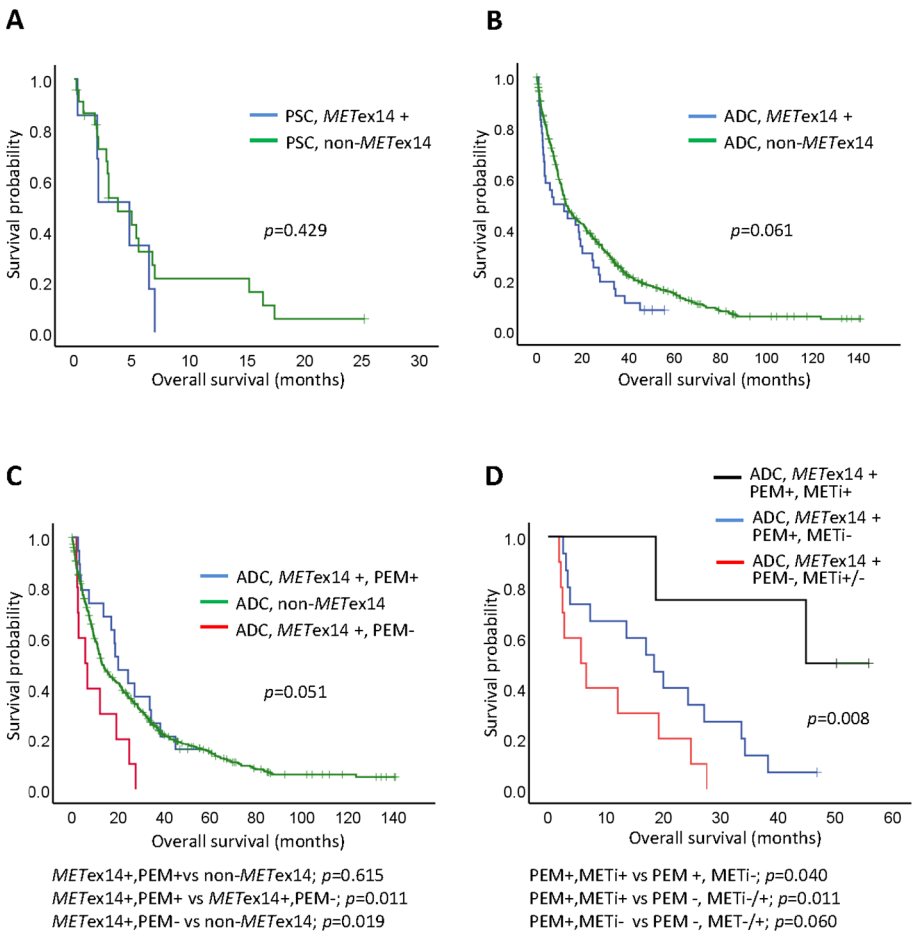

FIGURE 3
Kaplan-Meier curves of overall survival (OS) for Stage IV lung cancers. **(A)** Pulmonary sarcomatoid carcinoma (PSC) in *MET*ex14 positive (*MET*ex14+) or non-*MET*ex14 patients; **(B)** Adenocarcinoma (ADC) patients with *MET*ex14 (*MET*ex14+) or non-*MET*ex14 patients; **(C)** ADC patients with *MET*ex14 who received pemetrexed (PEM+), ADC patients with *MET*ex14 who did not received pemetrexed (PEM-), and ADC with non-*MET*ex14 patients; pairwise comparisons for *p*-value were shown below the panel; **(D)** ADC patients with *MET*ex14 who received sequential pemetrexed (PEM+) and MET inhibitor (METi+), who received pemetrexed (PEM+) without MET inhibitor (METi-), and who did not receive pemetrexed (PEM-) and with or without MET inhibitor (METi+/-); pairwise comparisons for *p*-value were shown below the panel.

months in those from the Surveillance, Epidemiology, and End Results (SEER) database (30). Patients who received anti-cancer chemotherapy still had a short mOS of only 6 months (31). A comparable and dismal survival outcome was observed in the current study; the mOS was <5 months for the advanced-stage patients with *MET*ex14-positive and *MET*ex14-negative PSC. None of the patients with *MET*ex14-positive PSC received targeted therapy, and most were not chemotherapy responders. This observation was consistent with the findings of a previous study that described the characteristics of chemoresistance and early progression in PSC (32). At present, evidence of treatment efficacy for *MET*ex14-positive PSC is inconclusive, and immune checkpoint inhibitors (ICIs) have been reported to show good efficacy in limited cases (33, 34). However, a pooled analysis of published data demonstrated that the *MET*ex14 mutation was not associated with tumor response (35). The impact of MET TKIs on OS in advanced PSC is also still unclear and variable since most reports either had small patient populations or were case reports, and described patients treated with different MET inhibitors (15, 17, 36). Lu et al. recently reported that 25 stage III or IV PSC patients with the *MET*ex14 mutation who were treated with savolitinib, a selective MET TKI, showed promising results with a response rate of 40% and a median PFS of 5.5 months (35), providing a beacon of hope for such dismal cases.

We identified several potential prognostic factors that predicted the OS in stage IV ADC patients harboring *MET*ex14 mutations. Pathological factors, including the staining score and the distribution of c-MET IHC staining, may help predict the OS. In particular, strong c-MET IHC staining with a score of 3+ was consistently associated with a short OS in both univariate and multivariate analyses for patients who received anti-cancer therapy. Awad et al. had previously reported that c-MET IHC staining in stage IV *MET*ex14-positive NSCLC was significantly stronger than that in stage I to III NSCLC with the *MET*ex14 mutation (27). The observation that strong MET expression could predict shorter OS in NSCLC (37, 38) suggests that a high MET IHC staining score may be an indicator of aggressive behavior in *MET*ex14-positive ADC (16, 39).

Among several treatment modalities, lung radiotherapy was associated with a longer mOS in both univariate and multivariate analyses. Treatment of primary lung tumors with thoracic radiotherapy has been reported to effectively ameliorate clinical respiratory symptoms, reduce tumor size, control recurrence, and prolong survival in patients with advanced NSCLC (40, 41). In our study, among 10 patients treated with lung radiotherapy, 9 patients received lung and/or mediastinal RT and other systemic therapies (chemotherapy or MET TKI) at different times and showed an mOS of 33.7 months (95% CI, 15.9-51.5), which was significantly longer than that in patients who received systemic therapy alone (mOS, 12.1 months; 95% CI, 2.1-22.0). Although MET activation plays an important role in conferring resistance to ionizing radiation by altering intracellular DNA damage response pathways in various cancer types (42), the underlying biological mechanism for prolonged OS in *MET*ex14-positive lung ADC patients receiving a combination of systemic therapy and local RT remains unclear. Radiotherapy may have diminished the resistance to systemic therapy and chemotherapy or TKI treatment may have enhanced radiosensitivity, thereby prolonging the treatment period and improving survival outcomes (43).

In multivariate analysis, initial brain metastasis was an independent risk factor for poor survival outcome in stage IV lung ADC patients harboring the *MET*ex14 mutation. The frequency of initial brain metastasis in this patient population was 30% (11 of 36), and the median OS was only 2.8 months for all 11 patients and 3.1 months for the eight patients who received anti-cancer therapy. Among them, two patients received crizotinib and six patients received brain RT plus standard chemotherapy. This mOS was inferior to that described in a previous report, which demonstrated a 6-month median OS in NSCLC with brain metastasis at initial presentation (44). The short OS in our cohort may be associated with the lack of effective treatment in most cases. Currently, the highly selective and potent MET inhibitors capmatinib and tepotinib are recommended and approved for the treatment of such patients because of their ability to cross the blood–brain barrier. A rapid partial response and impressive duration of response were reported with these inhibitors in patients with the *MET*ex14 mutation (45), and further follow-up data may be necessary to determine whether campatinib or tepotinib can prolong the OS in such patients.

Pemetrexed treatment is another strong predictor of OS for stage IV ADC patients with the *MET*ex14 mutation, which may be partly explained as a small population with MET TKI therapy in this study. However, pemetrexed–carboplatin plus pemetrexed maintenance regimen is currently used as initial chemotherapy for advanced NSCLC without targetable driver mutations (46), and may play a role in the treatment of advanced-stage ADC patients with the *MET*ex14 mutation when they are not able to receive specific MET TKIs or after MET TKI failure. Other chemotherapeutic agents, such as gemcitabine and taxanes, however, were not associated with favorable survival outcomes. Although *MET*ex14-positive ADC patients were shown to respond to platinum doublet therapy with a disease control rate of 80% in a study with small case number (47), we suggest that pemetrexed-based chemotherapy should be considered first if these patients need chemotherapy (48). Further prospective studies are needed to evaluate the role of pemetrexed in patients with advanced ADC with the *MET*ex14 mutation.

The present study had several limitations. Patient recruitment and data collection were retrospective, resulting in an inherent selection bias. Moreover, because of the relative rarity of *MET*ex14 in lung cancer, the imbalance in the number of mutation-positive and mutation-negative patients limited the viability of the analyses. In addition, intrinsic analysis of OS for stage IV ADC disease with the *MET*ex14 mutation may have been affected by the limited number of cases in each group. Finally, the type, combination, and sequence of chemotherapy, MET TKI, and immunotherapy varied among the study patients. Nevertheless, the present study provided crucial insights into the characteristics, associated factors, and survival outcomes of lung cancer patients with the *MET*ex14 mutation. Further large-scale prospective studies focusing on these prognostic factors are necessary to overcome these limitations.

5 Conclusion

In both lung ADC and PSC, patients with and without the *MET*ex14 mutation showed comparable survival outcomes. A higher frequency of *METex14* mutations was detected in PSC patients and these patients had poor overall survival. In lung ADC, pemetrexed-based chemotherapy (with or without MET TKI), strong c-MET ICH staining, brain metastasis at initial presentation, and lung radiotherapy were independent prognostic factors associated with survival outcomes. These findings provide information that can be expected to be important in clinical settings.

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 human participants were reviewed and approved by The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the National Taiwan University Hospital, Taipei, Taiwan (201509070RINA and 201103013RC). The patients/participants provided their written informed consent to participate in this study.

Author contributions

C-HG and J-YS designed the study and analyzed the data. M-SH contributed to pathological analysis. Y-LC and Y-NL performed genetic mutation analysis. C-HG wrote the manuscript. C-HG, M-

SH, Y-NL, S-GW, and J-YS edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

J-YS has served as an advisory board member for Amgen, AstraZeneca, Roche, Pfizer, Novartis, Merck Sharp, Dohme, Takeda, CStone Pharmaceuticals, Janssen, and Bristol-Myers Squibb; received speaking honoraria from ACT Genomics, Amgen, Chugai Pharma, CStone Pharmaceuticals, Bayer, AstraZeneca, Eli Lilly, Boehringer Ingelheim, Genconn Biotech, Roche, Novartis, TTY Biopharm, Pfizer, Orient EuroPharma, MundiPharma, Takeda, Janssen, Merck Sharp, Dohme, and Bristol-Myers Squibb; and received a grant from F. Hoffmann-La Roche Ltd.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1113696/full#supplementary-material

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Adjuvant EGFR-TKI therapy in resected EGFR-mutation positive non-small cell lung cancer: A real-world study

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Background: Although several clinical studies have laid the foundation for the adjuvant application of epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), some questions remain unresolved. This real-world study aimed to address questions such as the effect of adjuvant chemotherapy prior to adjuvant EGFR-TKI therapy on survival outcomes, and the duration of adjuvant EGFR-TKI therapy, *etc.*

Methods: Between October 2005 and October 2020, 227 consecutive patients with non-small cell lung cancer (NSCLC) who underwent complete pulmonary resections were included in this retrospective study. Patients received postoperative adjuvant chemotherapy followed by EGFR-TKI or adjuvant EGFR-TKI monotherapy. The disease-free survival (DFS) and overall survival (OS) were evaluated.

Results: Of the total 227 patients, 55 (24.2%) patients underwent 3-4 cycles of chemotherapy prior to receiving adjuvant EGFR-TKI therapy. The 5-year DFS rate was 67.8%, while the 5-year OS rate was 76.4%. The stages were significantly associated with both DFS (P<0.001) and OS (P<0.001), while no significant differences were observed in the DFS (P=0.093) and OS (P=0.399) between the adjuvant chemotherapy followed by EGFR-TKI and adjuvant EGFR-TKI monotherapy groups. A longer duration of EGFR-TKI therapy was associated with better DFS (P<0.001) and OS (P<0.001) benefit. Additionally, pTNM stage and duration of EGFR-TKI therapy were considered independent prognostic factors for long-term survival (All P<0.05).

Conclusions: This study supports the use of EGFR-TKI as a postoperative adjuvant treatment for patients with stage II-IIIA EGFR-mutation positive NSCLC. Additionally, patients with stage I who had pathological risk factors were also suitable for receiving adjuvant EGFR-TKI therapy. Postoperative EGFR-TKI based, chemotherapy-free adjuvant regimen may be a potential therapeutic option for patients with EGFR-mutation positive NSCLC.

KEYWORDS

adjuvant treatment, EGFR-TKI, non-small cell lung cancer, EGFR-mutation positive, adjuvant chemotherapy

Introduction

Lung cancer is the leading cause of cancer-related deaths both in China and in other countries worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer types, and adenocarcinoma is currently the most common lung cancer subtype (2). For resectable (stage I-IIIA) lung cancer, surgical resection is the mainstay of treatment; however, 40%-60% of patients relapse within 5 years after surgery, especially patients with stage IIIA NSCLC, with a median disease-free survival (DFS) of less than 1 year (3). Therefore, it is imperative to establish an appropriate adjuvant therapy modality for these patients.

A previous meta-analysis showed that platinum-containing two-drug adjuvant chemotherapy can only increase the 5-year survival rate of patients with resectable NSCLC by 5% (4), which is recommended for stage IB-IIIA NSCLC based on the NCCN guidelines (5). However, the hematological toxicity of the platinum-containing regimen usually leads to treatment delay, dose reduction, and eventually treatment discontinuation. In recent years, the discovery of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) has greatly improved the efficacy of patients with advanced NSCLC harboring EGFR mutation (6). To date, several studies have shown that EGFR-TKI treatment result in a higher response rate and longer progression-free survival in advanced NSCLC compared with platinum-containing two-drug regimens (7–9).

More recently, four published randomized clinical studies laid the foundation for EGFR-TKI postoperative adjuvant application (10–12). The ADJUVANT study (10) was a phase III randomized controlled trial, which included Chinese patients with stage II-IIIA EGFR-positive NSCLC. Patients in the experimental arm were administered gefitinib once a day for 2 consecutive years after surgery, while the control arm received four cycles of vinorelbine plus cisplatin regimen postoperatively. Results showed that a significantly longer DFS was observed with adjuvant gefitinib therapy compared with vinorelbine plus cisplatin in patients with completely resected stage II-IIIA (N1-N2) EGFR-mutant NSCLC. Similarly, patients with EGFR-mutant NSCLC postoperatively receiving other EGFR-TKIs (erlotinib, osimertinib or icotinib) also observed a significantly improvement in DFS compared to chemotherapy or placebo, with a better tolerability profile (11, 12). Nevertheless, several problems were encountered in the application of EGFR-TKI after surgery, including the right stage for application, application duration, and timing of chemotherapy.

Herein, we aimed to present the results of a real-world study on postoperative adjuvant EGFR-TKI therapy in patients with EGFR-mutation positive NSCLC, with a focus on evaluating the overall survival (OS) and DFS, to address these questions.

Materials and methods

Study design and patients

Patients with NSCLC who underwent various curative pulmonary resections from the Fourth Hospital, Hebei Medical University were included in this retrospective study between October 2005 and October 2020. The EGFR mutations status was assessed by the ADx-ARMS EGFR Five Mutations Detection Kit (Amoy Diagnostics, Xiamen, China). Eligible patients met the following criteria (1): aged ≥18 years (2); who underwent curative pulmonary resections for NSCLC (3); with postoperative histopathological diagnosis of stage I-IIIA NSCLC based on the American Joint Committee on Cancer (AJCC) criteria, 8th edition (4); with stage IA and IB cancers who had pathological risk factors such as invasion of visceral pleura, poor differentiation, spread through air spaces (STAS), nerve or vessel invasion, and micropapillary type (5); with NSCLC harboring an EGFR-sensitive mutations (exon 19 deletion or exon 21 L858R point mutation) (6); who received adjuvant EGFR-TKI treatment with or without chemotherapy prior to adjuvant EGFR-TKI therapy (7); EGFR-TKIs initiated within 4 weeks to 16 weeks after surgery (8); with adequately functioning hematological system, liver, and kidney (9); with an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1; and (10) with a postoperative survival duration of >6 months were included in the study.

Patients were excluded if they had (1) underwent palliative pulmonary resections for NSCLC (2), underwent wedge pulmonary resections with a biopsy intent (3), had concomitant cancers in other organs (4), used EGFR-TKI for less than 6 months due to certain reasons, and (5) received preoperative neoadjuvant therapy.

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethic Committee of Fourth Hospital, Hebei Medical University. All patients provided written informed consent.

Adjuvant use of EGFR-TKI after complete pulmonary resection

Adjuvant EGFR-TKI therapy was initiated within 4 weeks to 16 weeks after various pulmonary resections with or without chemotherapy prior to EGFR-TKI therapy. Patients were administered with oral first-generation EGFR-TKIs including erlotinib (Roche Pharmaceuticals) 150 mg once a day, gefitinib (AstraZeneca Pharmaceuticals Ltd) 250 mg once a day, or icotinib (Betta Pharmaceuticals) 125 mg thrice a day according to the availability of these drugs in the hospital at different times. Patients received the above EGFR-TKIs continued treatment until disease progression, unacceptable toxicity, or from the study at the clinician's discretion. Patients received postoperative chemotherapy (3-4 cycles, paclitaxel+platinum [TP] or vinorelbine+platinum [NP] regimens) before adjuvant EGFR-TKI therapy or adjuvant EGFR-TKI monotherapy. For the TP regimen, paclitaxel at a dose of 75 mg/m^2 was administered *via* intravenous drip on day 1, while cisplatin at a dose of 15 mg/m^2 was administered *via* intravenous drip from days 1–5, which was repeated every 3–4 weeks. For the NP regimen, vinorelbine at a dose of 25 mg/m^2 was administered intravenously on days 1–8, while cisplatin at a dose of 75 mg/m^2 was administered intravenously on day 1, which was repeated every 21 days.

Follow-up

All patients were followed up every 3 months, and the computed tomography (CT) scan of the chest and upper abdomen, and the biomarkers of NSCLC were routinely examined at each follow-up visit. Brain or/and spinal magnetic resonance imaging, skeleton scan, positron emission tomography-CT scan, and even needle biopsy were performed if indicated. The patients were followed up until death or December 31, 2020. Any patient who failed to respond to two consecutive follow-up reminders was defined as "lost to follow-up." These individuals were considered to be dead at the date of the first follow-up reminder when calculating the survival outcomes. DFS was defined as the time from surgery to the time of disease recurrence. Local or nodal recurrence and metastatic disease were considered to indicate primary tumor recurrence. OS was defined as the time from surgery to the time of death from any cause, with censoring at the longest follow-up.

Statistical analysis

SPSS 22.0 (IBM, Armonk, NY, USA) was used to perform all statistical analyses. Continuous variables were expressed as mean ± standard deviation. Categorical variables were expressed as frequency (percentage). In the univariate analysis, DFS and OS were calculated by the Kaplan–Meier method with the log-rank test applied for comparison. Multivariate analysis using the Cox proportional hazard model (forward stepwise regression) was performed for DFS and OS. $P<0.05$ was considered significant.

Results

Patients' and clinical data

We retrospectively analyzed 227 consecutive patients who used EGFR-TKI after undergoing complete pulmonary resections for NSCLC between October 2005 and October 2020. Of the 227 patients (84 males, 143 females; mean age, 60.8 ± 8.1 years), 96 patients had stage IA and IB NSCLC, 55 patients received 3-4 cycles of chemotherapy (TP regimen, n=32; NP regimen, n=23) prior to adjuvant EGFR-TKI therapy. The demographic and clinical data are described in Table 1.

Survival

In the present study, the median follow-up was 35.23 months. Of the study patients, 36 were followed for more than 5 years, while 103 were followed for more than 3 years. The 5-year DFS rate was 67.8% (median survival time [MST] not reached), while the 5-year OS rate was 76.4% (MST not reached).

The DFS and OS were calculated according to the disease stage. The stages were significantly associated with both DFS ($\chi^2 = 15.499$,

TABLE 1 Patient's demographic and clinical data.

	n=227 (%)
Sex	
Male	84 (37.0)
Female	143 (63.0)
Age (mean ± SD) years	60.8 ± 8.1
Smoke history	
Yes	23 (10.1)
No	204 (89.9)
Operation	
RUL	66 (29.1)
RML	16 (7.0)
RLL	48 (21.1)
RP	1 (0.4)
LUL	59 (26.0)
LLL	34 (15.0)
LP	3 (1.3)
Pathological type	
Adenocarcinoma	224 (98.7)
Squamous cell carcinoma	3 (1.3)
pTNM stage	
IA1	5 (2.2)
IA2	34 (15.0)
IA3	33 (14.5)
IB	24 (10.6)
IIA	3 (1.3)
IIB	47 (20.7)
IIIA	73 (32.2)
IIIB	8 (3.5)
EGFR mutation	
exon 19 deletion	113 (49.8)
exon 21 L858R	114 (50.2)
Chemotherapy before EGFR-TKI	
Yes	55 (24.2)
No	172 (75.8)
EGFR-TKI	
Erlotinib	9 (4.0)
Gefitinib	12 (5.3)
Icotinib	206 (90.7)

RUL, right upper lobectomy; RML, right middle lobectomy; RLL, right lower lobectomy; RP, right pneumonectomy; LUL, left upper lobectomy; LLL, left lower lobectomy; LP, left pneumonectomy; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitors; TNM, tumor node metastasis.

P<0.001) and OS (χ^2 = 22.924, P<0.001) (Figure 1). The 5-year DFS rates were 74.0% (MST not reached) in stage I subgroup, 61.2% (MST not reached) in stage II subgroup, and 41.4% (MST: 43.1 months [95% CI: 32.7-53.6]) in stage III subgroup. The 5-year OS rates were 94.4% (MST not reached) in stage I subgroup, 85.7% (MST: 72.5 months [95% CI: 45.3–99.8]) in stage II subgroup, and 59.2% (MST: 69.3 months [95% CI: 52.0–86.7]) in stage III subgroup.

To investigate the effect of the duration of EGFR-TKI therapy on long-term survival, the patients were divided into three subgroups, including less than 12 months subgroup, 12-36 months subgroup, and more than 36 months subgroup. A longer duration of EGFR-TKI therapy was associated with better DFS (χ^2 = 29.787, P<0.001) and OS (χ^2 = 29.585, P<0.001) benefit (Figure 2). The 5-year DFS rates were 24.3% (MST: 21.1 months [95% CI: 4.1–43.5]) in patients who used EGFR-TKIs for less than 12 months, 59.0% (MST not reached) in those who used EGFR-TKIs for 12-36 months, and 72.6% (MST not reached) in those who used EGFR-TKIs for more than 36 months. The 5-year OS rates were 48.3% (MST: 35.4 months [95% CI not estimable]) in patients who used EGFR-TKIs for less than 12 months, 60.4% (MST: 71.2 months [95% CI: 49.2-93.3]) in those who used EGFR-TKIs for 12-36 months, and 92.4% (MST not reached) in those who used EGFR-TKIs for more than 36 months.

Adjuvant chemotherapy was administered before adjuvant EGFR-TKI therapy in 55 patients, and 172 patients received adjuvant EGFR-TKI monotherapy. No significant differences were observed in the DFS (χ^2 = 2.859, P=0.093) and OS (χ^2 = 0.710, P=0.399) between the two groups (Figure 3). The 5-year DFS rates were 64.0% (MST not reached) in the adjuvant EGFR-TKI monotherapy subgroup and 59.6% (MST not reached) in the adjuvant chemotherapy followed by EGFR-TKI subgroup. The 5-year OS rates were 76.5% (MST not reached) in the adjuvant EGFR-TKI monotherapy subgroup and 72.6% (MST not reached) in the adjuvant chemotherapy followed by EGFR-TKI subgroup.

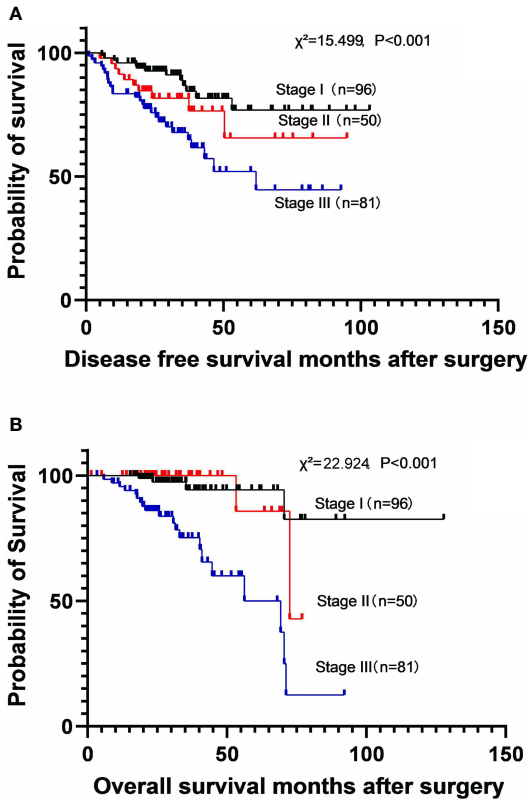

FIGURE 1
Kaplan-Meier survival curves for disease-free survival **(A)** and overall survival **(B)** according to the disease stage.

Univariate and multivariate analysis of DFS and OS

In the univariate analysis of DFS, age (≤60 *vs.* >60 years), smoking, pathological type (adenocarcinoma vs. squamous cell carcinoma), EGFR mutations status (exon 19 deletion *vs.* exon 21 L858R), different durations of EGFR-TKI therapy, and chemotherapy prior to the adjuvant EGFR-TKI therapy were not associated with DFS. Sex, disease stage, and duration of EGFR-TKI therapy (<1 year *vs.* 1-3 years vs. >3 years) were significantly associated with DFS. Sex, disease stage, and duration of EGFR-TKI therapy (<1 year *vs.* 1-3 years *vs.* >3 years) were included in the multivariate analysis. Pathological TNM stage and duration of EGFR-TKI therapy were independent prognostic factors for DFS (Table 2).

In the univariate analysis of OS, age (≤60 *vs.* >60 years), smoking status, pathological type (adenocarcinoma vs. squamous cell carcinoma), EGFR mutations status (exon 19 deletion *vs.* exon 21 L858R), different durations of EGFR-TKI therapy, and chemotherapy prior to the adjuvant EGFR-TKI therapy were not associated with OS. However, sex, disease stage, and the duration of EGFR-TKI therapy (<1 year *vs.* 1-3 years *vs.* >3 years) were significantly associated with OS. Sex, disease stage, and duration of EGFR-TKI therapy (<1 year *vs.* 1–3 years *vs.* >3 years) were included in the multivariate analysis. Pathological TNM stage and duration of EGFR-TKI therapy were independent prognostic factors for OS (Table 2).

Discussion

Four clinical studies, including ADJUVANT, EVEN, ADAURA, and EVIDENCE, examined the efficacy of postoperative adjuvant EGFR-TKI treatment in EGFR-mutant NSCLC, which provided clinicians with several useful informations, and changed the therapeutic modes of EGFR-TKI in the clinical setting to a certain extent. However, these studies had not addressed all the issues in this field, and many questions had remained hot topics for controversies such as the effect of adjuvant chemotherapy prior to adjuvant EGFR-TKI therapy on survival outcomes, and the duration of adjuvant EGFR-TKI therapy, *etc.* As a real-world study, this research may help deal with these leftover issues.

The first point of contention is whether to use adjuvant TKI alone or adjuvant chemotherapy prior to adjuvant EGFR-TKI

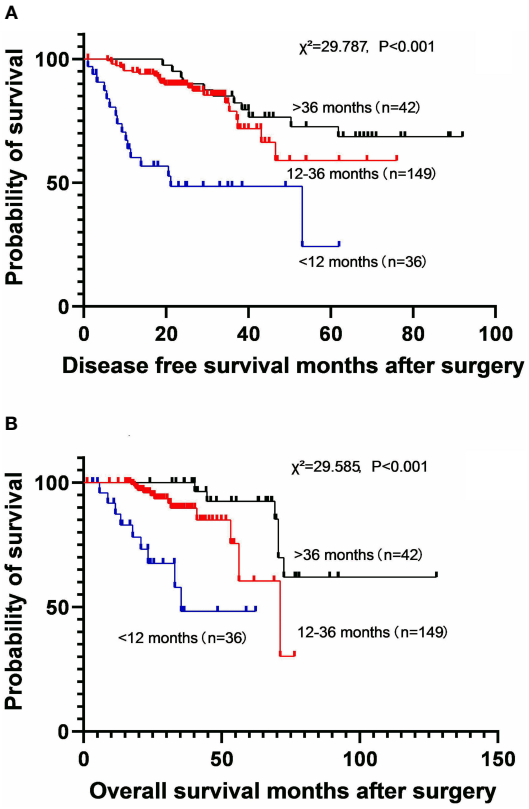

FIGURE 2
Kaplan-Meier survival curves for disease-free survival **(A)** and overall survival **(B)** according to the different durations of EGFR-TKI application.

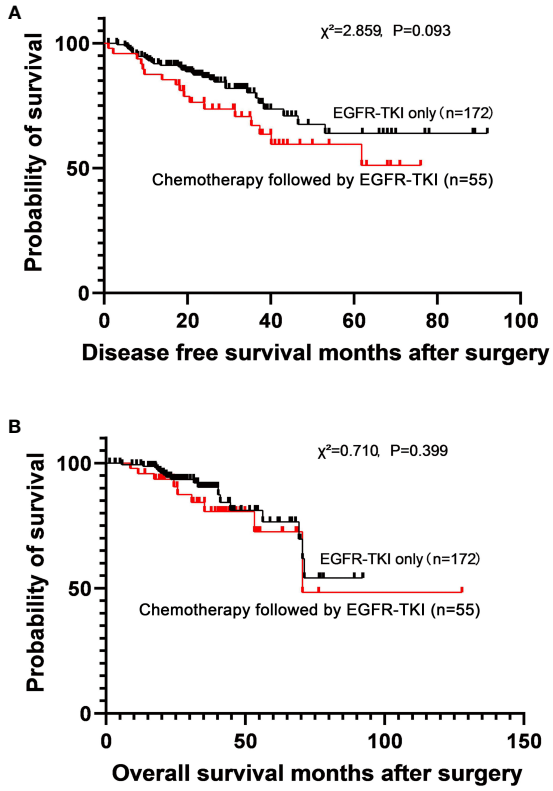

FIGURE 3
Survival curves for disease-free survival **(A)** and overall survival **(B)** in patients who used adjuvant EGFR-TKI only and adjuvant chemotherapy followed by EGFR-TKI.

therapy. Adjuvant chemotherapy after surgical resection would appear to be the logical approach in order to reduce disease recurrence and improve survival (13). However, results from the EVIDENCE trial showed that adjuvant icotinib significantly improves DFS in patients with EGFR-mutant stage II-IIIA NSCLC after complete tumor resection compared with adjuvant chemotherapy (47.0 vs. 22.1 months) (12). The ADAURA trial also confirmed the DFS advantage of adjuvant osimertinib over placebo in enrolled postoperative patients, the great mass of which had received prior chemotherapy (14). Notably, adding adjuvant chemotherapy before adjuvant EGFR-TKI was not beneficial among osimertinib-based patients according to cross-arm comparison of ADAURA trial by Liang et al. (15). Similarly, the addition of 3 to 4 cycles of adjuvant chemotherapy prior to adjuvant EGFR-TKI therapy did not significantly improve the DFS (5-year DFS rates: 59.6% vs. 64.0%, P=0.093) and OS (5-year OS rates: 72.6% vs. 76.5%, P=0.399) compared with adjuvant EGFR-TKI alone in the present study. Based on these findings, adjuvant EGFR-TKI appears to be superior to adjuvant chemotherapy in terms of DFS and can result in a further improvement in DFS for patients previously treated with postoperative adjuvant chemotherapy. Besides, there seems to be no additional survival benefit of adding adjuvant chemotherapy prior to adjuvant EGFR-TKI in patient with EGFR-mutation positive NSCLC. Certainly, considering the heterogeneity of patients, further screening of the precise beneficiary population for adjuvant chemotherapy and adjuvant EGFR-TKI therapy based on biomarkers (e.g., molecular residual disease [MRD] detection) may be required to enable patients to choose the optimal individualized treatment regimen while ensuring survival.

The second focus of controversy is the duration of adjuvant EGFR-TKI therapy. Previous studies had reported that the duration of adjuvant EGFR-TKI therapy had ranged from 0.5-3 years (10–12, 16). In the ADJUVANT study, the recurrence-free survival curve showed a significant downward trend after 2 years, which might be explained by the discontinuation of EGFR-TKI. Another randomized control trial conducted by Lyu et al. showed that the 2-year adjuvant icotinib therapy observed a significantly improved DFS and OS without an increase incidence of toxicity versus comparator 1-year therapy (17). In addition, the recurrence dynamics of the resected NSCLC display a multi-peak pattern, with the first peak occurring 7-9 months postoperatively irrespective of gender, the second peak occurring earlier in men (18-20 months) than in women (24-26 months), and the third peak occurring during the fourth year (18, 19). Thus, the ideal duration of medication therapy should cover these three periods of recurrence peaks. In the present study, the 5-year OS rates for patients treated with adjuvant EGFR-TKIs for less than 12 months, 12-36 months and more than 36 months were 48.3% (MST: 35.4 months [95% CI not estimable]), 60.4% (MST: 71.2 months [95%

TABLE 2 Results of univariate and multivariate analyses of disease-free survival and overall survival.

	Disease-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Sex: male vs. female	0.441 (0.255-0.764)	0.015	0.573 (0.321-1.021)	0.101	0.470 (0.220-1.003)	0.045	0.507 (0.221-1.165)	0.110
Age: <60 vs. ≥60 years	1.005 (0.580-1.742)	0.985			1.478 (0.695-3.142)	0.210		
Smoke history: yes vs. no	1.071 (0.421-2.727)	0.886			0.938 (0.216-4.078)	0.932		
Pathologic type: adenocarcinoma vs. squamous	0.049 (0.000-3612.560)	0.472			0.048 (000-796629.128)	0.592		
pTNM stage: I vs. II vs. III	1.876 (1.348-2.610)	0.000	1.915 (1.367-2.683)	0.000	3.390 (1.922-5.978)	0.000	3.315 (1.864-5.898)	0.000
EGFR mutation: exon 19 vs. exon 21	1.382 (0.942-2.027)	0.092			1.645 (0.906-2.987)	0.119		
Chemotherapy before TKI vs. TKI only	1.176 (0.966-1.431)	0.093			1.121 (0.857-1.467)	0.399		
EGFR-TKI: erlotinib vs. gefitinib vs. icotinib	0.748 (0.481-1.165)	0.178			0.680 (0.416-1.111)	0.123		
Duration of TKI use: <1 vs. 1-3 vs. >3 years	0.399 (0.254-0.627)	0.000	0.318 (0.150-0.676)	0.000	0.246 (0.132-0.458)	0.000	0.217 (0.109-0.432)	0.000

TNM, tumor node metastasis; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitors.

CI: 49.2-93.3]), and 92.4% (MST not reached), respectively. This finding indicated that survival advantage of patients seemed to increase with longer duration of EGFR-TKIs administration, offering more benefits in long-term outcomes. Moreover, the duration of TKI therapy was considered an independent prognostic factor by multivariate analysis in the present study. Accordingly, we also have to concern whether lifelong use of EGFR-TKIs is necessary and whether presence of patients who had been cured from surgery. Currently, Zhang et al. reported the prognostic value of MRD detection in patients with NSCLC after surgery, suggesting that patients with longitudinal undetectable MRD represent a potentially cured population and might not benefit from adjuvant therapy (20). Given the above evidence, the necessity to continue adjuvant EGFR-TKI therapy can be identified in the future studies by screening the patient population with long-term MRD detection (≥18 months).

The third focus of attention is determining the NSCLC stage that is suitable for adjuvant EGFR-TKI therapy. Due to the results of the four clinical studies mentioned above, the postoperative adjuvant EGFR-TKI therapy should be incontrovertible in patients with stage II–IIIA NSCLC. The focus of attention is whether patients with stage I EGFR-mutant NSCLC should receive adjuvant EGFR-TKI therapy. Goldstraw et al. reported a 5-year survival rate of 73% for patients with stage IB NSCLC (21), which implied that 27% of patients with stage IB will die due to locoregional recurrence or systemic spread within 5 years. Nevertheless, adjuvant chemotherapy for patients with stage I NSCLC who had risk factors such as lymphovascular or pleural invasion was still able to obtain a survival benefit (22, 23). This suggested a survival benefit for postoperative adjuvant therapy even in patients with stage I NSCLC with risk factors such as lymphovascular or pleural invasion. Furthermore, adjuvant osimertinib therapy had been shown to provide a survival benefit in patients with stage IB by the ADAURA trial. In the present study, 5-year OS rate for patients with stage I had achieved 94.4%, which was comparable with the historical data (21). Notably, all patients with stage I included in this study had pathological risk factors such as invasion of visceral pleura, poor differentiation, STAS, nerve or vessel invasion, and micropapillary type. Overall, this study supports adjuvant EGFR-TKI therapy for patients with stage I NSCLC who had pathological risk factors (invasion of visceral pleura, poor differentiation, or STAS, *etc.*).

There were several limitations to this study. Our study was a retrospective study conducted in a single-center, selection bias was inevitable due to the inherent limitations of single-center, non-randomized and retrospective design. Besides, this non-global study with data was only conducted in China, which might affect the generalizability of the results to a broader population. Therefore, more prospective studies and longer follow-up are required to validate the efficacy and safety of EGFR-TKI based, chemotherapy-free adjuvant regimen in patients with EGFR-mutation positive NSCLC.

Conclusion

In conclusion, this study supports the use of EGFR-TKI as a postoperative adjuvant treatment for patients with EGFR-mutation positive stage II-IIIA NSCLC. Additionally, patients with stage I who had pathological risk factors were also suitable for receiving adjuvant EGFR-TKI therapy. Postoperative EGFR-TKI based, chemotherapy-free adjuvant regimen may be a potential therapeutic option for patient with EGFR-mutation positive NSCLC. However, a multi-center randomized control trial is warranted to validate these findings.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethic Committee of Fourth Hospital, Hebei Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

J-FL: Conception and design; X-SS: Providing materials and samples; J-HY and X-EX: Data collection; J-FL: Data analysis and interpretation; J-FL: Drafting article; J-FL: Administrative support. 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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Association of smoking and ALK tyrosine-kinase inhibitors on overall survival in treatment-naïve ALK-positive advanced lung adenocarcinoma

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Introduction: Anaplastic lymphoma kinase (ALK) fusion mutation is more common in younger and never-smoking lung cancer patients. The association of smoking and ALK-tyrosine kinase inhibitors (TKIs) on overall survival (OS) of treatment-naïve ALK-positive advanced lung adenocarcinoma remains unclear in real-world.

Methods: This retrospective study evaluated all 33170 lung adenocarcinoma patients registered in the National Taiwan Cancer Registry from 2017 to 2019, of whom 9575 advanced stage patients had ALK mutation data.

Results: Among the 9575 patients, 650 (6.8%) patients had ALK mutation with the median follow-up survival time 30.97 months (median age, 62 years; 125 [19.2%] were aged ≥75 years; 357 (54.9%) females; 179 (27.5) smokers, 461 (70.9%) never-smokers, 10 (1.5%) with unknown smoking status; and 544 (83.7%) with first-line

ALK-TKI treatment). Overall, of 535 patients with known smoking status who received first-line ALK-TKI treatment, never-smokers and smokers had a median OS of 40.7 months (95% confidence interval (CI), 33.1-47.2 months) and 23.5 months (95% CI, 11.5-35.5 months) (P=0.015), respectively. Among never-smokers, those who received first-line ALK-TKI treatment had a median OS of 40.7 months (95% CI, 22.7-57.8 months), while those ALK-TKI not as first-line treatment had a median OS of 31.7 months (95% CI, 15.2-42.8 months) (P=0.23). In smokers, the median OS for these patients was 23.5 months (95% CI, 11.5-35.5 months) and 15.6 months (95% CI, 10.2-21.1 months) (P=0.026), respectively.

Conclusions and relevance: For patients with treatment-naïve advanced lung adenocarcinoma, the ALK test should be performed irrespective of smoking status and age. Smokers had shorter median OS than never-smokers among treatment-naïve-ALK-positive patients with first-line ALK-TKI treatment. Furthermore, smokers not receiving first-line ALK-TKI treatment had inferior OS. Further investigations for the first-line treatment of ALK-positive smoking advanced lung adenocarcinoma patients are needed.

KEYWORDS

lung cancer, TKI - tyrosine kinase inhibitor, smoking, ALK (anaplastic lymphoma kinase), non-small cell lung cancer, ALK non-small cell lung cancer

1 Introduction

Lung cancer is the leading cause of cancer death worldwide (1, 2). Mutations over several driver genes, such as epidermal growth factor receptor (*EGFR*), echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (*EML4-ALK*) fusion mutations, Kirsten rat sarcoma viral oncogene homolog, and human *EGFR* 2, are known to be involved in the initiation and maintenance of lung adenocarcinoma (3). Different kinds of driver gene mutations result in different clinical characteristics. *EML4-ALK* translocation is more common in younger patients and never-smokers (4, 5). Further, the mutation can be detected in approximately 3-5% of non-small cell lung cancer (NSCLC) patients (6). Several ALK tyrosine kinase inhibitors (TKIs), if administered as first-line treatment, can effectively suppress the oncogenic activity of ALK rearrangement and improve the outcomes of advanced ALK-positive lung cancer patients (7–11). Crizotinib was the first agent approved as it improved the progression-free survival (PFS) compared with platinum-based chemotherapy (10). Subsequently, several next-generation ALK-TKIs including alectinib, brigatinib and lorlatinib showed better PFS and intracranial efficacy compared with crizotinib in treatment-naïve setting (7, 8, 12). Therefore, these were preferred first line therapy in treatment-naïve patients. However, no clinical trials direct compare second- and third- generation ALK-TKIs and no treatment sequence after first-line therapy was suggested. How to make the right choice is based on factors including systemic and intracranial efficacy of the ALK-TKIs, various EML4-ALK variants, mechanisms of resistance as well as the toxicity profile.

Smoking is not only associated with lung cancer incidence, but also influences the efficacy of lung cancer treatment (13). ALK-TKIs as first-line (10, 11, 14) or second-line (15) treatments show similar benefits of PFS in never-smokers and smokers, but overall survival (OS) is immature for most trials (7–11). Meanwhile, the association of smoking and ALK-TKIs on OS of treatment-naïve ALK-positive advanced lung adenocarcinoma patients in the real world remains unclear. Thus, this study aimed to explore the epidemiology, clinical characteristics, and OS of treatment-naïve ALK-positive advanced lung adenocarcinoma patients, focusing on smoking status and ALK-TKIs treatment, using a nationwide cancer registry database in Taiwan.

2 Methods

2.1 Study design and patients

This retrospective cohort study used data from the National Taiwan Cancer Registry. The database stores standardised records of characteristics and clinical information of all cancer patients in Taiwan since 1979 (16–19). Detailed information on the smoking status and first-line treatment modalities and regimens for lung cancer patients has been recorded in the database starting since 2011, and ALK mutation data were added since 1 January 2017.

The current study analysed the data of treatment-naïve ALK-positive advanced lung adenocarcinoma patients recorded in the database between 01 January 2017 and 31 December 2019. In Taiwan, as Ventana immunohistochemistry (IHC) ALK (D5F3)

detection test in lung adenocarcinoma was reimbursed by National Health Insurance Administration (NHIA), so most of the ALK gene fusion was detected by this method. The inclusion criterion was cytological or pathological evidence of lung cancer and a clear classification of adenocarcinoma subtype. The National Taiwan Cancer Registry did not have ALK fusion data in squamous cell carcinoma of lung. The data were not available in this study. The association of smoking status, ALK-TKIs treatment, and clinical characteristics with OS was evaluated in ALK-positive advanced lung adenocarcinoma patients. The survival follow-up was until 31 December 2020.

The study protocol was approved by the Institutional Review Board of the National Health Research Institutes in Taiwan (approval number: EC1080506-E). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for observational studies was used to report the findings of this article (20).

2.2 Data collection

Data included age at diagnosis, sex, histological types, tumour stage, smoking status, Eastern Cooperative Oncology Group performance status (ECOG PS), ALK mutation status, and survival status. Patients were classified as never-smokers if they had never smoked in their lifetime; otherwise, they were classified as smokers. Before 2018, lung cancer staging in the registry was according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging system; and thereafter, according to the AJCC 8th edition (21, 22).

2.3 Statistical analysis

OS was calculated from the date of diagnosis to the date of death or the last follow-up. Survival status, which was evaluated using the National Death Certificates Database from the Department of Statistics, Ministry of Health and Welfare, Taiwan, was updated until 31 December 2020. Records were excluded if the date of death was unknown. The chi-square test was used to evaluate the association between categorical variables. OS curves were generated using the Kaplan–Meier method and compared using the log-rank test. The association between clinicopathological variables and outcomes was assessed using Cox proportional hazard regression models. Hazard ratios (HR) and 95% confidence intervals (CIs) were calculated using univariate and multivariable models. A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) software.

3 Results

A total of 46,897 patients were diagnosed with lung cancer during the study period, and 33170 (70.7%) had lung adenocarcinoma (Supplement Table 1A). Among patients with advanced stage (stages IIIB to IV) lung adenocarcinoma, 9575 had ALK mutation data; of these, 650 were ALK mutation positive with the median follow-up survival time 30.97 months. The 650 patients median age was 62 years, 125 (19.2%) patients were aged ≥75 years, 357 (54.9%) were female, and 640 and 10 (1.5%) had known and unknown smoking status, respectively. Overall, 179 (27.5) and 461 (70.9%) patients were smokers and never-smokers; 554 (85.2%) had ECOG PS 0-2. There were 592 patients (91.1%) with stage IV disease, and 313 (48.2%) had primary tumours in the upper lobes. Five hundred forty-four patients (83.7%) received ALK-TKI as first-line treatment (Supplement Table 1B).

Among the 9575, 5742, 3725, and 108 patients with known mutation data, never-smokers, smokers, and unknown smoking status, the ALK mutation rates were 6.8% (n=650), 8.0% (n=461), 4.8% (n=179), and 9.3% (n=10), respectively (Table 1). There were significantly more females among never-smokers (72.0%), whereas there were more males (90.5%) among smokers (P<0.001). Meanwhile, there were no differences in age distribution, ECOG PS, stage, primary tumour location, and use of first-line ALK-TKI treatment between never-smokers and smokers (Table 2). Concerning OS among ALK mutation-positive patients (n=640), univariate and multivariable analyses showed that those aged <65 years, never-smokers, those with better ECOG PS, and with stage IIIB or IIIC disease had significantly better OS outcomes. OS did not differ according to sex and use of first-line ALK-TKI treatment. Primary tumour location over the lower lobe had better OS in univariate, but no difference in multivariable analyses (Supplement Table 2).

With respect to OS according to smoking status, univariate analysis showed that in never-smokers with aged <75 years, male sex, ECOG PS 0-2, stage IV disease, primary tumour location over the upper lobe, and ALK-TKI treatment had better OS than did their smoker counterparts (Table 3A). Meanwhile, OS did not differ between smokers and never-smokers among those aged ≥75 years, female sex, ECOG PS 3-4, stage IIIB or IIIC disease, and primary tumours over lower lobes. Table 3B shows the multivariable analysis of influencing factors of OS in smokers and never-smokers were performed separately. In never-smokers with ALK mutation, age <65 years, and ECOG PS 0-2 had better OS, while there were no differences in OS according to sex, disease stage, primary tumour location, and first-line ALK-TKI treatment. In smokers, ALK-positive patients aged <65 years and with first-line ALK-TKI treatment had better OS, while there were no differences in OS according to sex, ECOG PS status, disease stage, and primary tumour location.

Among the 535 patients with known smoking status who received first-line ALK-TKI treatment, never-smokers had higher median OS than did smokers (40.7 months; 95% CI, 33.1-47.2 months vs. 23.5 months, 95% CI, 11.5-35.5 months; P=0.015; Figure 1). For OS according to the treatment of ALT-TKI among never-smokers, those who received first-line ALK-TKI treatment had longer median OS than those who did not receive ALK-TKI in the first-line setting, but the difference was not significant (40.7 months; 95% CI, 22.7-57.8 months vs. 31.7 months; 95% CI, 15.2-42.8 months; P=0.23; Figure 2A). Meanwhile, for smokers, those who received first-line ALK-TKI treatment had significantly higher

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TABLE 1 ALK-positive rates.

	Stage IIIB/IIIC		Stage IV		Total (%)	
	n	%	n	%	n	%
Never-smokers (n=5742)						
ALK mutation (+)	39	(12.0%)	422	(7.8%)	461	(8.0%)
ALK mutation (-)	285	(88.0%)	4996	(92.2%)	5281	(92.0%)
Smokers (n=3725)						
ALK mutation (+)	17	(4.7%)	162	(4.8%)	179	(4.8%)
ALK mutation (-)	341	(95.3%)	3205	(95.2%)	3546	(95.2%)
Unknown smoking status (n=108)						
ALK mutation (+)	2	(20.0%)	8	(8.2%)	10	(9.3%)
ALK mutation (-)	8	(80.0%)	90	(91.8%)	98	(90.7%)
Total (n=9575)						
ALK mutation (+)	58	(8.4%)	592	(6.7%)	650	(6.8%)
ALK mutation (-)	634	(91.6%)	8291	(93.3%)	8925	(93.2%)

AJCC 7TH edition before 2018.
AJCC 8th edition since 2018.
ALK, anaplastic lymphoma kinase.

median OS than those who did not receive ALK-TKI in the first-line setting (23.5 months; 95% CI, 11.5-35.5 months vs. 15.6 months; 95% CI, 10.2-21.1 months; P=0.026; Figure 2B).

4 Discussion

The association of smoking and ALK-TKIs on OS in treatment-naïve ALK-positive advanced lung adenocarcinoma patients is yet to be elucidated. This study using 2017-2019 data from the National Taiwan Cancer Registry database found that among patients who received first-line ALK-TKI treatment, the median OS was shorter among smokers than among never-smokers. Furthermore, in smokers, patients with ALK mutation who did not receive ALK-TKI as the first-line treatment had inferior median OS. These findings support the urgent need to consider new first-line treatment modalities for ALK-positive advanced lung adenocarcinoma patients who are smokers. The Taiwan

TABLE 2 Characteristics of the patients with known smoking status.

Patient characteristics	Total (n=640, 100%)		Never-smokers (n=461, 72.0%)		Smokers (n=179, 28.0%)		P value
	n	%	n	%	n	%	
Age, years							
<65	358	55.9%	250	54.2%	108	60.3%	0.36
65-74	158	24.7%	117	25.4%	41	22.9%	
≥75	124	19.4%	94	20.4%	30	16.8%	
Sex							
Male	291	45.5%	129	28.0%	162	90.5%	<0.001
Female	349	54.5%	332	72.0%	17	9.5%	
ECOG performance status							
0-2	547	85.5%	393	85.2%	154	86.0%	0.75
3-4	43	6.7%	33	7.2%	10	5.6%	
Unknown	50	7.8%	35	7.6%	15	8.4%	

(Continued)

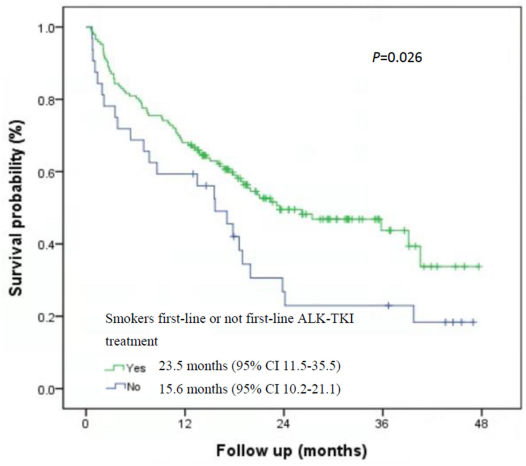
TABLE 2 Continued

Patient characteristics	Total (n=640, 100%)		Never-smokers (n=461, 72.0%)		Smokers (n=179, 28.0%)		P value
	n	%	n	%	n	%	
Stage							
IIIB or IIIC	56	8.8%	39	8.5%	17	9.5%	0.68
IV	584	91.2%	422	91.5%	162	90.5%	
Tumour location							
Upper lobes	311	48.6%	215	46.6%	96	53.6%	0.26
Lower lobes	290	45.3%	218	47.3%	72	40.2%	
Others	39	6.1%	28	6.1%	11	6.1%	
First-line ALK TKI							
Yes	535	83.6%	388	84.2%	147	82.1%	0.80
No	105	16.4%	73	15.8%	32	17.9%	

AJCC 7TH edition before 2018.
AJCC 8th edition since 2018.
Upper lobe includes the right middle lobe; others include bilateral lung lesions, trachea lesions, and main bronchus lesions.

TABLE 3A Univariate analysis of overall survival in different subgroups between smokers and never-smokers.

	Smokers vs. Never-smokers			P-value
	HR	(95% CI)		
Age, years				
<65	1.531	1.066	2.200	0.021
65-74	2.121	1.307	3.440	0.002
≥75	1.538	0.967	2.446	0.069
Sex				
Male	1.805	1.259	2.586	0.001
Female	1.584	0.833	3.013	0.161
ECOG performance status				
0-2	1.524	1.157	2.007	0.003
3-4	1.175	0.557	2.477	0.672
Unknown	1.849	0.843	4.054	0.125
Stage				
IIIB or IIIC	1.709	0.687	4.255	0.249
IV	1.501	1.165	1.935	0.002
Tumour location				
Upper lobes	1.851	1.335	2.567	<0.001
Lower lobes	1.226	0.820	1.833	0.321
Others	0.684	0.222	2.105	0.510
First-line ALK TKI				
Yes	1.409	1.068	1.860	0.015
No	1.501	1.165	1.935	0.023



No. at risk					
First-line	147	100	43	14	2
Not first-line	32	19	7	6	1

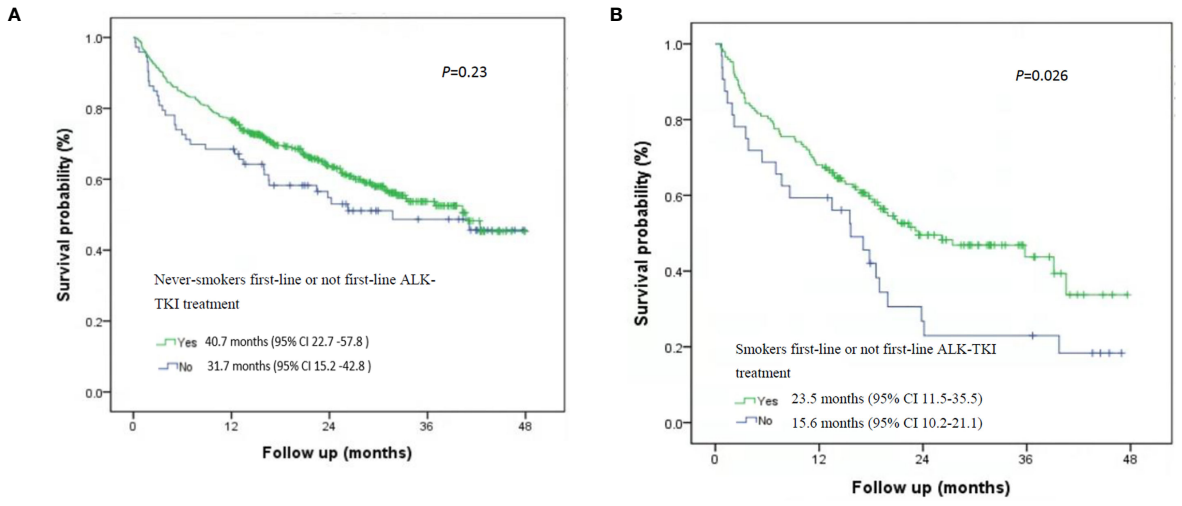
FIGURE 1
Kaplan-Meier curves of overall survival by smoking status among the 535 patients with first-line ALK-TKI treatment. ALK-TKI, anaplastic lymphoma kinase tyrosine kinase inhibitors.

nationwide database allows for the evaluation of OS according to smoking status and ALK-TKIs treatment. Also, it provides detailed information about clinical features that can influence OS.

EML4-ALK translocation is more common in never-smokers and younger patients. However, among the 650 patients in this study, approximately 30% of patients were current or former smokers, and 20% were aged >75 years. As such, it is necessary that all patients are evaluated for ALK gene rearrangements, irrespective of their smoking status and age. In Taiwan, three evaluation methods were allowed for ALK-TKIs reimbursement. The most commonly used is a fully automated immunohistochemistry assay (Ventana IHC, Ventana, Tucson, AZ) with the prediluted Ventana anti-ALK (D5F3) Rabbit monoclonal primary antibody as previously described (4). The other two methods, namely, next-generation sequencing and fluorescence *in situ* hybridization, are used less frequently.

Randomised clinical trials (23–25) of lung cancer patients with EGFR-sensitive mutation showed that first-line treatment with EGFR-TKIs achieved similar PFS benefits to chemotherapy in smokers and never-smokers. Meanwhile, a meta-analysis showed that never-smokers had better PFS benefits than did smokers (26). Several trials showed similar PFS benefits over chemotherapy using ALK-TKIs between smokers and never-smokers for patients with treatment-naïve advanced stage ALK mutation-positive lung cancer (10, 11, 14). However, in ALK mutation-positive patients, there are no clinical trial data on OS differences between smokers and never-smokers.

A real-world study evaluated 121 stage IV ALK mutation-positive NSCLC patients diagnosed between 2011 and 2016 and showed that never-smoking was the only independent prognostic factor associated with better OS (HR: 0.499, 95% CI: 0.265-0.941, P=0.032). The use of alectinib or lorlatinib in any treatment line improved OS (P=0.022) (27). Similar findings were observed in the current study, wherein crizotinib was the most common first-line treatment for ALK mutation-positive lung cancer patients, which may be explained by the reimbursement guidelines on ALK-TKIs by the Taiwan National Health Insurance Administration (Supplementary Table 3). The reimbursement for crizotinib use as a first-line treatment began in November 2017, but it has been available as a second-line treatment since 2015. A proportion of the patients had used crizotinib as the first-line treatment before 2017.



No. at risk					
First-line	388	296	159	47	4
Not first-line	73	50	31	19	1

No. at risk					
First-line	147	100	43	14	2
Not first-line	32	19	7	6	1

FIGURE 2
Kaplan-Meier curves of overall survival stratified by smoking status and ALK-TKI treatment line in ALK-positive patients. **(A)** Never-smokers and first-line or not first-line ALK-TKI treatment (n=461). **(B)** Smokers and first-line or not first-line ALK TKI treatment (n=179). ALK-TKI, anaplastic lymphoma kinase tyrosine kinase inhibitors.

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TABLE 3B Multivariable analysis of influencing factors of overall survival in smokers and never-smokers.

Patient characteristics	Never-smokers				Smokers			
	HR	(95%CI)		P value	HR	(95%CI)		P value
Age, years								
<65	1.0 (reference)				1.0 (reference)			
65-74	1.464	1.016	2.109	0.041	2.256	1.322	3.849	0.003
≥75	2.916	2.043	4.162	<0.001	4.338	2.512	7.491	0.000
Sex								
Male	1.0 (reference)				1.0 (reference)			
Female	1.159	0.822	1.634	0.401	0.843	0.412	1.723	0.639
ECOG performance status								
0-2	1.0 (reference)				1.0 (reference)			
3-4	3.019	1.890	4.825	<0.001	2.017	0.980	4.153	0.057
Unknown	1.533	0.888	2.644	0.125	2.039	0.979	4.249	0.057
Stage								
IIIB or IIIC	1.0 (reference)				1.0 (reference)			
IV	1.496	0.806	2.779	0.202	1.962	0.925	4.160	0.079
Tumour location								
Upper lobe	1.0 (reference)				1.0 (reference)			
Lower lobe	1.000	0.743	1.347	1.000	0.708	0.442	1.136	0.153
Others	0.857	0.461	1.590	0.624	0.658	0.229	1.892	0.438
First-line ALK TKI								
No	1.0 (reference)				1.0 (reference)			
Yes	1.014	0.627	1.638	0.956	0.392	0.204	0.752	0.005

HR, hazard ratio; CI, confidence interval.
AJCC 7^{TH} edition before 2018.
AJCC 8^{th} edition since 2018.
Upper lobe includes the right middle lobe; others include bilateral lung lesions, trachea lesions, and main bronchus lesions.

This explains the high percentage of first-line ALK-TKI use (83.6%) in this study. The same observations were noted for ceritinib and alectinib use.

The current study found that among patients with first-line ALK-TKI treatment, never-smokers had better median OS than smokers. In smokers, OS was significantly better in ALK-positive patients who received first-line ALK-TKI treatment than those who did not receive. This could be because first-line ALK-TKI treatment was less optimal in smokers than in never-smokers; however, clinical trials showed that it is still better than chemotherapy in smokers (10, 11, 14).

The possible mechanisms about the inferior OS in smoking ALK-positive patients remain unclear. In our previous study, the presence of ALK V3a/b subtype independently predicted a worse overall survival in patients receiving ALK inhibitors, however, the incidences of V3 subtype were similar between smokers and never-smokers (5). Smokers suffered more mutations than never-smokers and this could complicate the drug efficacy. Among smokers, TP53 mutation is the most common (28). There is a high rate of TP53 comutation in ALK-positive lung cancer patients, which has shown a significantly worse prognosis (29, 30).Additional studies are required to clarify the potential mechanisms.

There are limitations to this study. OS data from the PROFILE-1014 trial differ based on the access to subsequent ALK-TKIs after crizotinib failure, with a 5-year survival rate of 75% vs. 28% in patients who did and did not receive subsequent ALK-TKI, respectively (31, 32). First, we did not have detailed data on treatment after failure of first-line treatment. However, as ALK-TKIs were subsequently reimbursed in Taiwan (first-line crizotinib began in November 2017 and second-generation ALK-TKIs as the second line since September 2017), most patients who failed first-line ALK-TKIs treatment in the study would have had the chance to receive ceritinib or alectinib. Some even received brigatinib or lorlatinib in subsequent treatments. Second, our ALK mutation-positive patients had shorter OS than those reported in clinical trials. This could be because not all patients received alectinib or lorlatinib as subsequent treatment. Lorlatinib was not reimbursed as the second line until June 2020 and was limited to disease

progression to the brain after ceritinib or alectinib failure. The use of more ALK-TKI lines and the use of alectinib or lorlatinib in any treatment line are positively correlated with OS (27). Other limitations include a high proportion of patients older than 75 years and with poor PS.

5 Conclusion

For patients with treatment-naïve advanced lung adenocarcinoma, the ALK test should be performed irrespective of smoking status and age. For ALK mutation-positive patients with first-line ALK-TKI treatment, smokers have shorter median OS than never-smokers. In smokers, the survival would be inferior if they did not receive first-line ALK-TKIs treatment. Further investigations for the first-line treatment of ALK-positive smoking advanced lung adenocarcinoma patients are needed.

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 authors.

Ethics statement

The study protocol was approved by the Institutional Review Board of the National Health Research Institutes in Taiwan (approval number: EC1080506-E). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

G-CC and J-YH had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Z-RZ, H-YK, and K-CC contributed equally to this work as the co-first authors. G-CC and J-YH contributed equally to this work. Concept and design: Z-RZ, H-YK, K-CC, J-YH, and G-CC. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Z-RZ, H-YK, K-CC, J-YH, and G-CC. Critical revision of the manuscript for important intellectual content: C-JC, Chen, W-CL, T-WL, J-YH, and G-CC. Statistical analysis: Z-RZ, H-YK, C-JC, and G-CC. Obtained funding: T-WL and G-CC. Administrative, technical, or material support: Z-RZ, H-YK, Chen, C-JC, W-CL, T-WL, J-YH, and G-CC. Supervision: RZ, H-YK, Chen, J-YH, and G-CC. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1063695/full#supplementary-material

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New prognostic system specific for epidermal growth factor receptor-mutated lung cancer brain metastasis

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Introduction: Brain metastases (BM) from lung cancer are heterogeneous, and accurate prognosis is required for effective treatment strategies. This study aimed to identify prognostic factors and develop a prognostic system exclusively for epidermal growth factor receptor (EGFR)-mutated lung cancer BM.

Methods: In total, 173 patients with EGFR-mutated lung cancer from two hospitals who developed BM and received tyrosine kinase inhibitor (TKI) and brain radiation therapy (RT) were included. Univariate and multivariate analyses were performed to identify significant EGFR-mutated BM prognostic factors to construct a new EGFR recursive partitioning analysis (RPA) prognostic index. The predictive discrimination of five prognostic scoring systems including RPA, diagnosis-specific prognostic factors indexes (DS-GPA), basic score for brain metastases (BS-BM), lung cancer using molecular markers (lung-mol GPA) and EGFR-RPA were analyzed using log-rank test, concordance index (C-index), and receiver operating characteristic curve (ROC). The potential predictive factors in the multivariable analysis to construct a prognostic index included Karnofsky performance status, BM at initial lung cancer diagnosis, BM progression after TKI, EGFR mutation type, uncontrolled primary tumors, and number of BM.

Results and discussion: In the log-rank test, indices of RPA, DS-GPA, lung-mol GPA, BS-BM, and EGFR-RPA were all significant predictors of overall survival (OS) ($p \leq 0.05$). The C-indices of each prognostic score were 0.603, 0.569, 0.613, 0.595, and 0.671, respectively; The area under the curve (AUC) values predicting 1-year OS were 0.565 (p=0.215), 0.572 (p=0.174), 0.641 (p=0.007), 0.585 (p=0.106), and 0.781 (p=0.000), respectively. Furthermore, EGFR-RPA performed better in terms of calibration than other prognostic indices.BM progression after TKI and EGFR mutation type were specific prognostic factors for EGFR-mutated lung cancer BM. EGFR-RPA was more precise than other models, and useful for personal treatment.

KEYWORDS

EGFR, lung cancer, brain metastases, TKI, radiotherapy

1 Introduction

Lung cancer remains the most common cause of cancer and cancer-related mortality worldwide (1),Brain metastases (BM) are common in patients with lung cancer, 20-25% of non-small-cell lung cancer (NSCLC) patients are estimated to have BM at initial diagnosis (2, 3). Around 40- 60% of the patients diagnosed with NSCLC develop BM during the course of their disease, and this cumulative risk increases up to 70% in patients with epidermal growth factor receptor (EGFR) mutation (4). Mutations that constitutively activated the EGFR kinase domain are present in 10-15% of patients with lung adenocarcinoma in North America and up to 60% of patients in Asia (5). In the past, survival after the diagnosis of BM in NSCLC patients was uniformly poor, and its management was futile (6, 7). However, with advances in systemic treatment and technology, including molecularly targeted therapies and stereotactic radiosurgery (SRS), survival from BM has improved (8). Extensive efforts have focused on predicting outcomes for the considerable heterogeneity of patients with BM. An accurate and easy diagnosis-specific tool for clinicians is urgently required to improve their ability to assess patient prognosis and create clinical risk groups for informing treatment or patient stratification by disease severity in clinical trials.

Considerable research efforts have focused on predicting outcomes for the extremely heterogeneous population of patients with BM. Gaspar et al. (9)presented RPA prognostic system, Lorenzoni et al. (10) proposed BS-BM, and Sperduto et al. (11) developed GPA. However, these BM prognostic indices included various tumor types. Sperduto et al. (12) recognized the variability of the prognostic factors according to primary diagnosis and constructed a new prognostic index named DS-GPA. Based on the effect of gene alterations on survival in patients with lung cancer, Sperduto et al. (12)proposed lung-mol GPA that included the addition of gene status. The limitation of previous studies on BM was the inconsistency in treatment methods, especially those considering EGFR mutations. Tyrosine kinase inhibitors (TKIs) and brain radiation therapy (RT), which are the most important treatments for patients with EGFR-mutated BM. Recently, the use of TKIs for treating BM in patients who are EGFR-TKI naïve has been demonstrated to have a central nervous system (CNS) objective response rate of 91% (Osimertinib) and 68% (Gefitinib or Erlotinib) (13). Inconsistency in treatment methods may affect the construction of a BM model. Whether the existing BM indices were applicable was unknown in an era of lung cancer with targeted therapies. Therefore, in this study, only the patients with EGFR-mutated NSCLC BM who received TKIs and brain RT were included in order to reduce the risk of bias. We evaluated previous BM indices and established a new prognostic index EGFR recursive partitioning analysis (RPA) referring to the RPA model based on a reasonable combination of EGFR mutation-specific predictors.

2 Methods

2.1 Study design and patients

Patients with EGFR-mutated NSCLC who were diagnosed with BM at any point of the disease course and treated with EGFR-TKI and brain radiotherapy from January 2008 to December 2018 at the Fudan University Shanghai Cancer Center and the People's Hospital Affiliated to Jiangsu University were identified. Since this study aimed to retrospectively evaluate prognostic factors for OS and construct a new prognostic grading system for NSCLC, the following patients were included: (i) those histologically diagnosed with lung adenocarcinoma, (ii) those who presented with EGFR mutations in the primary tumor or metastatic brain lesions, (iii) those with confirmed BM using computed tomography and (or) magnetic resonance imaging, and (iv) those who received first-generation and second- generation EGFR TKIs and brain radiotherapy, including whole brain radiation therapy (WBRT) and SRS. Patients who received EGFR-TKI for less than 1 month and patients lost to follow-up were excluded. Patient data included detailed clinical data, follow-up examination results and death dates (if applicable). Patients were followed up *via* clinic visits and telephone interviews. OS was calculated from the date of diagnosis of BM to the date of death owing to cancer or by patient censoring on the date of the last follow up. All patients were followed up until death or April 2020 (end of follow-up). The study was conducted according to the Helsinki Declaration and the study protocol was approved by the Ethics Review Board of the Fudan University Shanghai Cancer Center and the People's Hospital Affiliated to Jiangsu University.

2.2 Analyses of prognostic factors

To evaluate prognostic factors for OS for EGFR-mutated lung cancer BM patients, data on the following variables were gathered for the analysis: sex, age, Karnofsky performance score (KPS) at the time of BM, stage at initial diagnosis, whether patients were symptomatic because of BM, whether there was BM progression after TKI, presence or absence of extracranial metastases concurrent with the BM, EGFR mutation site, symptoms related to BM, control of the primary tumor, number of BM, and type of RT delivered. The dates of the initial cancer diagnosis, BM diagnosis, intracranial progression, RT treatments, systemic therapy treatments, most recent follow-up, and death were also recorded. These variables were included in the univariate analysis which was performed using the Kaplan–Meier method plus the log-rank test. The variables that were significant in the univariate analysis ($p<0.05$) were evaluated for independent associations with survival in the multivariate analysis (Cox proportional hazards model).

2.3 Construction of a new prognostic index

By referring to the RPA scoring system (9), we established a new BM scoring system named EGFR mutation-specific RPA (EGFR-RPA) based on the multivariate analysis results. The variables significantly associated with survival ($p<0.05$) in the Cox proportional hazard analysis were incorporated in the *EGFR*-RPA.

2.4 Analyses and assessment of prognostic stratification

To evaluate the prognostic factors for *EGFR*-mutated NSCLC BM patients receiving *EGFR*-TKI and brain RT using the prognostic grading systems, patients were stratified according to RPA, DS-GPA, BS-BM, lung-mol GPA and *EGFR*-RPA.

2.5 Statistics

The Kaplan-Meier analysis was used to estimate the OS, from the date of diagnosis of BM to the date of death or last follow-up. The univariate Cox proportional hazards analysis examined the factors associated with an increased risk of death. With the significant variables obtained in the univariate analysis, multivariate Cox regression analysis was performed to determine the new model for predicting survival. Log-rank testing was used to compare the adjacent classes with OS for five prognostic indices. The AUC and C-index were used to estimate the discriminative ability with the five existing indices. All analyses were performed using SPSS version 17.0 (IBM Corporation, Chicago, IL) and R version 3.5.1. (The R Foundation, Vienna, Austria).

3 Results

3.1 Patient characteristics

From January 2008 to December 2018, a total of 173 patients were included in this retrospective study conducted in two hospitals. The process of screening eligible patients is provided in the Supplementary Materials (Supplement Figure 1). The median follow-up time for these patients was 67 months (range, 1-112 months). The median age was 57 years (range, 31-84 years). The patients were predominantly ≤70 old years (91.3%), were males (77.8%), had a KPS score ≥70 (72.3%), BM ≤3 (64.2%), had extracranial metastases (ECM) (72.8%), had symptomatic BM (59.5%), had metachronous BM (52.6%), and received upfront or concurrent WBRT or SRS (79.2%). Patients' characteristics at baseline are shown in Table 1.

3.2 Prognostic factors for outcomes of OS

The median OS was 30 (95% confidence interval [CI], 26-34, Supplement Figure 2) months. In the univariate analysis, a significantly shorter OS was observed in patients with KPS <70 (p=0.000), BM at initial diagnosis (p=0.001), BM progression after TKI (p=0.000), extracranial metastases (p=0.007), *EGFR* mutation type that was not exon 19 deletion (p=0.007), uncontrolled primary tumor (p=0.000), and number of BM >3 (p=0.016). In addition, we observed that the patients who underwent SRS or surgical resection

TABLE 1 Clinical characteristics of the 173 patients.

Variables	No.	(%)
Sex		
Female	100	57.8
Male	73	42.2
Age(years)		
<50	44	25.4
50 -<60	57	32.9
60 -<70	57	32.9
>=70	15	8.7
KPS		
<70	48	27.7
70-80	96	55.5
>=90	29	16.8
Stage at diagnosis		
I-III	51	29.5
IV	122	70.5
BM at initial diagnosis		
Synchronous	82	47.4
Metachronous	91	52.6
Extracranial metastases		
Yes	126	72.8
No	47	27.2
EGFR site		
19 deletion	90	52.0
21 mutation	72	41.6
Others	11	6.4
Symptomatic BM		
Yes	103	59.5
No	70	40.5
Control of primary tumor		
Yes	122	70.5
No	51	29.5
Numbers of BM		
1	72	41.6
2-3	39	22.5
>=4	62	35.8
EGFR-TKIs		
Gefitinib	82	47.4
Erlotinib	41	23.7

(Continued)

TABLE 1 Continued

Variables	No.	(%)
Icotinib	47	27.2
Afatinib	3	1.7
Radiotherapy technology		
WBRT	122	70.5
SRS	25	14.5
WBRT+SRS	17	9.8
Surgeon+WBRT	6	3.5
Surgeon+SRS	3	1.7
Timing of radiotherapy		
Upfront or concurrent WBRT or SRS	137	79.2
Upfront EGFR-TKI	36	20.8

KPS, Karnofsky performance status; BM, Brain metastases; EGFR, Epidermal growth factor mutation; WBRT, Whole brain radiation therapy; SRS, Stereotactic radiosurgery; TKI, Tyrosine kinase inhibitors.

with or without WBRT tended to have a longer OS than those who underwent only WBRT; however, the difference was not statistically significant (p= 0.063). Further, there was no significant difference observed in the patients with respect to sex, age, stage at initial diagnosis, symptomatic BM, and timing of RT. In the multivariate analysis using multiple Cox proportional hazards models, we observed that the performance status (KPS<70, p= 0.006), BM at the time of initial lung cancer diagnosis (p= 0.024), BM progression after TKI (p=0.000), *EGFR* mutation (p=0.023), uncontrolled primary tumor (p=0.002), and more than three BM (p=0.005) were the independent prognostic factors for OS (Table 2).

3.3 Prognostic indices and a new model

The prognostic values of the five indices examined are presented in Figure 1 and Supplement Table 1. In the log-rank test, the indices of RPA, DS-GPA, lung-mol GPA, and BS-BM were all significant predictors of OS. However, they did not demonstrate superiority of their predictive effect. In the multivariate analysis using multiple Cox proportional hazards models, age and extracranial metastases were not found to be the independent prognostic factors for OS. BM at the time of the initial diagnosis of lung cancer (p= 0.024), BM progression after TKI (p=0.000), and *EGFR* mutation type (p=0.023) were independent prognostic factors (Table 2); however, they were not associated with the four prognostic indices. Therefore, referring to the RPA model, we established a new BM scoring system named *EGFR*-RPA based on the results of the multivariate analysis (Figure 2). The first node split by BM progression after TKI indicated that the survival difference between patients was greater than the difference between any other subset among them. Among the patients with non-TKI advanced BM, the most significant split was the number of prognostic factors. Patients who met 5 prognostic factors or developed BM progression after TKI had the worst survival (Class I). The best survival was observed

TABLE 2 Univariable and multivariable analyses of covariables associated with OS.

	Univariable Analysis					Multivariable Analysis			
Variable	Median OS (month)	BE	95%CI		*P*	HR	95%CI		*P*
Gender					0.115				
Male	29	2.325	24.444	33.556					
Female	31	5.086	21.031	40.969					
Age					0.192				
<70	31	2.258	26.574	35.426					
>=70	27	7.059	13.164	40.836					
KPS					0.000	1.787	1.182	2.700	0.006
<70	19	2.411	14.274	23.726					
>=70	36	3.329	29.474	42.526					
Stage at diagnosis					0.964				
I-III	30	2.188	25.711	34.289					
IV	27	13.064	1.395	52.605					
BM at initial diagnosis					0.001	0.599	0.383	0.935	0.024
Synchronous	24	4.272	17.700	30.300					
Metachronous	40	3.193	31.600	48.400					
BM progrssion after TKI					0.000	2.529	1.557	4.111	0.000

(Continued)

TABLE 2 Continued

	Univariable Analysis					Multivariable Analysis			
Variable	Median OS (month)	BE	95%CI		*P*	HR	95%CI		*P*
Yes	10	1.829	6.415	13.585					
No	35	3.715	27.718	42.282					
Extracranial metastases					0.007				
Yes	27	2.092	22.900	31.100					
No	47	10.344	26.726	67.274					
EGFR site					0.007	1.498	1.058	2.122	0.023
19 deletion	35	3.981	27.197	42.803					
others	17	7.707	1.895	32.105					
Symptomatic BM					0.108				
Yes	27	3.426	28.285	41.715					
No	35	3.501	20.138	33.862					
Control of primary tumor					0.000	0.530	0.352	0.797	0.002
Yes	37	4.013	29.134	44.866					
No	23	2.866	17.383	28.617					
Numbers of BM					0.016	1.689	1.170	2.437	0.005
<=3	34	4.078	26.007	41.993					
>3	17	4.354	8.466	25.534					
Radiotherapy technology					0.063				
WBRT	27	3.091	20.941	33.059					
SRS/Surgeon± WBRT	32	3.383	25.370	38.630					
Timing of radiotherapy					0.249				
Upfront or concurrentWBRT or SRS	31	2.798	25.516	36.484					
Upfront EGFR-TKI	25	7.944	9.429	40.571					

KPS, Karnofsky performance status; BM, Brain metastases; TKI, Tyrosine kinase inhibitors; EGFR, Epidermal growth factor mutation; WBRT, Whole brain radiation therapy; SRS, Stereotactic radiosurgery; CI, Confidence interval.

in patients who had either no or only one prognostic factor (Class III). All the other patients had two to four prognostic factors, forming a middle stage (Class II). The median OS for Class I, Class II, and Class III were 11 months (95% CI, 7-15), 32 months (95% CI, 27-37), and 52 months (95% CI, 34-69), respectively (p=0.000 Figure 1 and Supplement Table 1). The 3-year OS rates for Class I, Class II, and Class III were 12%, 40%, and 63%, respectively. The 5-year OS rates for Class I, and Class II, and Class III were 0%, 19%, and 36%, respectively.

3.4 Comparison of predictive accuracy for overall survival between prognostic indices

The ROC and C-indices were used to compare the prognostic validity. The AUC values for 1-year OS were 0.565 for RPA (p=0.214), 0.752 for DS-GPA (p=0.175), 0.641 for lung-mol GPA (p=0.007), 0.585 for BS-BM (p=0.106), and 0.781 for *EGFR*-RPA (p=0.000). The C-indices for the survival probability prediction were 0.603, 0.569, 0.613, 0.595, and 0.671, for each scoring system, respectively. These results suggested that the *EGFR*-RPA model presented with the best AUC values and C-indices (Tables 3, 4). Furthermore, the calibration plot for the probability of 1- year OS presented a good correlation between the *EGFR*-RPA prediction and actual observation. (Supplement Figure 3).

4 Discussion

To the best of our knowledge, this is the first analysis of patients with *EGFR*-mutated NSCLC who developed BM after receiving all the effective treatments, including first-generation TKIs as first line treatment, Osimertinib as subsequent therapy and brain RT. In this study, we observed that KPS, BM at the time of initial diagnosis, BM progression after TKI, *EGFR* mutation type, uncontrolled primary tumor and the number of BM were the independent prognostic

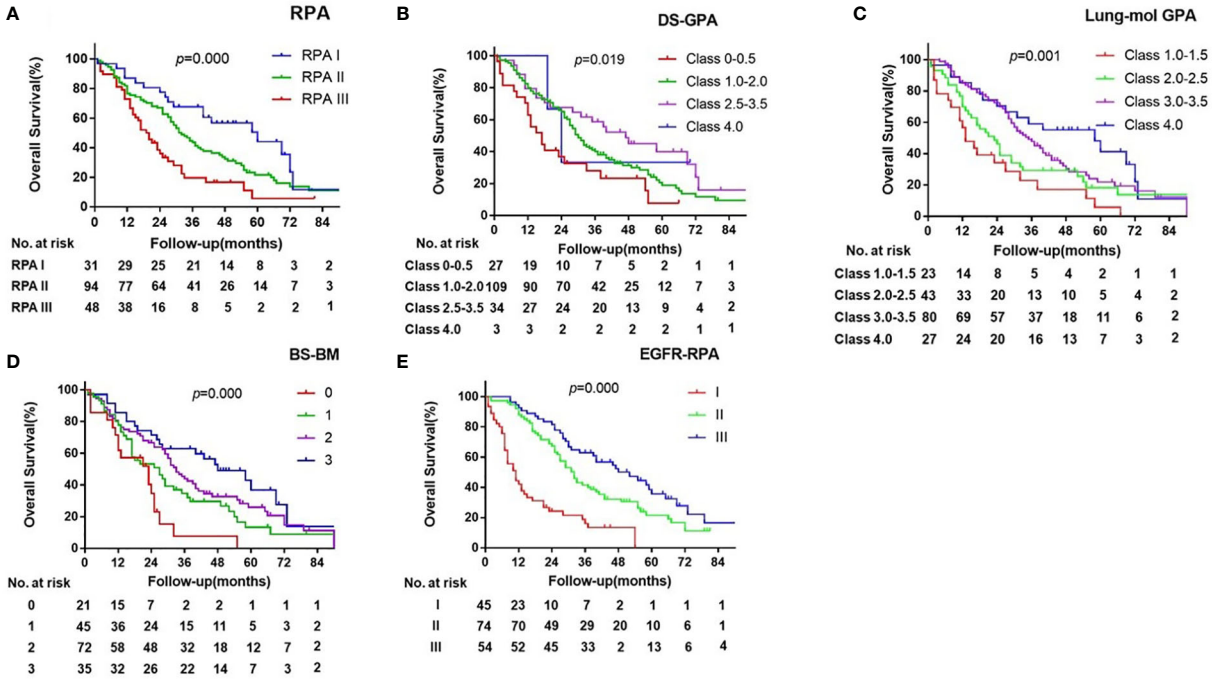

FIGURE 1
Kaplan-Meier Curves of overall survival showing Survival by the RPA **(A)**, DS-GPA **(B)**, BS-BM **(C)**, lung-mol GPA **(D)** and EGFR-RPA **(E)** for EGFR-mutated lung cancer BM.

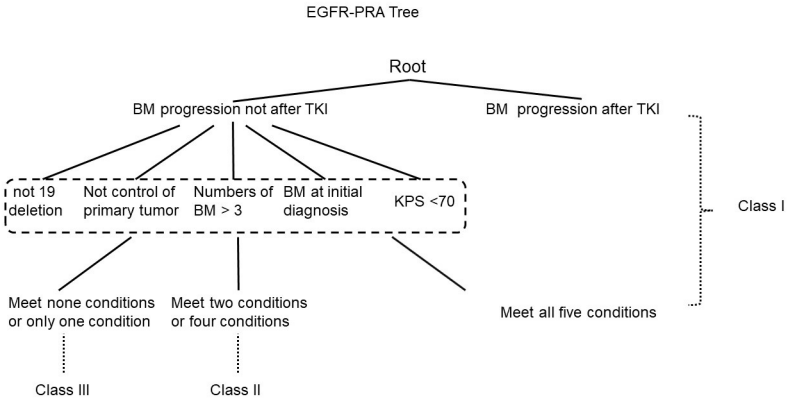

FIGURE 2
Recursive tree for the new specific prognostic system for epidermal growth factor receptor-mutated lung cancer brain metastases.

factors for OS in real-world practice. Moreover, our finding confirms that BM progression after TKI presented significantly worse outcome, with a median survival of only 10 months. Therefore, it was necessary to establish a new prognostic index specific for patients with *EGFR*-mutated NSCLC who developed BM and BM progression after TKI should be brought into the index. Compared with the previous models of BM, the new prognostic system (*EGFR*-RPA) can accurately classify or categorize patients according to their prognosis, which can be used to determine optimal and personalized management of patients with *EGFR*-mutated NSCLC who develop BM.

Currently, the scoring systems for BM include RPA, BS-BM, DS-GPA and Lung-mol GPA. The differences between them mainly existed in the selection and management of the prognostic factors. The selection of prognostic factors was based on population differences selected at the time of establishment of each scoring system. KPS plays a decisive role in RPA scoring system. The prognostic factors were equivalent in BS-BM, DS-GPA, and lung-mol GPA, and patient outcomes were stratified by scoring methods. The new model differs from the previous model such that age was not an independent prognostic factor, which is consistent with the results of a study conducted on Chinese patients with BM from

TABLE 3 AUC of each scoring model to predict 1 year survival.

Scores	AUC	95% CI	*P* value
RPA	0.565	0.466 - 0.665	0.215
DS-GPA	0.572	0.466 - 0.677	0.174
Lung-mol GPA	0.641	0.538 - 0.744	0.007
BS-BM	0.585	0.483 - 0.687	0.106
EGFR-RPA	0.781	0.693 - 0.868	0.000

AUC, Area under the curve; RPA, Recursive partitioning analysis; DS-GPA, Diagnosis specific graded partitioning analysis; lung-mol GPA, lung-molecular graded prognostic assessment; BS-BM, Basic score for brain metastases; EGFR-RPA, EGFR-recursive partitioning analysis; CI, Confidence interval.

TABLE 4 Predictive value analyses of the 5 scoring systems (C-index).

Score	Classes	No. of patients	C-index
RPA	I/II/III	31/94/48	0.603
DS-GPA	0-0.5/1.0-2.0/2.5-3.5/4.0	27/109/34/3	0.569
Lung-mol GPA	1.0-1.5/2.0-2.5/3.0-3.5/4.0	23/43/80/27	0.613
BS-BM	0/1/2/3	21/45/72/35	0.595
EGFR-RPA	I/II/III	45/74/54	0.671

RPA, Recursive partitioning analysis; DS-GPA, Diagnosis specific graded partitioning analysis; lung-mol GPA, lung-molecular graded prognostic assessment; BS-BM, Basic score for brain metastases; EGFR-RPA, EGFR-recursive partitioning analysis.

EGFR-mutated lung cancer (14). We speculated that older patients could tolerate targeted therapy well and, thus, benefit from SRS. Further, Sperduto et al. (15) observed that patients with BM from *EGFR*-mutated NSCLC presented with significantly different survival prognosis with different genetic status, thus, introducing *EGFR* gene mutation status and establishing the lung-mol GPA scoring system. However, the type of *EGFR* mutation was not distinguishable in this system. It has been confirmed that *EGFR*-mutated NSCLC is a genetically heterogeneous disease (16). The most common *EGFR* mutations (exon 19 deletion or L858R mutations) predict sensitivity to *EGFR* TKIs. However, patients with exon 19 deletions demonstrate improvement in OS and progression-free survival (PFS) compared to those harboring the L858R mutations following treatment with first-generation *EGFR* TKIs (17). Additionally, 10% of the patients have an uncommon *EGFR* mutation and are less responsive to *EGFR*-TKI therapy compared to the patients with either of the common mutations (18). In our study, the *EGFR* mutation subtype was an independent predictor for prognosis stratification. Finally, focusing on the *EGFR*-mutated NSCLC BM system, this study observed that BM resistance after TKI, which was not accounted for in the previous BM scoring systems, could identify the patients with the worst outcomes.

In this study, BM progression after TKIs was extremely poor prognostic factor for EGFR-RPA for patients with EGFR mutations.BM progression after TKIs belongs to metachronous BM. However, previous studies (19, 20) and our study have demonstrated that patients with metachronous BM have a better prognosis compared to patients who subsequently develop brain metastases. Most importantly, the other validated prognostic indices, such as KPS, *EGFR* mutation type, control of primary tumor, and the number of BM, were similar between groups with and without BM progression after TKI. The current findings suggest that the poor OS observed in patients with BM after TKI is not secondary to selection bias or differences between patient cohorts, albeit due to the prognostic factor itself. Similarly, Kimberly et al. (21)reported that patients treated with TKI prior to BM diagnosis presented worse outcomes than patients who did not receive targeted therapy prior to BM diagnosis (OS: 9 versus 19.6 months). However, unlike the current study in which the groups included 173 patients and the median follow-up was 67 months, only 54 patients were evaluated with a median follow-up at 8.6 months.

In this study, the second-line treatments for patients with BM progression after TKIs included bevacizumab combined with chemotherapy, Osimertinib targeted therapy, and salvage brain RT. In general, the traditional chemotherapeutic agents used to treat NSCLC do not cross the blood-brain barrier (BBB); Therefore, their effect on CNS metastases is limited (22). Recently, Wu (23) observed that the T790 mutation showed low consistency between cerebrospinal fluid (CSF) and plasma in the study of CSF genotyping in *EGFR*-mutated NSCLC, which could explain the poor response to Osimertinib in patients with T790 mutations detected in plasma. In this study, patients with BM progression after TKIs were treated with salvage RT, and the effect was poor. Performing RT for BM after TKI resistance worsened the occurrence of cerebral radiation necrosis in patients treated with TKIs (24).This may also be one of the reasons for the poor survival rate. Therefore, the presence of BM after TKIs indicates drug resistance, and currently, there is currently a lack of effective treatment.

Despite significant results, our study had limitations. First, the study had the limitations inherent to a retrospective analysis. Second, the potential toxicities associated with RT and their impact on the quality of life were not assessed. Last, all the patients received first- or second-generation *EGFR*-TKIs, but did not receive third-generation TKIs, which have a greater ability to penetrate the BBB than that of

first- or second-generation *EGFR* TKIs (25)and could reduce the risk of CNS progression versus standard *EGFR*-TKI (13).

5 Conclusions

In conclusion, this study presented that BM progression after TKI and *EGFR* mutation type were specific prognostic factors for *EGFR*-mutated lung cancer BM. The new index, whose ROC and C-index were better than those of previous indices, was more prognostic and divisive than the previous indices. According to the *EGFR*-RPA index, the worst median survival was 10 months, whereas the best median survival was 52 months.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Ethics statement

The study was conducted according to the Helsinki Declaration and the study protocol was approved by the Ethics Review Board of the Fudan University Shanghai Cancer Center and the People's Hospital Affiliated to Jiangsu University. Written informed consent from the patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

X-WF provided direction and guidance throughout the preparation of this manuscript. L-HZ wrote and edited the manuscript. L-HZ, LS, T-TN, and Y-QL collected and prepared the related papers. X-WF, L-HZ, K-LW, and C-YW reviewed and made significant revisions to 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1093084/full#supplementary-material

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Real-world risk of brain metastases in stage III non-small cell lung cancer in the era of PET and MRI staging

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Objective: The 2-year incidence of brain metastases (BrMs) in stage III non-small lung cell cancer (NSCLC) has been estimated to be around 30%. However, recent clinical trials have demonstrated considerably lower BrMs rates in this patient population. In this study, we aimed to review the real-world incidence, surveillance, and treatment patterns of BrMs in stage III NSCLC.

Materials and methods: Using a retrospective single-center study design, we identified patients with stage III NSCLC who received radiation with curative intent over a 10-year period. Outcome variables included BrMs incidence, overall survival (OS), and survival from date of BrMs. Additionally, we assessed patterns of BrMs surveillance in stage III NSCLC and treatment.

Results: We identified a total of 279 stage III NSCLC patients, of which 160 with adequate records were included in the final analyses [adenocarcinoma (n = 96), squamous cell carcinoma (n = 53), other histology subtype (n = 11)]. The median OS for the entire cohort was 41 months (95% CI, 28-53), while the median time from BrMs to death was 19 months (95% CI, 9-21). Twenty-three patients (14.4%) received planned surveillance brain MRIs at 6, 12, and 24 months after completion of treatment. The remaining 137 patients (85.6%) received brain MRIs at systemic recurrence (restaging) or when neurologically symptomatic. A total of 37 patients (23%) developed BrMs, with a 2-year cumulative BrMs incidence of 17% (95% CI, 11-23). A higher incidence of BrMs was identified in patients with adenocarcinoma relative to those with squamous cell carcinoma ($p < 0.01$). Similarly, a higher 2-year BrMs incidence was observed in patients who received planned surveillance brain MRI relative to those who did not, although statistical significance was not reached. Stereotactic radiosurgery (SRS) treated 29 of BrMs patients (78.4%) and was preferred over WBRT, which treated only 3 patients (8.1%).

Conclusions: At our center, BrMs incidence in stage III NSCLC patients was lower than historically reported but notably higher than the incidence described in recent clinical trials. Routine BrMs surveillance potentially allows earlier detection of asymptomatic BrMs. However, asymptomatic BrMs were mostly detected on restaging MRI at the time of recurrence.

KEYWORDS

non-small cell lung cancer, brain metastases, incidence, surveillance, MRI brain

Introduction

Lung cancer is the leading cause of cancer death worldwide, accounting for 23% and 24% of all deaths by cancer in females and males respectiely (1). Non-small cell lung cancer (NSCLC) accounts for the majority of cases, constituting approximately 76% of all lung cancers (2). Patients with stage III NSCLC have no brain metastases (BrMs) at the time of diagnosis but are at high risk for developing BrMs during their disease course. Despite recent treatment advances, BrMs are a common manifestation of recurrence in stage III NSCLC and often associated with a decreased quality of life and poor prognosis (3–5). The incidence of BrMs in stage III NSCLC has historically been estimated to be around 30% at 2-years (4, 6). However, recent clinical trials have demonstrated considerably lower BrMs rates in enrolled stage III NSCLC patients. Specifically, the PACIFIC trial (PD-L1 inhibitor, Durvalumab vs. placebo for stage III NSCLC) reported a BrMs rate of 11.8% in the placebo arm and 6.3% in the treatment arm at a median follow-up period of 25.2 months (7). It is worth noting however that in this multi-center international clinical trial, fewer brain magnetic resonance imaging (MRI) may have possibly been performed relative to standard practice in US-based health care systems.

With recent advances in diagnostic imaging and rapid accessibility to brain MRI, the true incidence of BrMs in stage III NSCLC in a real-world (outside the strictly controlled clinical trials) setting remains unclear. Given that up to one third of stage III NSCLC patients often develop BrMs over their disease course, our local practice has evolved to performing surveillance brain MRI at 6, 12, and 24 months after completion of initial treatment. Nonetheless, the support for routine asymptomatic BrMs surveillance in this patient population remains to be demonstrated.

In this study, we aimed to determine the real-world incidence of BrMs in stage III NSCLC and analyze the surveillance and treatment patterns of BrMs in this patient population.

Materials and methods

Patients and data collection

In this single-center retrospective study, we identified 279 patients who were seen at the Stanford Cancer Center from 2008-2018 and had a confirmed diagnosis of stage IIIA/B/C NSCLC (AJCC 8th edition staging criteria). All patients whose treatment plan included radiation with curative intent were included, regardless of treatment completion. The following information was collected: age at diagnosis of stage III NSCLC, diagnostic imaging findings [including computerized tomography (CT) and whole-body positron emission tomography (PET) scans, and brain MRI], pathology results, disease staging, radiation, and treatment plans [including whether prophylactic cranial irradiation (PCI) was utilized for BrMs prevention], and available follow-up visit information within the prior 6 months, or date of death. A total of 160 stage III NSCLC had complete data and were included in the final analyses of this study. Patients who completed treatment and surveillance elsewhere, did not have available follow-up data, or had a second primary cancer with high potential for brain metastatic disease (e.g., triple negative breast cancer), were excluded (see Figure 1).

Ethics

This retrospective study was approved by Stanford University IRB (reference: IRB-44962).

Data and statistical analysis

The following time to event outcomes were assessed and analyzed for the entire cohort as well as for each NSCLC histology subtype. The time to overall survival (OS) outcome was summarized using Kaplan-Meier curves. The time to OS was defined as the time from date of diagnosis until death from any cause, and patients who were still alive at the completion of analysis were censored at the date of the last follow up. The time to BrMs outcome was analyzed using competing risk methods and summarized using cumulative incidence curves. Time to BrMs was defined as the time from date of diagnosis until date of BrMs with death as a competing event. Patients who experienced neither BrMs nor death were censored at the date of last follow up. The cumulative incidence of BrMs was calculated over a 2-year period. The OS from date of BrMs was defined as the time from date of BrMs until death from any cause, and patients who were still alive

were censored at the date of the last follow up. The "number needed to scan" to detect asymptomatic BrMs was calculated as the total number of patients needed to get surveillance brain MRI to detect asymptomatic BrMs for the entire cohort and for patients with adenocarcinoma subtype (the most common histopathology subtype).

Descriptive statistics were utilized to summarize the characteristics of the study cohort. Chi-square tests were applied to assess outcome differences between histology subtypes. All tests were two-sided with an alpha level of 0.05. All analyses were performed in SAS v9.4 (SAS Institute Inc, Cary, NC).

Results

A total of 160 patients (93 male and 67 females) with stage III NSCLC were included in the final analyses of this study, see Figure 1. Baseline characteristics of the study cohort are described in Table 1. The median age at diagnosis of stage III NSCLC was 67 years (range, 34-90 years). Adenocarcinoma was the most common histopathology subtype and was identified in 96 patients (60%), with squamous cell carcinoma in 53 patients (33%). The remaining 11 patients (7%) had other histology subtypes, including large cell and neuro-endocrine tumor. Based on staging work-up, 90 patients (56%) had stage IIIA, 67 patients (42%) had stage IIIB, and 3 patients (2%) had stage IIIC.

The median OS from time of diagnosis for the entire cohort was 41 (95% CI, 28-53) months. In patients with adenocarcinoma, the median OS was 51 (95% CI, 36-68) months. Meanwhile, the median OS was 22 (95% CI, 14-43) months for those with squamous cell carcinoma and 21 (95% CI, 10-NR) months for those with other histology subtypes. The higher OS in patients with adenocarcinoma relative to patients with squamous cell carcinoma was statistically significant ($p < 0.01$; see Figure 2).

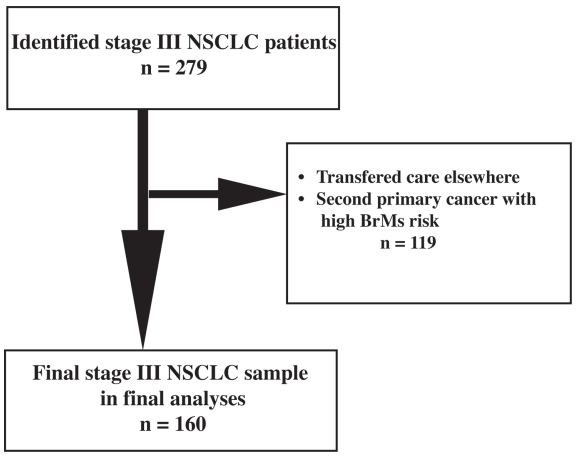

FIGURE 1
Flowchart indicating the selection process of the final study sample of stage III NSCLC. BrMs: brain metastases.

Prophylactic cranial irradiation and surveillance for BrMs in stage III NSCLC

Of the entire cohort, only two patients (1%) received PCI at an outside institution prior to establishing care at our cancer center. Neither of these two patients developed BrMs. Institutionally, we offer surveillance brain MRI at 6, 12, and 24 months after completion of treatment. Twenty-three patients (14.4%) received planned surveillance brain MRI to screen for asymptomatic BrMs. For the remaining patients (n = 137, 85.6%), in accordance with current guidelines (8, 9), no planned BrMs surveillance was carried out. Instead, brain MRI was only obtained at systemic recurrence for restaging or on development of neurological symptoms.

Incidence of BrMs in stage III NSCLC

A total of 37 patients (23%) developed BrMs. Of those, 20 (54.1%) were asymptomatic. On initial BrMs identification, 18 patients (48.7%) had one brain metastasis. Meanwhile, 8 patients (21.6%) had 2-3 BrMs and 11 (29.7%) had >3 BrMs. The estimated 2-year cumulative incidence of BrMs was 17% (95% CI, 11-23) for the entire cohort, see Figure 3A.

Based on histology subtype, 29 patients with adenocarcinoma (total n = 96; 30.2%) developed BrMs. Of the 53 patients with squamous cell carcinoma, 6 (11.3%) developed BrMs. Meanwhile, in the 11 patients with other histology subtypes, BrMs was identified in 2 (18%) patients. The higher incidence of BrMs in patients with adenocarcinoma [2-year BrMs incidence was 21.5% (13.7-30.4)], compared to squamous cell carcinoma [2-year BrMs incidence was 7.9% (3.5-17.6)], was statistically significant ($p < 0.01$), see Figure 3B.

The median time from BrMs development to death was 19 months (95% CI, 9-21) for all patients. No significant differences were noted in time from BrMs to death between NSCLC histology subtypes (see Table 1).

BrMs treatment modality

In patients with BrMs (n = 37), stereotactic radiosurgery (SRS) was utilized to treat 29 patients (78.4%) and whole brain radiation therapy (WBRT) was used to treat 3 patients (8.1%). The remaining 5 patients (13.5%) were treated with systemic therapies alone, without any form of radiation therapy, and were clinically monitored.

"Number needed to scan" to detect asymptomatic BrMs in stage III NSCLC

Among patients who received no BrMs surveillance (n = 137), 31 developed BrMs (22.6%), 17 of whom were asymptomatic. Meanwhile, among the 23 patients who received surveillance brain MRI, 6 developed BrMs (26.1%), of whom 3 were asymptomatic (see Table 2). There was no statistically significant

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TABLE 1 Demographic and baseline characteristics of included stage III NSCLC patients.

	Number (%)
Gender: number (%)	
Male	93 (58%)
Female	67 (42%)
Age at diagnosis: median [range] in years	67 [34-90] years
Stage: number (%)	
IIIA	90 (56%)
IIIB	67 (42%)
IIIC	3 (2%)
Histology subtype: number (%)	
Adenocarcinoma	96 (60%)
Squamous cell carcinoma	53 (33%)
Other (including large cell and neuro-endocrine tumor)	11 (7%)
Received PCI: number (%)	
Yes	2 (1%)
No	158 (99%)
Received surveillance brain MRI: number (%)	
Yes	23 (14.4%)
No	137 (85.6%)
Patients who developed BrMs: number (%)	
All histology subtypes	37 of 160 (23%)
Adenocarcinoma	29 of 96 (30%)
Squamous cell carcinoma	6 of 53 (11%)
Other histology types	2 of 11 (18%)
Symptomatic	17 (45.9%)
Asymptomatic	20 (54.1%)
Number of BrMs	18 (48.7%)
1	8 (21.6%)
2-3	11 (29.7%)
>3	
OS: median (95% CI) in months	
All histology subtypes	41 (28-53)
Adenocarcinoma	51 (36-68)*
Squamous cell carcinoma	22 (14-43)
Other histology types	21 (10-NR)
Time from BrMs to death: median (95% CI) in months	
All histology subtypes	19 (9-21)
Adenocarcinoma	20 (7-21)
Squamous cell carcinoma	16.5 (1-NR)
Other histology types	64.5 (15-NR)

BrMs, brain metastases; NR, not reached; MRI, magnetic resonance imaging; OS, overall survival; PCI, prophylactic cranial irradiation. *p < 0.01 (adenocarcinoma vs. squamous cell carcinoma subtype).

difference in the incidence of BrMs between patients who received BrMs surveillance and those who did not. However, a trend of higher 2-year incidence of BrMs was noted in those received BrMs surveillance [2-year BrMs incidence was 28.5% (11.1-48.9)], compared to patients who did not [2-year BrMs incidence was 15.0% (9.6-21.7)], indicating earlier detection of BrMs (see Table 2).

Based on the total number of patients who did not develop BrMs (n = 123), the number of patients needed to receive surveillance brain MRI scans to detect 1 asymptomatic BrMs is 7 for all histology subtypes.

Focusing on adenocarcinoma patients (the most common histology subtype in stage III NSCLC, n = 96), 79 (82.3%) received no BrMs surveillance. Meanwhile, 17 (17.7%) patients received surveillance brain MRI according to our local practice. Among adenocarcinoma patients who received no BrMs surveillance (n = 79), 25 developed BrMs (31.6%), 13 of whom were asymptomatic. In the 17 adenocarcinoma patients who received surveillance brain MRI, 4 developed BrMs (23.5%), of whom 1 was asymptomatic (Table 3). Based on the total number of adenocarcinoma patients who did not develop BrMs (n = 67), the number of adenocarcinoma patients needed to receive surveillance brain MRI scans to detect 1 asymptomatic BrMs is 5.

Discussion

In this single-center study, we assessed the real-world incidence of BrMs in stage III NSCLC in the modern era of cancer staging and rapid access to advanced diagnostic imaging, including whole body PET/CT scans and brain MRI. The estimated 2-year incidence of BrMs in stage III NSCLC patients treated at our center was 17%. This estimated BrMs incidence is lower than historically described (4). Yet, it is remarkably higher than that reported in more recent clinical trials, including the PACIFIC trial (7). This new evidence of BrMs incidence in stage III NSCLC highlights the considerable difference between outcomes and surveillance practices in the real-world setting versus clinical trials settings.

Due to increasing life expectancy of cancer patients and advances in diagnostic imaging, the incidence of BrMs in all cancer patients (in particular asymptomatic BrMs) is generally increasing (4). Interestingly, based on more recent evidence, the incidence of BrMs in stage III NSCLC has however been declining over time (7). This has been attributed to advances in NSCLC treatments and availability of newer and targeted therapies that has better CNS penetration, such as next-generation tyrosine kinase inhibitors (TKIs), small molecules like pemetrexed, and checkpoint inhibitors (10, 11). More importantly, better access to neuroimaging in recent years has allowed earlier detection of asymptomatic BrMs at initial disease staging, minimizing the possibility of patients being classified as having stage III NSCLC at the time of diagnosis.

Although our sample was likely enriched for targetable mutations (such as EGFR and ALK rearrangements), it remains a close representation of the real-world stage III NSCLC patient population treated at specialized cancer centers, with adenocarcinoma and squamous cell carcinoma histology subtypes comprising 60% and 33% of our cohort respectively. The incidence

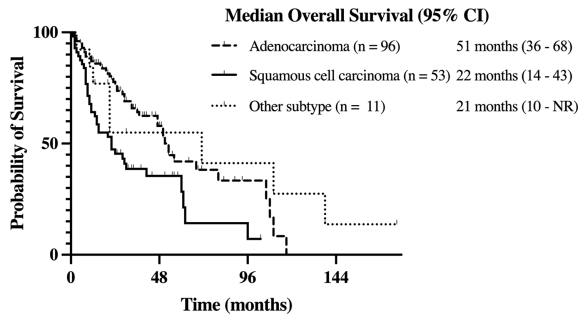

FIGURE 2
Kaplan-Meier estimate of overall survival (OS) in stage III NSCLC patients with adenocarcinoma, squamous cell carcinoma, and other history types.

of BrMs was higher in patients with adenocarcinoma relative to other histology subtypes, with 30% of our patients with adenocarcinoma subtype developing BrMs. This is in alignment with the evidence of higher BrMs rates in adenocarcinoma subtype, especially in those harboring targetable mutations, such as EGFR and ALK rearrangements (12–16). Likewise, the significantly higher OS we observed in our patients with adenocarcinoma compared to squamous cell carcinoma histology subtype is consistent with recent literature (12, 17).

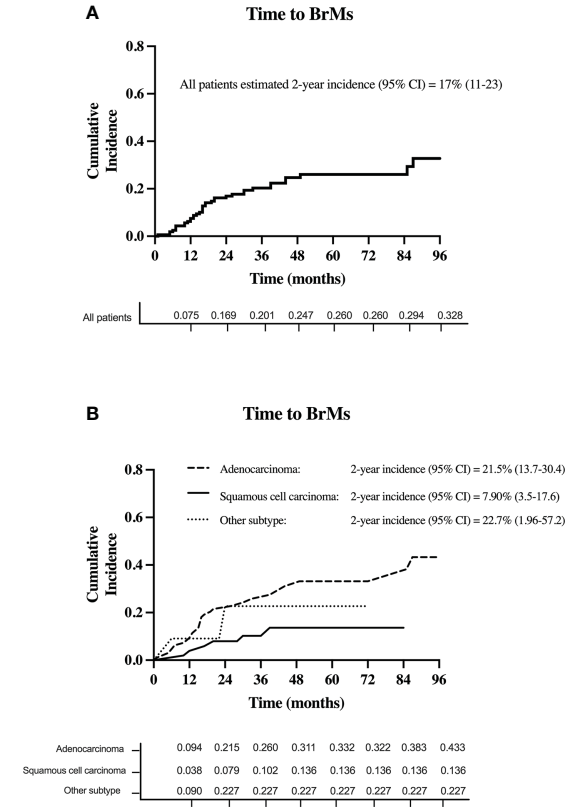

FIGURE 3
The cumulative incidence of BrMs in **(A)** all patients with stage III NSCLC and **(B)** for each stage III NSCLC histology subtype.

The clinical practice of BrMs surveillance in stage III NSCLC remains controversial. Surveillance brain MRIs are not explicitly recommended and current stage III NSCLC guidelines, including the 2020 National Comprehensive Cancer Network (NCCN) guidelines (9), recommend obtaining brain MRI to detect asymptomatic BrMs only at the time of initial diagnosis or systemic recurrence. It is unclear however how closely physicians adhere to these guidelines in the real-world setting (18). According to our local practice, 14.4% of (n = 23) of our stage III NSCLC patients received planned surveillance brain MRIs to screen for asymptomatic BrMs at 6, 12, and 24 months after completion of treatment. Most patients (85.6%) however were monitored clinically and only received surveillance brain MRI at the time of systemic recurrence as part of their re-staging process. The frequency of asymptomatic BrMs among patients who received BrMs surveillance was 13%, which was nearly equivalent to the frequency of asymptomatic BrMs among patients who did not receive surveillance brain MRI (12.4%). Although this is not surprising, a trend of earlier BrMs detection was noted in those who received BrMs surveillance. Interestingly, 50% of the patients who developed BrMs while undergoing BrMs surveillance required an additional brain MRI due to developing new neurological symptoms, indicating that regular surveillance was not entirely successful in detecting asymptomatic BrMs early. Overall, we estimated that 7 patients with stage III NSCLC "needed to be scanned" to detect one asymptomatic BrMs. Based on these observation, routine BrMs surveillance is likely to be challenged by its lack of superiority to clinical monitoring and potential cost-ineffectiveness, especially in less resourced areas and low-income countries. However, with ready access to, and growing affordability of, brain MRI, it can be argued that early BrMs detection is very pertinent to treatment planning and delaying BrMs-related complications. The benefit of BrMs surveillance might thus be justifiable in selected stage III NSCLC patients, such as patients with adenocarcinoma, those at high-risk of recurrence (including those harboring targetable mutations such as EGFR and ALK), and those with programmed cell death ligand 1 (PD-L1) expression on less than 1% of tumor cells (19, 20).

Recently, our group reviewed the landmark trials that investigated PCI in stage III NSCLC (10). Despite the fact that all, but one (21), of these trials were able to demonstrate a significant reduction in the incidence of BrMs in stage III NSCLC with PCI (22–28), they consistently demonstrated lack of significant benefit on OS. In the 2019 update to the Radiation Therapy Oncology Group (RTOG) 0214 phase 3 randomized clinical trial (RCT), the largest RTC of PCI in stage III NSCLC, the 5-year incidence of BrMs was 28.3% in the observation group compared to 16.7% in the PCI group (HR 0.43, p=0.003) (27). Despite this seemingly promising result, the findings unfortunately did not translate to an improvement in OS (neither 5-year, 10-year, nor median OS was significantly different between the two groups) (27). Interestingly, compared to our study, the 2-year incidence of BrMs in the NRG Oncology-RTOG 0214 Phase 3 RCT was 24.3% in the observation group (relative to 10.9% in the PCI group) (27). The higher 2-year BrMs incidence in the trial's observation group likely reflects the study accrual period between 2002 and 2007 and does not appear entirely consistent with

TABLE 2 Comparisons of stage III NSCLC patients who received surveillance brain MRI and those who did not.

	No Surveillance brain MRI (n = 137)	Surveillance brain MRI (n = 23)
Developed BrMs: number (%)		
Yes	31 (22.6%)	6 (26.1%)
No	106 (77.4%)	17 (73.9%)
Incidence of BrMs at 2-years: % (95% CI)	15.0% (9.6-21.7)	28.5% (11.1-48.9)^{ns}
BrMs: number (%)		
Symptomatic	14 (45.2%)	3 (50.0%)
Asymptomatic	17 (54.8%)	3 (50.0%)

BrMs, brain metastases; ns, non-significant.

contemporary evidence of lower BrMs incidence in stage III NSCLC or the findings of the current study (7). In our cohort, only 2 patients (1%) received PCI at other institutions prior to establishing care at our center. Neurological complications associated with WBRT are well documented and range from long-term cognitive dysfunction, gait and motor disturbance, and urinary incontinence (29, 30). These detrimental neurological complications and the lack of significant benefit on survival outcomes argue against PCI application in stage III NSCLC.

This study has several limitations. Firstly, this is a retrospective single center study and thus the results are subject to bias. Compared to clinical trials settings, however, we believe this retrospective study was less limited by the strict inclusion/exclusion criteria often applied in clinical trials and thus had more potential to estimate a closer real-world incidence of BrMs. For example, patients participating in the PACIFIC trial successfully completed chemoradiation without disease progression prior to enrollment, whereas patients in this cohort were included if their treatment plan had curative intention, regardless of completion. Secondly, molecular testing (such as EGFR/ALK mutations status) was not available for all patients in our cohort and thus this relevant information was not incorporated in our analyses. Future studies are strongly encouraged to include the results of targetable mutations testing when assessing the association between stage III NSCLC BrMs predisposition and clinical outcomes. Thirdly, we considered the fact that the American Joint Committee on Cancer (AJCC) Classification of NSCLC underwent edition changes during our study period which may affect classification of stage III NSCLC patients. Although, we do not believe this had largely impacted our data as most of the study period comprised the 7th edition classification system. Lastly, given that our center is a major academic center, there is a possibility that most of the patients who continued long-term follow-up represent a population of patients that are often doing clinically better than those who were lost to follow-up.

Conclusion

In this retrospective single-center study, the 2-year incidence of BrMs in stage III NSCLC was 17%. This BrMs incidence is lower than historically reported, but higher than that reported in recent clinical trials. Although, we found surveillance brain MRI not superior to clinical monitoring in detecting asymptomatic BrMs, a trend of earlier BrMs detection was noted. Therefore, surveillance brain MRI may still be appropriate in selected subgroups of stage III NSCLC patients, such as patients with adenocarcinoma and those with high risk of recurrence. At our institution, PCI is not performed for stage III NSCLC and there is a clear preference to deliver SRS over WBRT to treat BrMs.

TABLE 3 Comparisons of stage III NSCLC patients with adenocarcinoma subtype who received surveillance brain MRI and those who did not.

	No Surveillance brain MRI (n = 79)	Surveillance brain MRI (n = 17)
Developed BrMs: number (%)		
Yes	25 (31.6%)	4 (23.5%)
No	54 (68.4%)	13 (76.5%)
BrMs: number (%)		
Symptomatic	12 (48.0%)	3 (75.0%)
Asymptomatic	13 (52.0%)	1 (25.0%)

BrMs, brain metastases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SA, TL, RK, KT, BL, JN, SN: Conceptualization, methodology, writing, investigation, editing. RE, MD, SS, EP, AC, LV, HW, SP, KR: Writing, visualization, diting. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Publisher's note

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Clinical features and prognosis of pulmonary enteric adenocarcinoma: A retrospective study in China and the SEER database

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Objective: Pulmonary enteric adenocarcinoma (PEAC) is a rare subtype of pulmonary adenocarcinoma that lacks effective treatment. The purpose of this research was to investigate the clinical characteristics, treatment, and prognosis of PEAC, as well as the impact of relevant factors on survival, thus providing a reference for the clinical management of patients with this disease.

Methods: For this study, we gathered clinical data from 26 patients with PEAC in the Affiliated Cancer Hospital of Zhengzhou University from June 2014 to June 2021. We used SEER*Stat software V8.3.5 to download the PEAC patients from the Surveillance, Epidemiology, and End Results (SEER) database. In total, 20 patients were identified. Clinical data, including general information, imaging findings, and treatment protocols, were obtained, together with a follow-up of disease regression. The relevant clinical data were then analyzed.

Results: It included 12 males and 14 females out of 26 patients from China, whose mean age was (62.73 ± 11.89) years; 20 were in the lower lung, 11 were stage I-II, and 15 were stage III-IV. Five had EGFR mutations, and four had KRAS mutations. In terms of treatment, patients with stage I-II were primarily treated by surgery, and patients with stage III-IV were treated mostly by chemotherapy. We extended the follow-up date to January 2022. On completion of the follow-up visit, 11 patients died, and the remaining 15 patients survived. The overall survival (OS) of 26 patients was 2.0-76.0 months, while the mean was 53.1 months, and the median OS (mOS) was 38.0 months (95% CI:1.727-74.273). In the case of progression-free survival (PFS) times, it was 2.0-76.0 months, with a mean PFS of 31.0 months and a median PFS (mPFS) of 8.0 months (95% CI:4.333-11.667). The PFS of the 15 patients in stage III-IV was 2.0-17 months, while the mean PFS was 6.5 months and the mPFS was 6.0 months (95% CI:4.512-7.488). Out of the 20 patients identified in the SEER database, the average age was 69.9 years, with 14 males and 6 females. Of these patients, 8 were diagnosed with stage I-II, while the remaining 11 were diagnosed with stage III-IV. 10 underwent surgery, 4 received radiation therapy, and 9 received chemotherapy. The mean OS of the 20 patients was 67.5 months, mOS was 28.0 months (95% CI: 9.664- 46.336). For

patients diagnosed with stage III-IV, the mean OS was 14.8 months and mOS was 20 months (95% CI: 4.713-35.287).

Conclusion: PEAC is rare, and the prognosis is determined mainly by the stage; patients who undergo surgery in stage I-II have a better prognosis.

KEYWORDS

pulmonary enteric adenocarcinoma, treatment, prognosis, survival analysis, real word data

1 Introduction

Pulmonary enteric adenocarcinoma (PEAC) is a rarely seen pathological subtype of lung adenocarcinoma. Tsao and Fraser defined PEAC creatively for the first time in 1991 (1). The disease has subsequently been reported and researched by scholars in clinical practice. In 2015, the World Health Organization classified pulmonary enteric adenocarcinoma as one of the types of lung adenocarcinoma. It is defined as a primary lung tumor that shares histological and immunohistochemical features with colorectal cancer. The key diagnostic point of PEAC is to exclude gastrointestinal metastases, which should first contain >50% of the features similar to the cellular structure of colon adenocarcinoma, that is, tall columnar cells, with eosinophilic cytoplasm, formed into irregularly shaped glands, part of which may comprise necrotic material. Secondly, for at least one of the immunohistochemical markers of intestinal differentiation (CDX2: caudal type homeobox 2, CK20: cytokeratin 20, or MUC2: mucin 2), it is positive. It often expresses CDX2 and cytokeratin 7 (CK7), in contrast to thyroid transcription factor-1 (TTF-1) and CK20, which are usually negative, but any combination is possible (2). The morbidity of PEAC is not high; hence, most of the studies on the disease have been case reports over the years, leaving its prognosis unclear. There are no specific treatment guidelines for PEAC, and the current treatment strategy is similar to that for non-small cell lung cancer (NSCLC). In the early stages, surgery is the mainstay of treatment, while chemotherapy and radiotherapy may be used in the later stage. However, targeted therapy and immunotherapy are less commonly discussed.

In this study, we summarized and analyzed the clinical features and prognostic factors of this disease in our study center and the SEER database, seeking to improve the understanding and treatment of PEAC.

2 Materials and methods

2.1 Study population

Twenty-six patients were recruited for the project between June 2014 and June 2021.

2.1.1 Inclusion criteria

Patients with PEAC diagnosed by pathological examination.

2.1.2 Exclusion criteria

(1) Patients with concurrent primary malignancies of other systems within five years; (2) Combination of refractory or other serious life-threatening diseases. The research was reviewed and approved by the Ethics Committee of the Cancer Hospital of Zhengzhou University. The written informed consent waived by the ethics committee. Ethical Review No. (2019083002).

This study also collected PEAC patients from the SEER database. The samples were selected by downloading SEER Research Plus Data, 12 Registries, Nov 2021 Sub (1992-2019) from the SEER database using SEER*Stat software V8.3.5. Inclusion criteria: (1) Pathological diagnosis of PEAC (International Classification of Diseases for Oncology ICD-O-8144); (2) Primary focus limited to lung {Site and Morphology. Site recode ICD-O-3/WHO 2008} =' Lung and Bronchus' AND {Site and Morphology. ICD-O-3 Hist/Behav, malignant} =8144/3: Adenocarcinoma, intestinal type'. Twenty patients were included in the study.

2.2 Variable collections

Clinical information of the patient is recorded, including age, gender, tumor site, family history, personal history, clinical symptoms, imaging findings (including tracheoscopy, CT, MRI, PET/CT imaging, etc.), tumor stage, tumor markers, surgical situation, post-operative pathology results, recovery after surgery, treatment programs, as well as disease regression.

2.3 Follow-up

Follow-up was done by reviewing medical records and communicating *via* phone, with a deadline of January 2022 or patient death. Overall survival (OS) is considered to be the time interval from the diagnosis of PEAC to death or the end of follow-up, while progression-free survival (PFS) is considered to be the time to disease progression or the time to the end of follow-up.

TABLE 1 Clinical characteristics of 26 patients from China.

Variables	N=26
Age (years)	62.73 ± 11.89
≤65	15(57.7)
>65	11(42.3)
Gender, n (%)	
Male	12(46.2)
Female	14(53.8)
Clinical symptoms, n (%)	
Coughing and/or coughing up sputum	6(23.1)
Coughing up blood	6(23.1)
Shortness of breath and/or wheezing	4(15.4)
Chest or back pain	4(15.4)
Fever	1(3.8)
No symptom	4(15.4)
Axillary mass	1(3.8)
Family history of cancer, n (%)	8(30.8)
Smoking history, n (%)	6(23.1)
History of alcohol intake, n (%)	4(15.4)
Primary site, n (%)	
Upper lobe	3(11.5)
Middle lobe	1(3.8)
Lower lobe	20(76.9)
Laterality, n (%)	
Left	11(42.3)
Right	14(53.8)
Double-sided	1(3.8)
Clinical stage, n (%)	
Stage I	5(19.2)
Stage II	6(23.1)
Stage III	3(11.5)
Stage IV	12(46.2)
Genic mutation, n (%)	
EGFR	5(19.2)
KRAS	4(15.4)
TP53	2(7.7)
ERBB2	3(11.5)
Peripheral Blood Biomarkers, n (%)	
CEA, (0-4.7ng/mL)	10/18(55.6)
NSE, (0-16.3ng/mL)	6/18(33.3)
CYFRA 21.1, (0-3.3ng/mL)	13/18(72.2)
CA 19.9, (0-27U/mL)	2/2(100)
First-line treatment	
Surgery, n (%)	12(46.2)
Radiation, n (%)	3(11.5)
Chemotherapy, n (%)	19(73.1)
Targeted therapy, n (%)	3(11.5)
Untreated	1(3.8)
Death, n (%)	11(42.3)

EGFR, epithelial growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; TP53, tumor protein p53; ERBB2, human epidermal growth factor receptor 2; CEA, carcinoembryonic antigen; NSE, neuron-specific enolase; CYFRA 21.1, cytokeratin 19 fragment.

2.4 Statistical methods

①The collected clinical data were summarized and statistically described. ②The statistical analysis was carried out using SPSS 26.0 software: the Kaplan-Meier method was selected to compute the median survival, and graph survival curves; the Log-rank test was applied to make comparisons of between-group differences in survival curves, and univariate analysis was performed. A multiple-factor analysis of survival using a Cox proportional risk regression model (Cox model) to compare prognostic influences. For our test, a P value < 0.05 was accepted as statistical significance.

3 Results

3.1 Clinical characteristics

Table 1 summarizes the PEAC data for the 26 patients, including 46.2% males and 53.8% females. Their age range was 46 to 86 years, with a mean age of 62.73 years. The primary tumor was located on the left lung in 11 cases (42.3%), on the right lung in 14 cases (53.8%), in the lower lung in 20 cases (76.9%), as well as in the upper lung in three cases (11.5%). It was observed that a family history of cancer in 8 cases (30.8%), including three cases of direct relatives with a history of lung cancer. There were six cases with a smoking history (23.1%). The initial symptoms included cough, sputum, hemoptysis, shortness of breath or chest pain, etc. Moreover, 11 cases were in stage I-II (42.3%), 15 in stage III-IV (57.7%), while stage IV patients showed mainly lung, bone, liver, distant lymph nodes, and adrenal metastases. Besides, five cases (19.2%) had epidermal growth factor receptor (EGFR) mutation, and four (15.4%) had Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation. In terms of tumor markers, the Carcinoembryonic antigen (CEA) positivity rate was 55.6% within the range of 0.48 - 370.3 ng/mL, the neuron-specific enolase (NSE) positivity rate was 33.3% in the range of 9.99 - 23.46 ng/mL, and the cytokeratin 19 fragment (CYFRA211) positivity rate was 72.2% wide (1.53 - 130 ng/mL). In terms of treatment, 12 cases received first-line surgery, 19 received chemotherapy, two received radiotherapy, and three received targeted therapy.

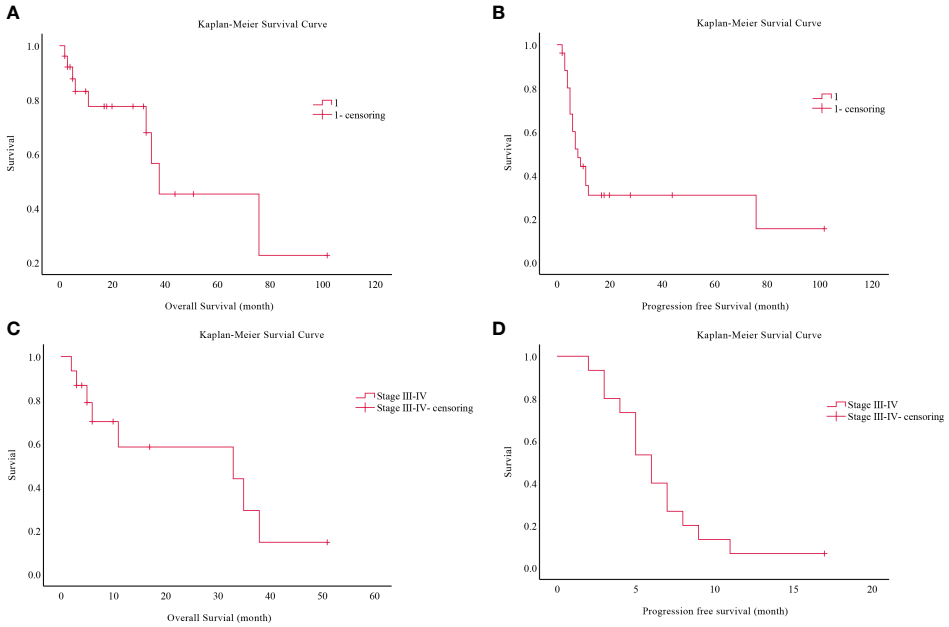

FIGURE 1
Survival analysis of 26 patients from China. **(A)** Overall survival analysis of 26 patients. **(B)** Progression free survival analysis of 26 patients. **(C)** Overall survival analysis of stage III-IV. **(D)** Progression free survival analysis of stage III-IV.

3.2 Prognostic analysis

Eleven of the 26 patients had passed away when the study ended, nine of disease progression, one of pulmonary infection, and one of post-operative bronchial stump fistula Figure 1. Despite this, four cases were lost to follow-up with no OS obtained. The OS for 26 patients was 2.0-76.0 months, with a mean OS of 53.1 months and a median OS (mOS) of 38.0 months (95% confidence interval (CI):1.727-74.273). In addition, PFS was 2.0-76.0 months, the average PFS was 31.9 months, and the median PFS (mPFS) was eight months (95% CI:4.333-11.667). After this, we performed a survival analysis of 15 patients in stages III-IV. We found that OS was 2-38.0months, for a mean OS of 25 months and a mOS of 33 months (95%CI: 0.0-83.085). Meanwhile, PFS was 2-11.0 months, with a mean PFS of 6.5 months and a mPFS of 6 months (95%CI: 4.512-7.488).

TABLE 2 Univariate analysis of PFS.

Variables	n	mPFS	t/χ^2	P	95%CI
Age (years)			1.255	0.263	
≤65	15	11			5.14-16.86
>65	11	7			3.98-10.02
Gender			2.581	0.108	
Male	12	7			3.88-10.11
Female	14	11			0.0-42.78
Family history of cancer			1.74	0.187	
Yes	8	11			0.0-73.83
No	18	1			5.0-8.98
Smoking history			0.321	0.571	
Yes	6	5			0.83-9.17
No	20	8			3.62-12.38

(Continued)

TABLE 2 Continued

Variables	n	mPFS	t/χ^2	P	95%CI
History of alcohol intake			1.197	0.274	
Yes	4	5			–
No	22	8			2.56-13.44
Primary site			0.938	0.333	
Lower lobe	20	6			1.62-10.38
Others	6	9			4.71-13.29
Laterality			0.29	0.865	
Left	11	8			0.45-15.55
Right	14	9			5.00-13.00
Double-sided	1	7			–
Clinical stage			12.603	0.000	
Stage I-II	11	76			0.00-169.26
Stage III-IV	15	6			4.51-7.48
Surgery			9.762	0.002	
Yes	12	76			0.00-170.03
No	14	6			4.19-7.82
Radiation			2.514	0.113	
Yes	3	5			–
No	23	11			6.65-15.35
Chemotherapy			0.114	0.735	
Yes	19	8			4.80-11.20
No	7	76			–

3.3 Univariate and multivariate prognostic analyses

To outline the factors associated with predicting the impact of PFS, we used univariate and multivariate COX regression models (Tables 2, 3). Univariate analysis revealed that two factors, tumor stage and whether the surgery had been operated on, were associated with PFS. Naturally, we added variables with P<0.2 to the multivariate analysis; nevertheless, the differences in all variables were not statistically significant.

3.4 Treatment

Ten of these stage III-IV cases received only chemotherapy as first-line treatment: six of them received pemetrexed + platinum-based regimens, two of them adopted paclitaxel-based + platinum-based protocols, and the remaining two were given gemcitabine + platinum-based chemotherapy. Survival analysis is shown in Figure 2. The mean PFS was 5.8 months with a mPFS of 5 months (95% CI: 1.399-8.601) for patients on the pemetrexed + platinum regimen, while the mean PFS was 10.5 months with a

TABLE 3 Multivariate Cox regression models associated with PFS.

Variables	B	SE	Wald	HR	95%CI	P
Gender	-0.605	0.539	1.259	0.546	0.190-1.571	0.262
Family history of cancer	0.119	0.691	0.030	1.126	0.291-4.362	0.863
Clinical stage	1.030	1.321	0.609	2.802	0.211-37.292	0.435
Surgery	-1.382	1.125	1.510	0.251	0.028-2.275	0.219
Radiation	0.012	0.694	0.000	1.013	0.260-3.948	0.986

mPFS of 4 months (95%CI: -) among patients on the paclitaxel-like + platinum regimen, compared to 8.5 months with gemcitabine + platinum regimen with a mPFS of 8 months (95%CI: -), p=0.446. There was no statistically meaningful difference.

It was also discovered that two cases with EGFR exon 19 mutations in stage III-IV patients, one treated with pemetrexed + carboplatin + bevacizumab + gefitinib with a PFS of six months, the other with six cycles of paclitaxel + carboplatin followed by maintenance treatment with osimertinib, which did not progress by the end of follow-up with a PFS of 17 months.

3.5 Additionally, we analyzed 20 cases screened by the SEER database

Their age ranged between 39 and 86 years, giving a mean age of 69.9 years. A total of 14 of them were male, and six were female. The tumor was situated in the upper lung in nine cases, in the lower lung in eight cases, in the left lung in six, and in the right lung in thirteen. Regarding the tumor stage, eight were in stages I-II, and the remaining 11 were in III-IV. Concerning treatment, 10 underwent surgery, four radiotherapies, and nine chemotherapy (Table 4). We carried out a survival analysis of 20 patients, which suggested a mean OS of 67.5 months and a mOS of 28.0 months (95% CI: 9.664-46.336), yet the deletion rate was 60%, so the conclusions were for reference only. To understand the mortality of late-stage patients, we abstracted the OS of patients with stages III-IV and came up with a mean OS of 14.8 months and a mOS of 20 months (95% CI: 4.713-35.287) (Figure 3).

4 Discussion

In this study, the age range at diagnosis was 46-86 years, with a mean age of 62.73 years for the 26 patients with PEAC and a ratio of 6:7 for males to females. The age at diagnosis ranged from 39 to 86 years in the 20 patients with PEAC in the SEER database, with a mean age of 69.9 years. The ratio of males to females was 7:3. Raffaele Palmirotta et al. (3) identified 295 patients (116 males and 90 females) in articles published up to January 25, 2020. As a result of the analysis, the patients' ages ranged from 25 to 81 years, with a mean of 63.24 years. It indicates that the average age of disease onset in patients is greater in the elderly, and the proportion of men and women with morbidity is unknown due to the small sample size. In the present investigation, the primary tumor occurrence ratio in the left lung to the right lung was 11:14, and that in the lower lung to the upper lung was 20:3. On the other hand, among the 20 patients in the SEER database, the ratio of the left lung to the right lung was 6:13, and that of the upper lung to the lower lung was 9:8. It was similar to the study by Haiyan Li et al. (4), who identified 103 patients with lesions mostly in the right lung tissue and a ratio of right lung lesions to left lung lesions of approximately 66:49. They found that lesions were mainly located in the upper lung, where the lesion sites were 59:40:5 in the upper lung: lower lung: middle lung. Our findings showed that the first symptoms of PEAC patients were cough, sputum, hemoptysis, chest tightness, shortness of breath, or chest pain. Moreover, six patients had a history of smoking. Remarkably, we found that eight of the 26 patients had a family history of cancer, with three having immediate family members who had lung cancer. The case report by Garajová et al. (5) recommended that their immediate family members were also diagnosed with PEAC, whereas the three immediate family members with lung cancer in this study were all deceased, so it is not known whether they were also PEAC or not. Thus, we included an immediate family member with a malignant neoplasm in the univariate analysis of the effect on PFS, although it was not statistically significant (p=0.187).

Five patients in this study had EGFR mutations, four of which were EGFR exon 19 mutations and one exon 21 mutation. KRAS mutation positivity was more frequent than tumor protein p53 (TP53) mutations and human epidermal growth factor receptor 2 (ERBB2/HER2). The most common gene mutations in the ten patients studied by Xie et al. (6) included TP53 (57%, 4/7) and KRAS (57%, 5/7) mutations. Tu, L. F. et al. (7) showed two patients with KRAS mutations, one patient with a

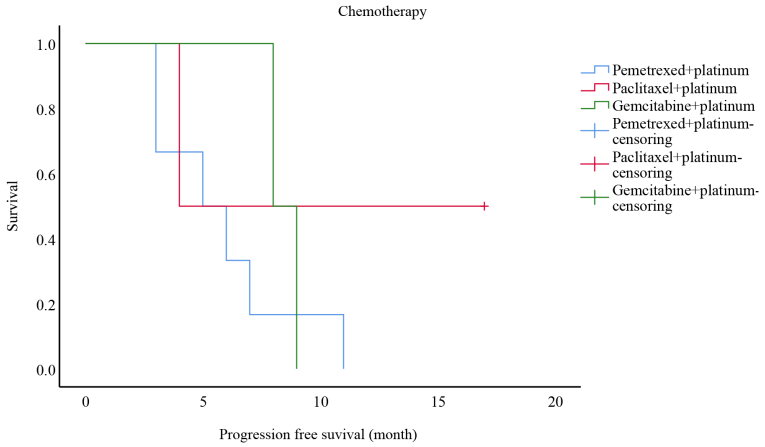

FIGURE 2
Progression free survival analysis of patients with different chemotherapy modalities in stages III-IV from China.

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TABLE 4 Clinical characteristics of 20 patients from SEER database.

Variables	N=20
Age (years)	69.9 ± 10.26
≤65	5(25.0)
>65	15(75.0)
Gender, n (%)	
Male	14(70.0)
Female	6(30.0)
Race	
White	15(75.0)
Black	5(25.0)
Primary site, n (%)	
Upper lobe	9(45.0)
Middle lobe	2(10.0)
Lower lobe	8(40.0)
Unknown	1(5.0)
Laterality, n (%)	
Left	6(30.0)
Right	13(65.0)
Unknown	1(5.0)
Clinical stage, n (%)	
Stage I	6(30.0)
Stage II	2(10.0)
Stage III	4(20.0)
Stage IV	7(35.0)
Unknown	1(5.0)
Surgery, n (%)	10(50)
Radiation, n (%)	4(20.0)
Chemotherapy, n (%)	9 (45.0)

KRAS missense mutation, and the other patient with a BRAC1 nonsense mutation and a KRAS missense mutation. It was also shown in a case report by Shimizu et al. (8) that PEAC carries rare BRAF G469V mutations. Wang et al. (9) discovered EGFR to be a critical driver mutation in PEAC, but its incidence was lower than that of classic lung adenocarcinoma, in contrast to ERBB2 and KRAS, which were more common in PEAC. Jurmeister et al. (10) observed TP53 mutations in 6 out of 7 samples and KRAS mutations in three cases. Jurmeister et al. (11) noted KRAS mutations in nine (60%) of 15 PEAC cases. Nottegar et al. (12) observed EML4-ALK rearrangements in 6/46 (13.0%) PEAC. 1/46 patients with PEAC had mutations in EGFR exon 19 (p.E746_S752) (2.2%), and 28 had the KRAS gene mutation at codon 12 (60.9%). There was no case showing BRAF mutation (0/46). According to Lin et al. (13), ALK/ROS1 point mutations were found in five cases (71.42%, 5/7) and MSH2/MSH6 point mutations in three cases (42.86%, 3/7). In contrast, all nine patients shown by Wang et al. (14) were EGFR and KRAS wild-type. Feng et al. (15) and Zhao et al. (16) found EGFR mutations in 13 (43.3%) of 30 patients, EGFR mutations in three (10.7%) of 28 patients, and KRAS mutations in ten cases 10/25 (40%), respectively. Nottegar et al. (17) assessed eight patients, 1/8 (12.5%) had both PIK3CA mutations and EML4-ALK translocations, while 4/8 (50%) had the KRAS gene mutation at codon 12. In contrast, NRAS, BRAF, and EGFR genes were all wildtypes. Accordingly, the most common mutations in PEAC are EGFR mutations and KRAS mutations, while TP53 mutations and ERBB2 amplifications, EML4-ALK rearrangements, and BRAF G469V mutations are less common.

To better identify the value of serum tumor markers in the diagnosis of PEAC, we examined the levels of tumor markers associated with lung cancer (CEA, NSE, and CYFRA 21.1) and

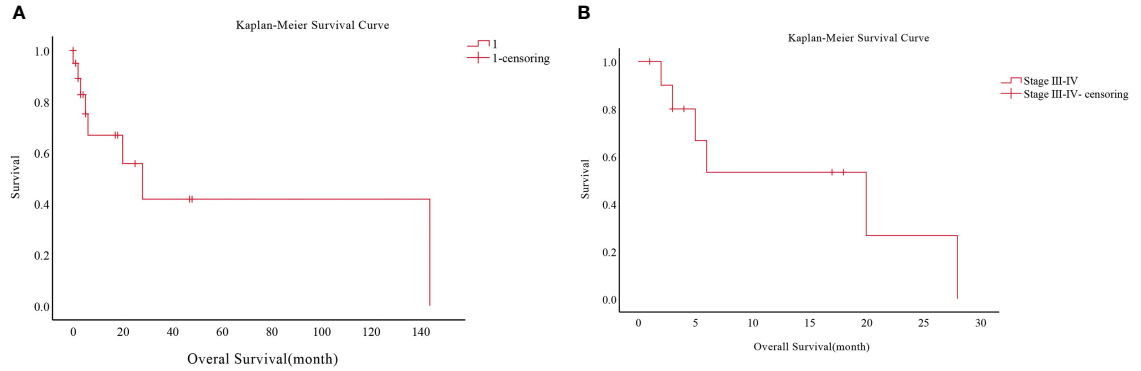

FIGURE 3
Survival analysis of 20 patients from the SEER database. **(A)** Overall survival analysis of 20 patients. **(B)** Overall survival analysis of stage III-IV patients.

the level of colorectal cancer-related tumor marker carbohydrate antigen (CA 19.9). Regrettably, CA 19.9 levels were detected at diagnosis in only two patients in this review, and one of them had serum CA 19.9 levels >1000 ng/ml. The positive rate of CEA and CYFRA 21.1 were more highly expressed than NSE expression in this report. Gu et al. (18) found that the positive rates of tumor markers CEA, CA19.9, and CA125 in PEAC were 71% (10/14), 50% (5/10), and 50% (5/10), respectively. Furthermore, Chen et al. (19) discovered that CEA and CA 19.9 were more abundant in primary cultured PEAC than CYFRA 21.1 and NSE. CA 19.9 was the richest expressed tumor marker, but NSE was barely expressed. CEA, CA 19.9, and CA125 were abnormally elevated in six PEAC cases shown by Tu et al. (7). CA 19.9 and CEA increased markedly over CA125. The highest values of CEA and CA 19.9 were 509 ng/mL and 1449.9 U/mL, separately. Both the NSE and CYFRA 21.1 were all normal. When diagnosing and monitoring lung cancer, physicians should look for CA 19.9 levels and lung cancer-related serum tumor markers. Eleven patients were in stages I-II and 15 patients were in stages III-IV at the time of diagnosis in this study. While 19 early-stage (stage I and II) patients and 9 stage III-IV patients were among the 28 patients with PEAC investigated by Zhao et al. (16). The most prevalent distant metastatic sites in patients with advanced stages were lung, bone, liver, distant lymph nodes, and adrenal metastases (6, 20), whereas skin and pancreatic metastases were rare (21, 22). Feng et al. (15) enrolled three patients (30%) in stages I and II and 27 (90%) in stages III-IV of the 30 patients included. Chen et al. (19) found 12 patients (67%) in early-stage (stage I-II) and 6 (90%) in stages III-IV of the 18 patients with PEAC. In the various small sample studies, the staging percentages at diagnosis were not found to have a regular pattern. Patients in stages I-II received mainly surgical treatment, while the main treatment in stages III-IV was chemotherapy, and radiotherapy and targeted therapy accounted for a small proportion. By the end of follow-up, 9 patients had died due to disease progression. It was found that 26 patients had an OS of 2.0-76.0 months, giving an average OS of 53.1 months as well as a mOS of 38.0 months (95% CI:1.727-74.273). Chen et al. (19) showed a median survival of 31 months (4-96 months) in 18 patients. The mOS of the 11 patients enrolled by Lin et al. (13) was nine months. Xie et al. (6) suggested that the median disease-free survival (DFS) of patients was 20.5 months (interquartile range, 16-28.3). Previous research has shown that the prognosis of patients with PEAC is directly associated with their clinical stage (23), with survival times ranging from 0 to 9 months for stage III or IV patients. In our research, the OS for stage III-IV patients was 2-38.0 months, the mean OS was 25 months, and the mOS was 33 months (95% CI: 0.0-83.085). 26 patients had a PFS of 2.0-76.0 months, with a mean PFS of 31.9 months as well as a mPFS of 8 months (95% CI: 4.333-11.667). A further analysis of stage III-IV patients then revealed that the PFS was 2-11.0 months, the mPFS was 6 months (95% CI: 4.512-7.488) and the mean PFS was 6 months. Furthermore, we analyzed 11 stages III-IV patients out of 20 cases from the SEER database, yielding a mean OS of 14.8 months and a mOS for 20 months (CI: 4.713-35.287). Lastly, the data from our study center were analyzed. We performed a univariate analysis of the factors of age, gender, family history of tumor, history of smoking, history of alcohol consumption, site, stage, and treatment, and concluded that tumor stage and whether surgery was associated with prognosis. No statistically significant differences were found in the multifactorial analysis, probably related to the small sample size of this study.

When it comes to systemic therapy, Teranishi et al. (24) reported a 68-year-old male with a stage IV B diagnosis, positive KRAS G12D mutation, and a tumor percentage score (TPS) of <1% for programmed cell death ligand 1 (PD-L1). Palliative radiotherapy and pembrolizumab + pemetrexed + carboplatin chemotherapy were administered, and the outcome was evaluated as partial response (PR). In comparison, Hu et al. (25) reviewed a 6 years old man with KRAS mutation in stage IV, who progressed rapidly after one cycle of paclitaxel + carboplatin + sintilimab. Tu et al. (7) reported that the four recipients received surgical treatment, curative knife treatment, and/or chemotherapy. The main chemotherapy regimens were pemetrexed + platinum (cisplatin or carboplatin) and paclitaxel + cisplatin, and the disease was controlled in all cases (efficacy evaluated as PR/stable disease (SD)). Patel et al. (20) covered a 60-year-old male patient treated with docetaxel + cisplatin after surgery. Six months later, the disease relapsed, then he received nivolumab, which remained effective for more than 14 months. As for targeted therapies and immunotherapy, scholars have described a case of a

patient given icotinib (a first-generation EGFR TKI) for over 1.5 months and then treated with volutumab (an immunotherapy drug) for more than 9.5 months (13). As the pathology of PEAC is characterized by intestinal differentiation, it is feasible to treat it with chemotherapy for colorectal cancer. Lin et al. (26) described a 53 years old female in stage IV who was initially treated with the XELOX (capecitabine plus oxaliplatin), followed by disease progression. The pulmonary achieved partial remission after four cycles of chemotherapy with the TP (paclitaxel plus cisplatin) regimen. However, the supraclavicular response to the drug was poor. After two cycles of the FOLFIRI (5-fluorouracil, leucovorin, and irinotecan) regimen, the disease progressed again, and the patient has finally treated with the DP (docetaxel plus cisplatin) regimen after palliative surgery. Garajova et al. (5) presented a case of a 68 male patient who underwent surgery and was later found to have bone metastases, so he was treated with XELOX and bisphosphonates, the disease progressed after two cycles, and four months later, the patient had a recurrence of multi-site osseous metastases and received four cycles of carboplatin + pemetrexed, none of which prevented the progression of the tumor. Succeeding chemotherapy with doxorubicin stabilized the progression after two cycles. Likewise, the patient's sister underwent a lobectomy. She was found to have stage IB PEAC, which progressed with pulmonary and adrenal metastases over 12 months. However, after receiving 6 cycles of carboplatin + pemetrexed and then pemetrexed alone, the disease stabilized. Qureshi et al. (27) reported a 61years old female with stable disease after four cycles of treatment with pemetrexed + carboplatin. According to Chen et al. (19), PEAC had a higher rate of TMB and MMR mutations than pulmonary adenocarcinoma (PAC). Manglaviti et al. (28) assessed PEAC data in ten cases with immune checkpoint inhibitors, yielding mPFS was 1.5 months (95% CI 0.2-2.8) and mOS was 17.3 months (95% CI 0.2-12.6). PR was 1 (10%) case, SD was 1 (10%) case, and PD was 8 (80%) cases. PEAC appears to have a poor response to immunotherapy, according to the research results. This suggests that PEAC is effectively treated with the pemetrexed/paclitaxel-based + platinum regimen, while it responds poorly with the XELOX/FOLFIRI regimen. In our center's study, ten patients with stage III-IV I had chemotherapy alone as their first-line treatment: six of platinum-based chemotherapy, and two had gemcitabine + platinum-based chemotherapy. Mean PFS was 5.8 months for pemetrexed + platinum, mPFS was five months (CI: 1.399-8.601), mean PFS was 10.5 months for paclitaxel + platinum, mPFS was four months (CI: -), and mean PFS was 8.5 months for gemcitabine + platinum, mPFS was eight months (CI. -), P=0.446. There were two phase III-IV patients with EGFR exon 19 mutations, one of whom was given treatment with pemetrexed + carboplatin + bevacizumab + gefitinib with a PFS of six months and one treated with paclitaxel + carboplatin after six cycles and maintenance treatment with osimertinib, which did not progress to the end of follow-up with a PFS of 17 months.

5 Limitations

Due to the small sample size, the difference in prevalence between men and women is not known. A proportion of patients had a family history of malignancy, particularly lung cancer, and the relationship between family history and incidence was uncertain because of the limitations of the sample size. This was a retrospective study and only 2 patients had examined CA 19.9 levels at the time of diagnosis, which prevented further assessment of the relationship between CA 19.9 and prognosis.

6 Conclusion

In conclusion, the onset of PEAC is most often seen in the elderly. Patients tend to seek treatment with chest complaints as the first symptom. The common genetic mutations are EGFR and KRAS mutations. CEA, CA 19.9, and CYFRA 21.1 levels must be monitored during diagnosis and follow-up. Surgery is often the mainstay of early treatment, while doublet chemotherapy with platinum-based is used in the late stages. Patients who can undergo surgery in the early stages have a better survival than those who cannot undergo surgery because of advanced cancer.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Cancer Hospital of Zhengzhou University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

QW and XS contributed to conception and design of the study. QW and LZ contributed to the acquisition of the data. QW, LZ, HLi and LL contributed to the analysis and interpretation of data. QW wrote first draft of the manuscript. XS and HLiu contributed to the revision of the manuscript. All the authors made substantial contributions to this work and approved it for publication.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1099117/full#supplementary-material

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Is cancer stage data missing completely at random? A report from a large population-based cohort of non-small cell lung cancer

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Introduction: Population-based datasets are often used to estimate changes in utilization or outcomes of novel therapies. Inclusion or exclusion of unstaged patients may impact on interpretation of these studies.

Methods: A large population-based dataset in Ontario, Canada of non-small cell lung cancer patients was examined to evaluate the characteristics and outcomes of unstaged patients compared to staged patients. Multivariable Poisson regression was used to evaluate differences in patient-level characteristics between groups. Kaplan-Meier estimates of survival and log-rank statistics were utilized.

Results: In our Ontario cohort of 51,152 patients with NSCLC, 11.2% (n=5,707) were unstaged, and there was evidence that stage data was not missing completely at random. Those without assigned stage were more likely than staged patients to be older (RR [95%CI]), (70-79 vs. 20-59: 1.51 [1.38-1.66]; 80+ vs. 20-59: 2.87 [2.62-3.15]), have a higher comorbidity index (Score 1-2 vs 0: 1.19 [1.12-1.27]; 3 vs. 0: 1.49 [1.38-1.60]), and have a lower socioeconomic class (4 vs. 1 (lowest): 0.91 [0.84-0.98]; 5 vs. 1 (lowest): 0.89 [0.83-0.97]). Overall survival of unstaged patients suggested a mixture of early and advanced stage, but with a large proportion that are probably stage IV patients with more rapid death than those with reported stage IV disease.

Conclusion: In this case study, evaluation of stage-specific health care utilization and outcomes for staged patients with stage IV disease at the population level may have a bias as a distinct subset of stage IV patients with rapid death are likely among those without a documented stage in administrative data.

KEYWORDS

missing data, non-small cell lung cancer, administrative data, population-based, cancer stage

Introduction

Population-based data are often used to explore stage-based outcomes of large groups of patients, and to describe treatment utilization rates for these groups in routine practice (1). However, many databases may be incomplete with less than 100% capture of variables such as stage (2).

Understanding the impact of missing stage information on studies estimating health care utilization or population-based outcomes in cancer patient data sets may be useful in interpreting various methods of estimating these rates and outcomes. Some databases may be missing stage information due to uniformly incomplete data collection of staged patients, while others may be missing stage information if patients are unstaged for medical reasons such as advanced rapidly progressive disease not amenable to active treatment. The latter condition represents data missing not completely at random, where the variable distribution (in this case stage) is different. Missing data in this case may be informative (3). If the act of being staged is associated with being 'fit' enough to receive treatment, then studies examining associated utilization rates or outcomes limited to patients with advanced disease with stage information may produce biased estimates compared to the true population value.

Here we provide a case study exploring patient characteristics and survival of patients stratified according to the presence of stage data. Given the high incidence and mortality of lung cancer, we explored this in a population-based sample of patients with non-small cell lung cancer (NSCLC) in the Canadian province of Ontario.

Methods

Study Design and Population

A population-based cohort of patients from the Ontario Cancer Registry (OCR) diagnosed with NSCLC between January 1, 2007, and December 31, 2016, were included. Ontario has a single-payer universal health care system with a population of over 14 million. We included patients with only one NSCLC diagnosis, with no history of previous chemotherapy, radiation therapy or surgery treatments. Patients were required to have a minimum of 5 years of continuous health insurance coverage prior to diagnosis to provide sufficient look back for comorbidity scoring, to be 20 years of age or older, and have a place of residence in Ontario. This study was approved by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

Data sources

ICES is an independent, non-profit research institute whose legal status under Ontario's health information privacy law allows it to collect and analyze health care and demographic data, without consent, for health system evaluation and improvement. These datasets were linked using unique encoded identifiers and analyzed at ICES.

Classification of independent variables

Stage was assigned on available data from Collaborative Stage in OCR and pathological/clinical stage in the Activity Level Reporting (ALR) data. This uses information derived from clinic-reported stage and manual chart review to assign stage based on the most reliable information (e.g. chart review data may be used in priority over cancer centre reported stage). Patient demographic data at the time of diagnosis were obtained from Ministry of Health administrative data. Comorbidity was assigned based on the Elixhauser comorbidity index (a validated algorithm to classify comorbidity using International Classification of Disease codes in administrative data) with a five-year lookback with Canadian Institute for Health Information Discharge Abstract Database (DAD) and Same Day Surgery (SDS) data (4). Diagnostic codes for lymphoma, metastatic cancer and solid tumours without metastasis were not included in the score. Neighbourhood income quintile was utilized as an area-level measure of socioeconomic status. Categorization of place of residence as urban, sub-urban or rural was based on the 2008 Rurality Index for Ontario (5). Chronic diseases (e.g., asthma and congestive heart failure) were identified with ICES-derived datasets based validated algorithms.

Classification of dependent variables

Overall survival and cancer-specific survival were measured from the date of diagnosis. Follow-up data were censored at 4 years for overall survival and 2 years for cancer-specific survival. Follow-up was shorter for cancer-specific survival as cause-specific death information from Ontario's Office of the Registrar General-Death (ORGD) is complete only up to December 31, 2018.

Statistical analyses

Demographic and general health data were summarized by stage (including unstaged information). Multivariable Poisson regression was used to evaluate the differences in the patient-level characteristics between the unstaged and staged groups. Kaplan-Meier estimates of survival were determined according to stage. Log-rank statistics were utilized. All analyses were performed using the SAS software 9.4 (SAS Institute, Cary NC).

Results

Of 51,152 NSCLC patients, 11.2% (5,707) were unstaged (Table 1). Unstaged patients were significantly more likely to be

TABLE 1 Demographic and general health characteristics for non-small cell lung cancer (NSCLC) patients in 2007-2016.

Patient Characteristics	Best Stage Information					
	I	II	III	IV	Unstaged	Total
	N=7,959	N=3,309	N=9,967	N=24,210	N=5,707	N=51,152
Year of diagnosis						
Mean ± SD	2011.84 ± 2.91	2011.87 ± 2.71	2011.21 ± 2.91	2011.55 ± 2.75	2010.71 ± 3.29	2011.46 ± 2.89
Median (IQR)	2012 (2009-2014)	2012 (2010-2014)	2011 (2009-2014)	2012 (2009-2014)	2009 (2008-2014)	2011 (2009-2014)
Age						
Mean ± SD	70.71 ± 10.13	70.27 ± 10.07	69.71 ± 10.60	69.58 ± 11.07	75.21 ± 11.33	70.45 ± 10.94
Median (IQR)	71 (64-78)	71 (63-78)	70 (62-78)	70 (62-78)	77 (68-84)	71 (63-79)
Age (categorized)						
20-59	1,117 (14.03%)	517 (15.62%)	1,779 (17.85%)	4,673 (19.30%)	587 (10.29%)	8,673 (16.96%)
60-69	2,292 (28.80%)	977 (29.53%)	2,942 (29.52%)	7,126 (29.43%)	1,043 (18.28%)	14,380 (28.11%)
70-79	2,905 (36.50%)	1,181 (35.69%)	3,322 (33.33%)	7,474 (30.87%)	1,782 (31.22%)	16,664 (32.58%)
80+	1,645 (20.67%)	634 (19.16%)	1,924 (19.30%)	4,937 (20.39%)	2,295 (40.21%)	11,435 (22.35%)
Sex						
Female	4,345 (54.59%)	1,535 (46.39%)	4,625 (46.40%)	11,258 (46.50%)	2,781 (48.73%)	24,544 (47.98%)
Male	3,614 (45.41%)	1,774 (53.61%)	5,342 (53.60%)	12,952 (53.50%)	2,926 (51.27%)	26,608 (52.02%)
Neighbourhood income quintile						
Missing	24 (0.30%)	9 (0.27%)	31 (0.31%)	93 (0.38%)	37 (0.65%)	194 (0.38%)
1 (Lowest)	1,888 (23.72%)	841 (25.42%)	2,510 (25.18%)	5,754 (23.77%)	1,450 (25.41%)	12,443 (24.33%)
2	1,762 (22.14%)	720 (21.76%)	2,236 (22.43%)	5,411 (22.35%)	1,258 (22.04%)	11,387 (22.26%)
3	1,535 (19.29%)	662 (20.01%)	1,930 (19.36%)	4,719 (19.49%)	1,124 (19.70%)	9,970 (19.49%)
4	1,452 (18.24%)	595 (17.98%)	1,722 (17.28%)	4,430 (18.30%)	975 (17.08%)	9,174 (17.93%)
5 (Highest)	1,298 (16.31%)	482 (14.57%)	1,538 (15.43%)	3,803 (15.71%)	863 (15.12%)	7,984 (15.61%)
Urban/rural residence						
NA/Missing	82 (1.03%)	33 (1.00%)	132 (1.32%)	287 (1.19%)	121 (2.12%)	655 (1.28%)
Urban (RIO<10)	4,994 (62.75%)	1,957 (59.14%)	5,972 (59.92%)	15,584 (64.37%)	3,117 (54.62%)	31,624 (61.82%)
Sub-urban (10≤RIO<40)	2,036 (25.58%)	875 (26.44%)	2,655 (26.64%)	5,923 (24.47%)	1,506 (26.39%)	12,995 (25.40%)
Rural (40≤RIO)	847 (10.64%)	444 (13.42%)	1,208 (12.12%)	2,416 (9.98%)	963 (16.87%)	5,878 (11.49%)
Place of residence						
Erie St. Clair	412 (5.18%)	188 (5.68%)	653 (6.55%)	1,537 (6.35%)	376 (6.59%)	3,166 (6.19%)
South West	546 (6.86%)	268 (8.10%)	893 (8.96%)	1,968 (8.13%)	517 (9.06%)	4,192 (8.20%)
Waterloo Wellington	311 (3.91%)	158 (4.77%)	474 (4.76%)	1,250 (5.16%)	264 (4.63%)	2,457 (4.80%)
Hamilton Niagara Haldimand Brant	996 (12.51%)	427 (12.90%)	1,344 (13.48%)	3,331 (13.76%)	698 (12.23%)	6,796 (13.29%)
Central West	313 (3.93%)	114 (3.45%)	356 (3.57%)	959 (3.96%)	208 (3.64%)	1,950 (3.81%)
Mississauga Halton	466 (5.86%)	183 (5.53%)	525 (5.27%)	1,406 (5.81%)	327 (5.73%)	2,907 (5.68%)
Toronto Central	626 (7.87%)	235 (7.10%)	605 (6.07%)	1,788 (7.39%)	333 (5.83%)	3,587 (7.01%)
Central	785 (9.86%)	242 (7.31%)	778 (7.81%)	2,336 (9.65%)	433 (7.59%)	4,574 (8.94%)
Central East	983 (12.35%)	394 (11.91%)	1,171 (11.75%)	2,936 (12.13%)	765 (13.40%)	6,249 (12.22%)
South East	459 (5.77%)	177 (5.35%)	599 (6.01%)	1,418 (5.86%)	322 (5.64%)	2,975 (5.82%)

(Continued)

TABLE 1 Continued

Patient Characteristics	Best Stage Information					
	I	II	III	IV	Unstaged	Total
	N=7,959	N=3,309	N=9,967	N=24,210	N=5,707	N=51,152
Champlain	984 (12.36%)	388 (11.73%)	1,170 (11.74%)	2,151 (8.88%)	481 (8.43%)	5,174 (10.11%)
North Simcoe Muskoka	348 (4.37%)	171 (5.17%)	425 (4.26%)	1,025 (4.23%)	309 (5.41%)	2,278 (4.45%)
North East	541 (6.80%)	271 (8.19%)	766 (7.69%)	1,563 (6.46%)	458 (8.03%)	3,599 (7.04%)
North West	189 (2.37%)	93 (2.81%)	208 (2.09%)	542 (2.24%)	216 (3.78%)	1,248 (2.44%)
Elixhauser comorbidity index[1]						
Mean ± SD	1.10 ± 1.76	0.93 ± 1.58	0.88 ± 1.56	0.76 ± 1.48	1.32 ± 1.90	0.91 ± 1.61
Median (IQR)	0.0 (0.0-2.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-2.0)	0.0 (0.0-1.0)
Elixhauser comorbidity index[1] (categorized)						
0	4,616 (58.00%)	2,031 (61.38%)	6,376 (63.97%)	16,596 (68.55%)	3,015 (52.83%)	32,634 (63.80%)
1-2	2,005 (25.19%)	808 (24.42%)	2,280 (22.88%)	4,861 (20.08%)	1,452 (25.44%)	11,406 (22.30%)
3+	1,338 (16.81%)	470 (14.20%)	1,311 (13.15%)	2,753 (11.37%)	1,240 (21.73%)	7,112 (13.90%)
Chronic disease						
Asthma	1,731 (21.75%)	615 (18.59%)	1,758 (17.64%)	3,409 (14.08%)	1,026 (17.98%)	8,539 (16.69%)
Chronic obstructive pulmonary disease	4,507 (56.63%)	1,813 (54.79%)	5,124 (51.41%)	10,334 (42.68%)	3,081 (53.99%)	24,859 (48.60%)
Hypertension	5,298 (66.57%)	2,115 (63.92%)	6,124 (61.44%)	14,411 (59.52%)	3,855 (67.55%)	31,803 (62.17%)
Congestive heart failure	1,108 (13.92%)	405 (12.24%)	1,239 (12.43%)	2,586 (10.68%)	1,173 (20.55%)	6,511 (12.73%)

SD, Standard deviation; IQR, Interquartile range; RIO, Rurality Index for Ontario.
1. Comorbidity is based on hospital visits in a 5-year lookback from NSCLC diagnosis. Total score excludes diagnostic codes for lymphoma, metastatic cancer and solid tumours without metastasis.

older (Relative Risk (RR) [95% Confidence Interval (CI)]: 70-79 vs. 20-59, 1.51 [1.38-1.66]; 80+ vs. 20-59, 2.87 [2.62-3.15]), reside in lower income neighbourhoods (RR [95% CI]: 4th vs. 1st quintile, 0.91 [0.84-0.98]; 5th vs. 1st quintile, 0.89 [0.83-0.97]) and rural areas (RR [95% CI]: urban vs. rural, 0.58 [0.54-0.61]; sub-urban vs. rural, 0.71 [0.66-0.77]), and have a higher comorbidity index (RR [95% CI]: 1-2 vs. 0, 1.19 [1.12-1.27]; 3+ vs. 0, 1.49 [1.38-1.60]) (Table 2). The occurrence of missing stage also changed over time, becoming increasingly less likely during the study period (RR year of diagnosis, per 1-year increase [95% CI]: 0.92 [0.91-0.92]). Among the unstaged group, 89.4% (5,102) died within 4 years from diagnosis. Earlier stage patients at diagnosis (stage I/II/III) comprised ~32.8% of deaths.

Survival curves are shown in Figures 1A, B. For stage III and IV patients, the one-year overall survival (OS) are 47.3% and 20.2% (Figure 1A), while the one-year cancer-specific survival (CSS) are 51.8% and 22.8%, respectively (Figure 1B). Noticeable in the Kaplan Meier curves is the different shape of the curve for unstaged patients, with a steeper initial drop than stage IV patients, but with a similar one-year survival to stage IV patients (one-year OS: 21.6% vs. 20.2%) and a higher survival in the tail of the curve (four-year OS: 10.6% vs. 3.9%).

Discussion

In this case study of a large population-based cohort of NSCLC patients, stage data is not missing completely at random. Evaluation of stage-specific health care utilization and outcomes for staged patients, particularly those with stage IV disease, at the population level may thus have a bias as a distinct subset of stage IV patients with rapid death are likely among those without a documented stage in administrative data.

Healthcare utilization differences between staged and unstaged groups was not evaluated in our study. However, it is known that costs (and therefore utilization) vary by lung cancer stage in Canada. A recent study found that unstaged patients with lung cancer had higher costs than stage I and II patients with lung cancer, likely due to the high costs of end-of-life care (6). Treatment receipt for both staged and unstaged groups is delivered based on accepted provincial, national, and international guidelines. These guidelines are based on important prognostic factors not fully available in our cohort, but both groups (staged and unstaged) would have access to fully reimbursed standard of care treatment options. The unstaged group accounted for approximately 11.2% of cases and approximately 12.1% of deaths. These patients have higher

TABLE 2 Comparison of demographic and general health characteristics according to stage information for non-small cell lung cancer (NSCLC) patients in 2007-2016[1].

Patient Characteristics	Adjusted Full Model			
	Unstaged vs. Stage I-IV		Unstaged vs. Stage IV	
	RR (95% CI)	p-value	RR (95% CI)	p-value
Year of diagnosis, per 1-year increase	0.91 (0.90-0.92)	<0.001	0.92 (0.91-0.92)	<0.001
Age (categorized)				
60-69 vs. 20-59	1.05 (0.96-1.16)	0.293	1.09 (0.99-1.20)	0.066
70-79 vs. 20-59	1.51 (1.38-1.66)	<0.001	1.57 (1.43-1.71)	<0.001
80+ vs. 20-59	2.87 (2.62-3.15)	<0.001	2.61 (2.39-2.85)	<0.001
Sex				
Male vs. Female	0.95 (0.91-1.00)	0.054	0.93 (0.89-0.98)	0.003
Neighbourhood income quintile				
2 vs. 1 (Lowest)	0.94 (0.87-1.00)	0.064	0.93 (0.87-0.99)	0.026
3 vs. 1 (Lowest)	0.94 (0.87-1.01)	0.083	0.93 (0.87-0.99)	0.035
4 vs. 1 (Lowest)	0.91 (0.84-0.98)	0.012	0.89 (0.83-0.96)	0.002
5 (Highest) vs. 1 (Lowest)	0.89 (0.83-0.97)	0.005	0.90 (0.83-0.97)	0.004
Urban/rural residence				
Urban (RIO<10) vs. Rural (40≤RIO)	0.58 (0.54-0.61)	<0.001	0.58 (0.54-0.61)	<0.001
Sub-urban (10≤RIO<40) vs. Rural (40≤RIO)	0.71 (0.66-0.77)	<0.001	0.73 (0.68-0.78)	<0.001
Elixhauser comorbidity index[2] (categorized)				
1-2 vs. 0	1.19 (1.12-1.27)	<0.001	1.23 (1.16-1.31)	<0.001
3+ vs. 0	1.49 (1.38-1.60)	<0.001	1.50 (1.40-1.60)	<0.001
Chronic disease				
Asthma, yes vs. no	1.01 (0.95-1.08)	0.765	1.09 (1.03-1.16)	0.004
Chronic obstructive pulmonary disease, yes vs. no	1.02 (0.97-1.07)	0.511	1.13 (1.08-1.19)	<0.001
Hypertension, yes vs. no	0.88 (0.83-0.93)	<0.001	0.92 (0.87-0.97)	0.003
Congestive heart failure, yes vs. no	1.19 (1.11-1.27)	<0.001	1.16 (1.09-1.23)	<0.001

RR, Relative risk; CI, Confidence interval; RIO, Rurality Index for Ontario.
1. Patients with missing responses from specified variables of interest were excluded.
2. Comorbidity is based on hospital visits in a 5-year lookback from NSCLC diagnosis. Total score excludes diagnostic codes for lymphoma, metastatic cancer and solid tumours without metastasis.

comorbidity, rurality, and age than staged patients. It is well known that variation in care and service delivery exists in a single-payer public universal health care system and are associated with patient-level characteristics (7, 8).

Missing stage was also more likely to occur earlier in the study period. Based on the shape of survival curves, we hypothesize that the group without stage data likely represents at least two populations; a rapidly dying advanced cancer cohort dying too quickly to be formally staged or treated in a cancer centre, as well as an earlier stage cohort with better survival with omitted staging due to technical, rather than clinical, reasons. This potential mixture of early and advanced cases argues against simply combining unstaged patients with stage IV patients in studies of stage IV management and outcome.

In population-based studies on palliative systemic therapy utilization in Ontario and possibly other jurisdictions, using a metric of the number of patients who received such therapy divided by all stage IV patients can overestimate utilization (9, 10). This is because the 'denominator' of database-recorded stage IV lung cancer may be lower than the 'true' number of stage IV patients in the population as a component of patients with true stage IV disease may be missing stage information. In certain populations, like the aged (80+), the bias may be significantly higher, as 40.2% of the unstaged patients were 80+, representing 20.1% of the lung cancers diagnosed in that group.

Cancer stage determination in Ontario is captured by the OCR who receive pathological and clinical (stage assigned by the managing physician) reporting from regional cancer centers

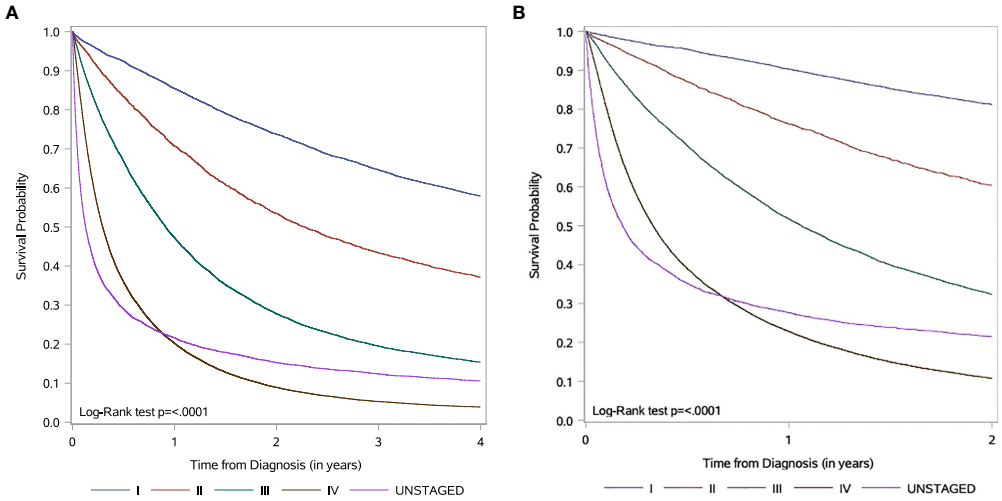

FIGURE 1
Kaplan-Meier survival curves according to stage information for non-small cell lung cancer (NSCLC) patients in 2007-2016 **(A)** Overall survival. Data is censored 4 years from diagnosis. **(B)** Cancer-specific survival. Data is censored 2 years from diagnosis.

across Ontario (11). This process often relies on OCR registrar staff to incorporate and assess clinical, pathological and post-therapy stage information. Other Canadian provincial cancer registries as well as large American cancer registries (National Cancer Database (NCDB) and Surveillance, Epidemiology, and End Results Program (SEER)) have collected stage information following similar processes to Ontario, using trained tumor registrars to abstract specified data elements from patient records in accordance with registry data standards (12).

Our study supports previous findings from other high-income countries of improved (decreasing) rates of missing stage data over time, likely due to improvements in coding standards and cancer registry quality (12, 13). However, as much as stage data capture is improving, it will never be entirely complete due to clinical (e.g., physician failing to assign a category) and data-registry (e.g., miscoded fields) level factors. In the NCDB, high levels of missing data were found for NSCLC and other major cancer sites that also appear not to be missing completely at random. (12). The SEER is also faced with similar challenges with regards to missing data (14). It is highly likely that the COVID-19 pandemic has compromised and continues to affect cancer stage recording and capture, as it has already impacted recent studies (15). Therefore, we expect the trend of decreasing missing cancer data to reverse, and further emphasize the importance of understanding the implications and nature of missing data.

While large databases and staging are helpful in determining real world utilization of palliative systemic therapy and real-world outcomes, there are factors that may bias data collection and interpretation and may lead to over- or under-estimation of treatment utilization. Using only staged patients with stage IV disease to determine palliative systemic treatment utilization in NSCLC may lead to different estimates of utilization in comparison to other methods, such as the 'lookback' method from death – which will miss those who have not died, but includes those who receive palliative therapy for unresectable or recurrent disease. Another approach is to look forward from the time of first palliative therapy, which will miss those who receive no palliative therapy, but may include those who had earlier stage disease and subsequently recurred, and those with incurable locally advanced disease (e.g., some stage IIIB). Each of these methods of estimating palliative systemic therapy utilization may lead to different estimates and should be seen as complimentary in determining the real 'real world' utilization.

Conclusion

In this case study, there was evidence that stage data was not missing completely at random. Evaluation of stage-specific health care utilization and outcomes for staged patients with stage IV disease at the population level may have a bias as a distinct subset of stage IV patients with rapid death are likely among those without a documented stage in administrative data.

Data availability statement

The dataset from this study is held securely in coded form at ICES. While legal data sharing agreements between ICES and data providers (e.g., healthcare organizations and government) prohibit ICES from making the dataset publicly available, access may be granted to those who meet pre-specified criteria for confidential access, available at www.ices.on.ca/DAS (email: das@ices.on.ca). The full dataset creation plan and underlying analytic code are available from the authors upon request, understanding that the computer programs may rely upon coding templates or macros that are unique to ICES and are therefore either inaccessible or may require modification.

Author contributions

AR: conceptualization, formal analysis, methodology, writing original draft, writing, review and editing. TH: funding acquisition, investigation, writing review and editing. PN: writing, review and editing, data curation, formal analysis, methodology. CG: funding acquisition, writing review and editing. MJ: writing, review and editing, interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

AR reports speaker fees from Astra-Zeneca, Merck and BMS, outside the submitted work.

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.

Publisher's note

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Reconsidering the T category for the T3 non-small cell lung cancer with additional tumor nodules in the same lobe: A population-based study

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Background: This study aimed to evaluate the prognosis of the T3 non-small cell lung cancer (NSCLC) patients with additional tumor nodules in the same lobe (T3-Add), and externally validate the current T category of this population.

Methods: NSCLC data deposited in the Surveillance, Epidemiology, and End Results (SEER) dataset was extracted. Survivals were estimated using the Kaplan-Meier method with a log-rank test. Propensity score matching (PSM) was performed to reduce bias. The least absolute shrinkage and selection operator (LASSO)-penalized Cox model was used to determine the prognostic factors.

Results: A total of 41,370 eligible cases were included. There were 2,312, 20,632, 12,787, 3,374 and 2,265 cases in the T3-Add, T1, T2, T3 and T4 group, respectively. The Kaplan-Meier curves demonstrated that the survivals of the T3-Add patients were superior to those of the T3 patients both before and after PSM. Additionally, the OS of the T3-Add patients were worse than that of the T2 patients, but the CSS differences between these two groups were not statistically significant. In the subset analyses, the survivals of the T3-Add patients were inferior to those of the T2a patients, but were comparable to those of the T2b patients (5-year OS rate: 54.3% vs. 57.2%, P = 0.884; 5-year CSS rate: 76.2% vs. 76.8%, P = 0.370). In the T3-Add & T2b matched pair, multivariable Cox analysis further confirmed that T category was not a prognostic factor for survivals.

Conclusion: T3-Add and T2b NSCLC patients had similar survivals, and we proposed that it is necessary to reconsider the T category of the patients with additional nodules in the same lobe in the forthcoming 9th edition of TNM staging manual.

KEYWORDS

non-small cell lung cancer, T3-Add, T3 category, T2b category, survival

Introduction

Lung cancer ranks first as the cause of cancer-related mortality worldwide (1, 2). Non-small cell lung cancer (NSCLC) accounts for about 85% of all the lung malignancies (3). The 8th edition of the International Association for the Study of Lung Cancer (IASLC) proposed tumor-node-metastasis (TNM) staging manual for NSCLC (4) is considered as the predominating prognostic factor for NSCLC patients' survivals, which guides treatment strategy selection. In recent years, much effort has been devoted to refine this staging manual to achieve a more accurate staging (5–8).

In the current TNM staging manual, tumors with additional nodules in the same lobe are assigned to T3 category (T3-Add) (4, 9). However, survival dispute arises over the T category of these patients (8), and more externally validations are needed. The previous report demonstrated that the overall survival (OS) of the T3-Add patients was superior to that of the remaining T3 patients, but was similar to that of T2b patients (8). Therefore, the authors proposed to reconsider these patients to T2b category (8). Given the great significance of the TNM staging manual, it is imperative to determine the proper T category of this population subset.

Against this background, the current study analyzed the resected NSCLC data recorded in the Surveillance, Epidemiology, and End Results (SEER) dataset and aimed to revealed the heterogeneity of prognosis between T3-Add and other patients. We hoped to answer the question that whether T3-Add patients should remain classified as T3 category.

Methods

Study population

From 2010 to 2015, a total of 360,702 lung malignancies cases were extracted from the SEER database. The patient selection flow chart is showed in Figure 1. The eligible cases satisfy the following criteria: [1] diagnosed as NSCLC and [2] received surgery. The exclusion criteria mandated that: [1] age < 18 years; [2] neoadjuvant radiotherapy; [3] N3 category; [4] M1 category; [5] location unknown; [6] grade unknown; [7] examined lymph nodes unknown; [8] positive lymph nodes unknown and [9] TNM stage unknown. According to the CS Site-Specific Factor 1 code, T3-Add patients were selected (code 010: separate tumor nodules in ipsilateral lung, same lobe). At last, the included cases were assigned to five groups: T3-Add, T1, T2, T3 and T4 groups. This study mainly focused on the T3-Add, T2 and T3 patients.

Ethic

This study was approved by the Ethics Committee of Peking University People's Hospital. Permission was obtained to retrieve SEER data files using the reference number: 12962-Nov2019. Given the anonymous patient data and retrospective design, this study was dispensed with the informed consent forms.

Data collection

The variables, including age, sex (male/female), tumor location (upper lobe/middle lobe/lower lobe), histology (adenocarcinoma/squamous cell carcinoma/other), grade (well/moderate/poor or undifferentiated), surgery (lobectomy/pneumonectomy/sublobectomy), radiotherapy (no and yes), chemotherapy (no and yes), examined lymph nodes, positive lymph nodes, tumor size, pathologic T category, pathologic N category, pathologic TNM stage, visceral pleural invasion (VPI), patient status, cause of death and survival time. The pathologic 8th edition of the TNM staging manual (4) was used in this study. A complete data analysis was conducted in this study.

Endpoints

Patients with exact survival time and definitive status were included. The primary endpoints of this study were OS and Cancer specific survival (CSS). OS was defined as the time period from the date of diagnosis to the date of death from any cause or the last follow-up. CSS was defined as the time period from the date of diagnosis to the date of death caused by NSCLC or the last follow-up. The median follow-up time was 33.0 months (range: 0.48-83.04 months).

Statistical analysis

SEER*Stat software version 8.3.4. was used to extract the NSCLC data. R version 4.1.1 (The R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org) and IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY, USA) were

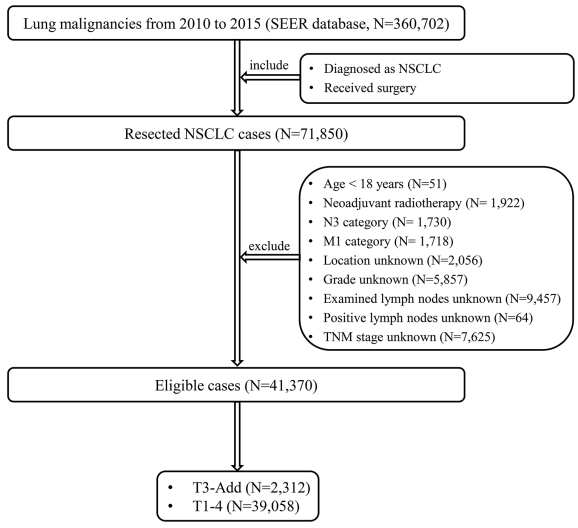

FIGURE 1
The patient selection flow chart. NSCLC, non-small cell lung cancer; TNM, tumor-node-metastasis; T3-Add, T3 non-small cell lung cancer with additional tumor nodules in the same lobe.

used to conduct statistical analyses. Categorical variables were presented as numbers and percentages, which were compared using the Pearson $\chi 2$ test or Fisher's exact test. Nonnormally distributed continuous variables were presented as median and range, which were compared using the Mann-Whitney U test. Survivals were analyzed using the Kaplan-Meier method with a log-rank test. A one to one propensity score matching (PSM) method was used to reduce the bias caused by the confounding variables in baseline characteristics using the R package "MatchIt" (10). The variables, including age, sex, histology, grade, surgery, radiotherapy, chemotherapy, N category and VPI, were included in the PSM models. The caliper distance of the T3-Add & T2 pair, T3-Add & T3 pair, T3-Add & T2a pair and T3-Add & T2b pair were 0.0001, 0.00001, 0.0001 and 0.00001, respectively. Least absolute shrinkage and selection operator (LASSO) regression model was used to minimize and select the potential prognostic factors using the R package "glmnet" (11). The variables, including age, sex, location, histology, grade, surgery, radiotherapy, chemotherapy, examined lymph nodes, positive lymph nodes, T category, N category, TNM stage and VPI, were included in the LASSO model. The LASSO-selected prognostic factors were further entered into a forward stepwise multivariable Cox model to determine the final independent prognostic factors. Two-sided P values < 0.05 was considered statistically significant.

Results

Patient characteristics

The aforementioned inclusion and exclusion criteria yielded a study population of 41,370 eligible cases. The baseline characteristics of the included patients are listed in Table 1. The median age was 69 years (range: 18-99 years). Over half of cases were female (52.8%). There were 2,312 cases, 20,632 cases, 12,787 cases, 3,374 cases and 2,265 cases in the T3-Add, T1, T2, T3 and T4 group, respectively. The incidence of the T3-Add patients was 5.6% (2,312/41,370). The 5-year OS rate of the T3-Add patients was 51.5%, and the 5-year CSS rate was 72.5%. When compared with the T2 group, more patients in the T3-Add group were female ($P < 0.001$), diagnosed with adenocarcinoma ($P < 0.001$), diagnosed with well differentiated diseases ($P < 0.001$), received radiotherapy ($P < 0.001$) and received chemotherapy ($P < 0.001$). In addition, less patients in the T3-Add group were suffered from VPI ($P < 0.001$). When compared with the T3 group, there were higher percentage of female ($P < 0.001$), upper lobe tumors ($P < 0.001$), adenocarcinoma ($P < 0.001$) and well differentiated diseases ($P < 0.001$) in the T3-Add group. More small-sized and early N category tumors were diagnosed in the T3-Add group (tumor size: $P < 0.001$; N category: $P < 0.001$). Less patients in the T3-Add group received pneumonectomy ($P < 0.001$), received radiotherapy ($P = 0.001$) and received chemotherapy ($P < 0.001$). After PSM, there were 1,623 cases, 650 cases, 1,381 cases and 788 cases in the T3-Add & T2 pair, T3-Add & T3 pair, T3-Add & T2a pair and T3-Add & T2b pair, respectively. The baseline characteristics were all balanced well after PSM (Tables S1, S2).

Survival analysis

Before PSM, a progressively reduced OS and CSS were observed depending on T category (OS: $P < 0.001$, Figure 2A; CSS: $P < 0.001$, Figure 2B). The OS of the T3-Add patients was better than that of the T3 patients (5-year OS rate: 51.5% vs. 46.1%, $P < 0.001$), but was inferior to that of T2 patients (5-year OS rate: 51.5% vs. 55.6%, $P = 0.002$). Similar results were also observed in the CSS comparisons (5-year CSS rate: T3-Add vs. T3 = 72.5% vs. 55.6%, $P < 0.001$; 5-year CSS rate: T3-Add vs. T2 = 72.5% vs. 75.6%, $P = 0.016$).

After PSM, considering the T3-Add & T2 matched pair, the OS of the T3-Add patients was still worse than that of the T2 patients (5-year OS rate: 53.9% vs. 58.0%, $P = 0.037$, Figure 3A). However, these two groups of patients had similar CSS (5-year CSS rate: 74.4% vs. 77.1%, $P = 0.121$, Figure 3B). Regarding the T3-Add & T3 matched pair, the survivals of the T3-Add patients were superior to those of the T3 patients (5-year OS rate: 54.8% vs. 50.4%, $P = 0.009$, Figure 3C; 5-year CSS rate: 75.3% vs. 71.2%, $P = 0.008$, Figure 3D). In the subset analyses, considering the T3-Add & T2a matched pair, T2a patients had longer survivals than T3-Add patients (5-year OS rate: 55.7% vs. 63.1%, $P = 0.001$, Figure 4A; 5-year CSS rate: 76.5% vs. 81.8%, $P = 0.004$, Figure 4B). Regarding the T3-Add & T2b matched pair, the survivals of the T3-Add patients were comparable to those of the T2b patients (5-year OS rate: 54.3% vs. 57.2%, $P = 0.884$, Figure 4C; 5-year CSS rate: 76.2% vs. 76.8%, $P = 0.370$, Figure 4D).

When stratified T3-Add patients based on tumor size, the data showed that T3-Add (0-30 mm) had the best survival rates (Both OS and CSS) than the remaining T3 patients (Figure S1, all $P < 0.001$). The OS of T3-Add (30-50 mm) was comparable to that of T3-Add (50-70 mm) patients ($P = 0.474$, Figure S1A), but was marginally better than that of T3-Add (> 70 mm) ($P = 0.047$, Figure S1A). T3-Add (50-70 mm) and T3-Add (> 70 mm) patients had similar OS ($P = 0.252$, Figure S1A). Considering CSS, diminishing CSS with increasing tumor size was observed except for the survival comparison between T3-Add (50-70 mm) and T3-Add (> 70 mm) patients ($P = 0.330$, Figure S1B).

LASSO-penalized multivariable Cox analysis

After PSM, regarding the T3-Add & T2a matched pair, 14 variables were entered into the LASSO models (OS: Figure S2A; CSS: Figure S2C). The results showed that age, sex, grade, positive lymph nodes, T category, N category and VPI were potential prognostic factors for OS (Figure S2B) and grade, positive lymph nodes, T category and N category were potential prognostic factors for CSS (Figure S2D). The LASSO-selected variables were further included in the multivariable Cox analysis. The multivariable Cox analysis further confirmed that age ($P < 0.001$), sex ($P < 0.001$), grade ($P < 0.001$), T category (adjusted HR: T3-Add vs. T2a = 1 vs. 0.775, $P < 0.001$), N category ($P = 0.004$) and VPI ($P = 0.028$) were the independent prognostic factors for OS (Table 2), and grade ($P < 0.001$), T category (adjusted HR: T3-Add vs. T2a = 1 vs. 0.717, $P = 0.001$) and N category ($P < 0.001$) were the independent prognostic factors for CSS (Table 2).

TABLE 1 The baseline characteristics of the included NSCLC patients.

Variables	Total (N= 41,370)	T3-Add (N=2,312)	T2 (N=12,787)	T3 (N=3,374)	*P1*[a]	*P2*[b]
Age, years					0.596[c]	0.921[c]
Median (range)	69 (18-99)	69 (24-91)	69 (19-96)	69 (18-95)		
Sex					< 0.001	< 0.001
Male	19,523 (47.2)	1,018 (44.0)	6,300 (49.3)	1,983 (58.8)		
Female	21,847 (52.8)	1,294 (56.0)	6,487 (50.7)	1,391 (41.2)		
Location					0.191	< 0.001
Upper lobe	24,586 (59.4)	1,411 (61.0)	7,643 (59.8)	1,919 (56.9)		
Middle lobe	2,448 (5.9)	114 (4.9)	744 (4.8)	132 (3.9)		
Lower lobe	14,336 (34.7)	787 (34.0)	4,400 (34.4)	1,323 (39.2)		
Histology					< 0.001	< 0.001
Adenocarcinoma	21,201 (51.2)	1,296 (56.1)	6,356 (49.7)	1,214 (36.0)		
Squamous cell carcinoma	10,264 (24.8)	396 (17.1)	3,455 (27.0)	1,449 (42.9)		
Other	9,905 (23.9)	620 (26.8)	2,976 (23.3)	711 (21.1)		
Grade					< 0.001	< 0.001
Well	8,028 (19.4)	485 (21.0)	1,632 (12.8)	259 (7.7)		
Moderate	18,906 (45.7)	1,032 (44.6)	6,031 (47.2)	1,260 (37.3)		
Poor/undifferentiated	14,436 (34.9)	795 (34.4)	5,124 (40.1)	1,855 (55.0)		
Surgery					0.027	< 0.001
Lobectomy	34,047 (82.3)	1,956 (84.6)	11,032 (86.3)	2,946 (87.3)		
Pneumonectomy	1,362 (3.3)	74 (3.2)	433 (3.4)	289 (8.6)		
Sublobectomy	5,961 (14.4)	282 (12.2)	1,322 (10.3)	139 (4.1)		
Radiotherapy					< 0.001	0.001
No	38,155 (92.2)	2,044 (88.4)	11,607 (90.8)	2,876 (85.2)		
Yes	3,215 (7.8)	268 (11.6)	1,180 (9.2)	498 (14.8)		
Chemotherapy					< 0.001	< 0.001
No	31,933 (77.2)	1,461 (63.2)	9,187 (71.8)	1,776 (52.6)		
Yes	9,437 (22.8)	851 (36.8)	3,600 (28.8)	1,598 (47.7)		
Examined lymph nodes					0.139[c]	< 0.001[c]
Median (range)	8 (1-90)	8 (1-90)	9 (1-82)	10 (1-90)		
Positive lymph nodes					0.044[c]	< 0.001[c]
Median (range)	0 (0-61)	0 (0-24)	0 (0-38)	0 (0-18)		
Tumor size, mm					< 0.001[c]	< 0.001[c]
Median (range)	25 (1-989)	25 (1-185)	35 (1-50)	58 (6-70)		
T category						
1	20,632 (49.9)					
2	12,787 (30.9)					
3	5,686 (13.7)					
4	2,265 (5.5)					
N category					0.090	< 0.001

(Continued)

TABLE 1 Continued

Variables	Total (N= 41,370)	T3-Add (N=2,312)	T2 (N=12,787)	T3 (N=3,374)	*P1*[a]	*P2*[b]
0	3,2585 (78.8)	1,658 (71.7)	9,364 (73.2)	2,211 (65.5)		
1	5,087 (12.30)	350 (15.1)	1,945 (15.2)	722 (21.4)		
2	3,698 (8.9)	304 (13.1)	1,478 (11.6)	441 (13.1)		
TNM stage						
IA	17,946 (43.4)					
IB	7,382 (17.8)					
IIA	1,982 (4.8)					
IIB	7,371 (17.8)					
IIIA	5,598 (13.5)					
IIIB	1,091 (2.6)					
VPI					< 0.001	< 0.001
Without	32,479 (78.5)	1,720 (74.4)	6,576 (51.4)	2,068 (61.3)		
With	8,891 (21.5)	592 (25.6)	6,211 (48.6)	1,306 (38.7)		

a T3-Add vs. T2.
b T3-Add vs. T3.
c Mann–Whitney U test.
NSCLC, non-small cell lung cancer; T, tumor; T3-Add, T3 tumors with additional nodules in the same lobe; N, node; TNM, tumor-node-metastasis; VPI, visceral pleural invasion.

Considering the T3-Add & T2b matched pair, the LASSO models selected 5 variables including age, sex, grade, positive lymph nodes and N category for OS, and 2 variables including grade and T category for CSS. In further analyses, the Cox models confirmed that age ($P < 0.001$), sex ($P < 0.001$), grade ($P < 0.001$) and N category ($P = 0.001$) were the independent prognostic factors for OS (Table 3), and grade ($P < 0.001$) and N category ($P < 0.001$) were the independent prognostic factors for CSS (Table 3).

Discussion

The current study evaluated the prognosis of the T3-Add NSCLC patients from a large public database. Our results suggested that the survivals of the T3-Add patients were superior to those of the T3 patients, but were comparable to those of the T2b patients after balancing the baseline characteristics. The LASSO-penalized Cox models further confirmed that the T category (T3-Add vs. T2b) was not a prognostic factor for the patients in the T3-Add & T2b matched pair. Therefore, we proposed that it is necessary to reconsider the T category of the patients with additional nodules in the same lobe in the forthcoming 9th edition of TNM staging manual. This gave us a hint, but more validations are still warranted.

In this study, the incidence of the T3-Add patients was 5.6% (2,312/41,370). The 5-year OS rate of the T3-Add patients was 51.5%, which was higher than the previous studies, where the authors reported the rate of 42.0% (8, 9). A possible explanation for this difference might be that a portion of T3-Add patients had not received surgery in their cohorts, whereas all included patients had undergone surgical resection in this study. To date, surgery is still the preferred treatment for these patients (12). We reported for

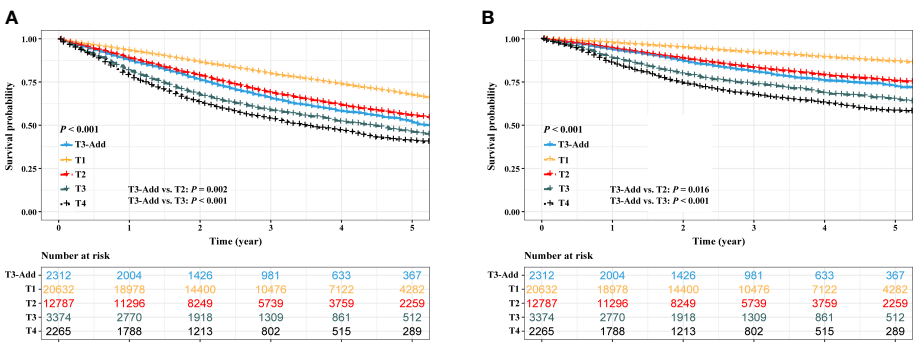

FIGURE 2
Kaplan–Meier estimates of survivals in the full analysis set. **(A)** overall survival: T3-Add vs. T1 vs. T2 vs. T3 vs. T4 and **(B)** cancer specific survival: T3-Add vs. T1 vs. T2 vs. T3 vs. T4. T3-Add, T3 non-small cell lung cancer with additional tumor nodules in the same lobe.

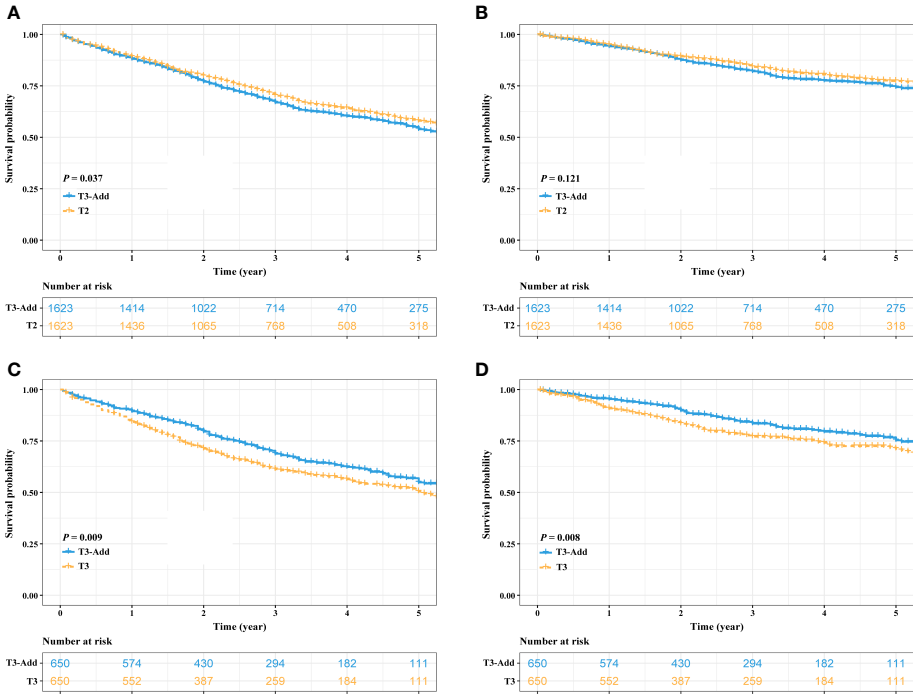

FIGURE 3
Kaplan–Meier estimates of survivals in the T3-Add & T2 pair and the T3-Add & T3 pair after PSM. **(A)** overall survival: T3-Add vs. T2; **(B)** cancer specific survival: T3-Add vs. T2; **(C)** overall survival: T3-Add vs. T3; **(D)** cancer specific survival: T3-Add vs. T3. PSM, propensity score matching; T3-Add, T3 non-small cell lung cancer with additional tumor nodules in the same lobe.

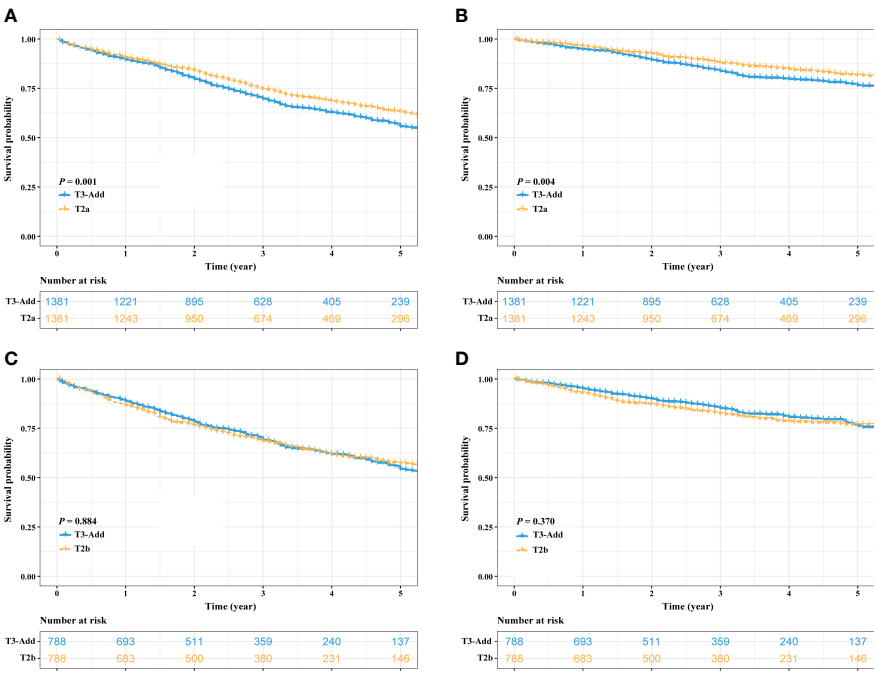

FIGURE 4
Kaplan–Meier estimates of survivals in the T3-Add & T2a pair and the T3-Add & T2b pair after PSM. **(A)** overall survival: T3-Add vs. T2a; **(B)** cancer specific survival: T3-Add vs. T2a; **(C)** overall survival: T3-Add vs. T2b; **(D)** cancer specific survival: T3-Add vs. T2b. PSM, propensity score matching; T3-Add, T3 non-small cell lung cancer with additional tumor nodules in the same lobe.

TABLE 2 LASSO-penalized multivariable Cox analysis of the T2a and T3-Add NSCLC patients after PSM.

Variables	OS[a]			CSS[b]		
	HR	95% CI	*P*	HR	95% CI	*P*
Age, years			< 0.001			
Continue	1.033	1.025-1.042				
Sex			< 0.001			
Male	1					
Female	0.656	0.573-0.751				
Grade			< 0.001			< 0.001
Well	1			1		
Moderate	1.605	1.282-2.008		2.223	1.503-3.288	
Poor/undifferentiated	2.261	1.800-2.840		3.587	2.426-5.302	
Positive lymph nodes			0.087			0.118
Continue	1.044	0.994-1.096		1.049	0.988-1.115	
T category			< 0.001			0.001
T3-Add	1			1		
T2a	0.775	0.678-0.886		0.717	0.588-0.875	
N category			0.004			< 0.001
0	1			1		
1	1.340	1.068-1.682		1.762	1.306-2.378	
2	1.632	1.192-2.235		2.171	1.440-3.271	
VPI			0.028			
Without	1					
with	1.179	1.018-1.365				

a Age, sex, grade, positive lymph nodes, T category, N category and VPI were included in the LASSO-penalized Cox model of OS.
b Grade, positive lymph nodes, T category and N category were included in the LASSO-penalized Cox model of CSS.
LASSO, least absolute shrinkage and selection operator; NSCLC, non-small cell lung cancer; PSM, propensity score matching; OS, overall survival; CSS, cancer specific survival; T, tumor; T3-Add, T3 tumors with additional nodules in the same lobe; N, node; VPI, visceral pleural invasion.

the first time that the 5-year CSS rate of the T3-Add patients was 72.5%, which was better than previously thought.

In the current TNM staging manual, T3 category descriptors include tumor size greater than 5 cm but less than or equal to 7 cm, tumors with additional nodules in the same lobe and tumors with parietal pleural, chest wall, pericardium or phrenic nerve invasion (4). In the analysis of the classification of lung cancer with separate tumor nodules, the results of the IASLC lung cancer staging project showed a trend toward longer OS in the T3-Add group when compared with other T3 groups, but the result was not statistically significant (9). Therefore, the project proposed that tumors with same-lobe tumor nodules should be classified as T3. However, controversy exists on the T category of these patients. A Netherland study analyzed the data of 683 pT3N0M0 NSCLC patients and demonstrated that the adenocarcinoma subgroup of the T3-Add patients and T2 patients had comparable survival rates, whereas the T3-Add patients in the squamous cell carcinoma subgroup should remain classified as T3 category (13).

To date, only one study had externally validated the current T2b and T3-Add category developed by IASLC (4). In the study by Kumar et al. (8), the authors reviewed 31,563 T2b-3N0-3M0 NSCLC patients and demonstrated that the T2b and T3-Add patients had similar 5-year OS rates (53.4% vs. 52.3%). So, they concluded that this finding should be taken into consideration in the forthcoming 9th edition of TNM staging manual. The strengths of their study were the large number of cases in several subgroups and the implementation of PSM method. However, clinical TNM stage was used in their study which could lead to bias. In addition, only OS was evaluated, which might limit clinical reference value. In our study, only resected patients were included to ensure the accuracy of staging. We also analyzed the CSS differences between these two groups of patients. It is known that the older patients have a high rate of comorbidities, which could lead to a high risk of competing non-cancer related events. In this study, the median age of included patients was 69 years. In addition, the 5-year CSS rate of the T3-Add patients was much better than the corresponding 5-year

TABLE 3 LASSO-penalized multivariable Cox analysis of the T2b and T3-Add NSCLC patients after PSM.

Variables	OS[a]			CSS[b]		
	HR	95% CI	*P*	HR	95% CI	*P*
Age, years			< 0.001			
Continue	1.037	1.025-1.048				
Sex			< 0.001			
Male	1					
Female	0.581	0.489-0.689				
Grade			< 0.001			< 0.001
Well	1			1		
Moderate	1.882	1.372-2.581		3.142	1.723-5.728	
Poor/undifferentiated	2.957	2.159-4.048		5.487	3.024-9.955	
Positive lymph nodes			0.082			
Continue	1.047	0.994-1.103				
N category			0.001			< 0.001
0	1			1		
1	1.322	1.006-1.737		1.864	1.329-2.616	
2	1.997	1.376-2.900		3.301	2.261-4.821	

a Age, sex, grade, positive lymph nodes and N category were included in the LASSO-penalized Cox model of OS.
b Grade and T category were included in the LASSO-penalized Cox model of CSS.
LASSO, least absolute shrinkage and selection operator; NSCLC, non-small cell lung cancer; PSM, propensity score matching; T3-Add, T3 tumors with additional nodules in the same lobe; OS, overall survival; CSS, cancer specific survival; N, node.

OS rate (72.5% vs. 51.5%). Therefore, it is necessary to explore the CSS differences between these two groups.

Our study is meaningful. Our study revealed that the survivals of the T3-Add patients was comparable to those of the T2b patients, which added to the evidence on the topic that it is necessary to reconsider the T category for the NSCLC patients with additional nodules in the same lobe. The accurate TNM staging is crucial to estimate patients' prognosis and drive subsequent treatments selection. Although the current National Comprehensive Cancer Network (NCCN) guideline (12) for NSCLC recommends nondifferential treatment modalities for the T3-Add and T2b patients, in the real-world setting, the former one is less likely to receive surgery than the latter one. It is reported that only half of the T3-Add patients were treated surgically as compared with up to 90% of the T2b patients (8). The reason behind the difference was that surgery was not included in the initial treatments for most of the T3-Add patient with non-operative management because the physicians did not recommend it (8). In our view, just like T2b patients, T3-Add patients do benefit from surgery, and these patients should be treated properly.

Our study had some limitations. First, the information about additional tumor nodules recorded in the SEER data set is not specific, which only includes a simple description that CS Site-Specific Factor 1 code 010: separate tumor nodules in the same lobe of ipsilateral lung. The rigor definition of T3-Add is that a solid lung tumor with at least one additional tumor nodule of similar imaging appearance and matching histologic appearance (14), and the information is not recorded in the SEER data set. However, due to the low incidence of the T3-Add tumors, it might be hard to draw a conclusion with strong statistical power from a single institute. Therefore, the superiority of large number of cases deposited in the SEER data set is reflected. Second, the common drawback of the public data set is the rough deposited data. In this study, other important prognostic variables for example comorbidity, surgical margin and genetic and molecular factors are lacking. Further efforts on multicenter study results collection and broader clinicopathological variables recruitment are encouraged. At last, although PSM method was used in this study, the retrospective design may have contributed to bias. We hoped future works could validate our results.

Conclusion

T3-Add and T2b NSCLC patients had similar survivals, and we proposed that it is necessary to reconsider the T category for the NSCLC patients with additional nodules in the same lobe in the forthcoming 9th edition of TNM staging manual.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession

number(s) can be found below: SEER database (https://seer.cancer.gov/).

Ethics statement

The studies involving human participants were reviewed and approved by Peking University People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

XW and FY: Conceptualization, Supervision, Writing-Reviewing and Editing. J-SC: Methodology, Software, Data curation, Writing-Original draft preparation. XW: Data curation. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1043386/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Kaplan–Meier estimates of survivals in T3-Add patients stratified by tumor size. **(A)** overall survival: T3-Add (0-30 mm) vs. T3-Add (30-50 mm) vs. T3-Add (50-70 mm) vs. T3-Add (> 50 mm); **(B)** cancer specific survival: T3-Add (0-30 mm) vs. T3-Add (30-50 mm) vs. T3-Add (50-70 mm) vs. T3-Add (> 50 mm). T3-Add, T3 non-small cell lung cancer with additional tumor nodules in the same lobe.

SUPPLEMENTARY FIGURE 2
Prognostic variables selection using the LASSO regression model in the T3-Add & T2a pair after PSM. LASSO coefficient profiles of 14 variables against the log (Lambda) sequence for overall survival **(A)** and cancer specific survival **(C)**. Tuning parameter (Lambda) selection in the LASSO model used 10-fold cross-validation *via* minimum criteria (overall survival: **(B)** cancer specific survival: **(D)**. LASSO, least absolute shrinkage and selection operator; PSM, propensity score matching.

SUPPLEMENTARY FIGURE 3
Prognostic variables selection using the LASSO regression model in the T3-Add & T2b pair after PSM. LASSO coefficient profiles of 14 variables against the log (Lambda) sequence for overall survival **(A)** and cancer specific survival **(C)**. Tuning parameter (Lambda) selection in the LASSO model used 10-fold cross-validation *via* minimum criteria (overall survival: **(B)** cancer specific survival: **(D)**. LASSO, least absolute shrinkage and selection operator; PSM, propensity score matching.

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Efficacy and safety of pembrolizumab versus sintilimab treatment in patients with advanced squamous lung cancer: A real-world study in China

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Importance: Both pembrolizumab and sintilimab have been approved by the Chinese State Drug Administration (NMPA) for the first-line treatment of patients with advanced squamous lung cancer. The differences of the two drugs in efficacy and safety are unclear.

Objectives: To compare the real-world efficacy and safety of first-line treatments in patients with advanced squamous lung cancer.

Materials and methods: This was a retrospective review of patients with advanced squamous carcinoma who received sintilimab or pembrolizumab in combination with chemotherapy as first-line therapy between June 2018 and April 2022 in the Chinese PLA Hospital. The primary objective was to compare the objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) between the two groups. Secondary objectives were to compare the disease control rate (DCR) and to analyze adverse events (AEs) between the two groups.

Results: A total of 164 patients were enrolled, including 63 patients (38.4%) in the sintilimab-combined chemotherapy group and 101 patients (61.6%) in the pembrolizumab-combined chemotherapy group. The ORR was 65.10% in the sintilimab group and 61.40% in the pembrolizumab group (P=0.634). The DCR was 92.10% and 92.10% in the sintilimab and pembrolizumab groups, respectively (P=0.991). The median PFS was 22.2 months for patients treated with sintilimab group compared with 16.5 months for patients treated with pembrolizumab group[hazard ratio (HR) = 0.743; 95% confidence interval (CI): 0.479-1.152; P = 0.599]. Patients treated with pembrolizumab did not achieve a median OS, and patients treated with sintilimab had a median OS of 30.7 months. In the sintilimab group, the incidence of all treatment-related adverse events

(TRAEs) was 92.1% (58/63), and the incidence of grade 3-4 TRAEs of 42.9% (27/63). In the pembrolizumab group, the incidence of all TRAEs was 90.1% (91/101), and the incidence of grade 3-4 TRAEs was 37.6% (38/101).

Conclusion: In the clinical treatment of Chinese patients with advanced squamous lung cancer, first-line treatment with sintilimab in combination with chemotherapy provided similar efficacy to pembrolizumab in combination with chemotherapy, and the treatment-related adverse effect profiles were comparable between the two groups, including similar rates of grade 3-4 and all adverse events.

KEYWORDS

squamous lung cancer, pembrolizumab, sintilimab, efficacy, safety

1 Introduction

Lung cancer is a malignant neoplastic disease with the highest mortality rate in the world today (1). Non-small cell lung cancer (NSCLC), the most common histologic type, accounts for more than 85% of all lung cancers. Squamous lung cancer cases account for approximately 17% of all NSCLC cases (2). Advanced squamous lung cancer patients have poor prognosis, who receive treatment with platinum-based regimens struggling to achieve the one-year overall survival time. Programmed cell death protein 1 (PD1) is one of the checkpoints that regulates the immune response. Currently, immune checkpoint inhibitors (ICIs), such as programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) inhibitors, have been widely used in clinical practice, showing good efficacy and safety in a variety of tumors (3). ICIs also bring a new opportunity for the treatment of patients with advanced squamous lung cancer. Studies have shown that immunotherapy in combination with chemotherapy can result in significant improvements in patients, which may be related to the immunological effects mediated by chemotherapeutic agents through direct and indirect stimulation of immune responses and increased tumor immunogenicity.

Pembrolizumab is a humanized monoclonal anti-PD-1 antibody that has been widely used in the clinical treatment of a variety of malignancies. The KEYNOTE-407 clinical trial demonstrated that pembrolizumab in combination with platinum-based therapies can be the standard of treatment in the first-line treatment of advanced squamous lung cancer, regardless of PD-L1 expression (4–6). Sintilimab is a fully human IgG4 monoclonal antibody, which has a unique PD-1 epitope that blocks the binding of PD-1 to PD-L1 and PD-L2 (7). Based on the ORIENT-12 clinical trial, sintilimab in combination with gemcitabine and platinum-based therapies was approved by the NMPA for the first-line treatment of nonsurgically resectable locally advanced or metastatic squamous lung cancer (8). The NMPA has approved platinum-based therapies for the first-line treatment of inoperable advanced or metastatic squamous lung cancer. According to the latest Chinese Society of Clinical Oncology (CSCO) 2022 guidelines on clinical practice of immune checkpoint inhibitors and CSCO guidelines on NSCLC, both pembrolizumab in combination with chemotherapy and sintilimab in combination with platinum-based chemotherapy are recommended as Class 1A first-line therapy for patients with advanced squamous lung cancer without driver mutations.

In KEYNOTE-407 and ORIENT-12 clinical trials, both pembrolizumab and sintilimab showed good efficacy and safety in the treatment of advanced squamous lung cancer, but the clinical trial populations were different, with pembrolizumab being used in a predominantly non-Asian population and sintilimab in a predominantly Chinese population. The binding sites and biological activities of pembrolizumab and sintilimab are different. There is a lack of real-world comparative studies on the efficacy and safety of different immunotherapeutic agents in patients with advanced squamous lung cancer. Therefore, we conducted a retrospective cohort study to compare the efficacy and safety of real-world treatment with sintilimab and pembrolizumab as first-line therapy in patients with advanced squamous lung cancer.

2 Materials and methods

2.1 Patient characteristics

This retrospective study was conducted in patients with advanced squamous lung cancer who received consecutive chemotherapy in combination with sintilimab or pembrolizumab as first-line treatment at the Chinese PLA general hospital (Beijing, China) between June 2018 and April 2022. The inclusion criteria were as follows: 1) pathologically definite diagnosis of squamous epithelial cell carcinoma of the lung; 2) patients with advanced squamous non-small cell lung cancer of stage IIIB-IV according to the International Association for the Study of Lung Cancer (IASLC) TNM Staging of Lung Cancer (8th edition) and relevant imaging; and 3) patients who received at least 2 cycles of sintilimab or pembrolizumab in combination with chemotherapy in first-line treatment; 4) patients with lesions available for imaging measurements and evaluation for

efficacy; and 5) ECOG score ≤ 2. Exclusion criteria were as follows: 1) lack of clear pathological diagnosis; 2) lung squamous carcinoma combined with other malignancies; and 3) patients who have received previous antineoplastic therapy. As this study was retrospective, a waiver of personal consent was allowed. All procedures performed in this study were in accordance with the Declaration of Helsinki (revised 2013).

2.2 Treatment options

Patients were treated with either pembrolizumab (200 mg every 3 weeks over 30 min IV infusion) or sintilimab (200 mg every 3 weeks IV infusion). The chemotherapeutic drug regimens were platinum-based dual drug regimen, including gemcitabine in combination with cisplatin or carboplatin and paclitaxel in combination with cisplatin or carboplatin, chosen by the clinician on a case-by-case basis. Chemotherapeutic agents were administered as follows: gemcitabine 1,250 mg/m^2, intravenously; albumin-bound paclitaxel 260 mg/m^2, intravenously; paclitaxel 175 mg/m^2, intravenously; cisplatin 75 mg/m^2, intravenously; carboplatin AUC 5 mg/ml/min, intravenously.

2.3 Assessment

Basic patient characteristics and clinical information were collected, including age, sex, smoking history, Eastern Cooperative Oncology Group physical status (ECOG-PS), tumor TNM stage, histologic type, metastases, PD-L1 expression, number of treatment cycles, time to progression, time to death, and adverse events. Computed tomography (CT) scans of the chest and abdomen and magnetic resonance imaging (MRI) of the head were collected and evaluated for efficacy according to the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) definition. Efficacy evaluation included complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The objective response rate (ORR) was defined as (CR+PR)/(CR+PR+SD+PD)×100%; the disease control rate (DCR) was defined as (CR+PR+SD)/(CR+PR +SD+PD)×100%. Progression-free survival (PFS) was defined as the time interval between the start of first-line treatment and disease progression or death; overall survival (OS) was defined as the time between the start of first-line treatment and death from any cause. PFS data or OS data were censored for patients who had not progressed, were lost to follow-up or were still alive at the end of the follow-up period. The follow-up cutoff date was August 24, 2022. Evaluation of all adverse events: Adverse reactions were evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (Class I-IV).

2.4 Statistical analysis

SPSS 26.0 was used for statistical analysis. Categorical variables were expressed as frequencies and percentages, and continuous variables were expressed as medians and ranges. Baseline characteristics and efficacy data of the two treatment groups were compared using the $\chi 2$ test or Wilcoxon rank sum test. Kaplan-Meier survival models were developed, and PFS and OS were compared between the two groups using the log-rank test. For subgroup analysis, PFS and OS were calculated using the same method after classifying patients by age, sex, smoking status, ECOG-PS, tumor TNM stage, pathological type, PD-L1 expression, and treatment strategy. Differences with p values < 0.05 were considered statistically significant differences.

3 Results

3.1 Patient baseline information characteristics

A total of 164 patients with advanced squamous lung cancer receiving pembrolizumab or sintilimab in combination with chemotherapy as first-line therapy were enrolled in this study. Table 1 shows the baseline characteristics of the patients. Sixty-three patients (38.4%) were in the sintilimab group, and 101 patients (61.6%) were in the pembrolizumab monotherapy group. The baseline characteristics of the patients in both groups were comparable.

The median age was 65 years (57-72 years) in the sintilimab group and 65 years (55-74 years) in the pembrolizumab group. The proportion of men was higher than that of women in both groups. 54 patients in the sintilimab group and 72 patients in the pembrolizumab group were past or current smokers. Stage IV patients were predominant in both groups, with 55 (87.3%) and 85 (84.2%) patients; there were 8 (12.7%) and 16 (15.8%) and IIIB/IIIC patients in the sintilimab and pembrolizumab groups, respectively. A total of 44 patients with squamous lung cancer had a family history, including 19 in the sintilimab group and 25 in the pembrolizumab group. There were 48 patients with distant metastases in the sintilimab group; 16 patients (25.4%) had bone metastases, 3 (4.8%) had brain metastases, 5 (7.9%) had liver metastases, 3 (4.8%) had adrenal metastases, and 6 (9.5%) had pleural metastases. There were 86 patients with distant metastases in the pembrolizumab group; 16 (15.8%) had bone metastases, 10 (9.9%) had brain metastases, 7 (6.9%) had liver metastases, 9 (8.9%) had adrenal metastases, and 6 (5.9%) had pleural metastases. A total of 79 patients underwent a PD-L1 (22C3) expression assay before treatment. In the sintilimab group, 7 patients had high PD-L1 expression (PD-L1 ≥ 50%), 12 patients had low PD-L1 expression (1% ≤ PD-L1 < 50%), and 7 patients had negative PD-L1 expression (PD-L1 < 1%). In the pembrolizumab group, 10 patients had high PD-L1 expression (PD-L1 ≥ 50%), 26 had low PD-L1 expression (1% ≤ PD-L1 < 50%), and 17 had negative PD-L1 expression (PD-L1 < 1%) (Table 1).

3.2 Recent results

In the sintilimab group, 41 (65.1%) patients achieved PR, 17 (27.0%) patients achieved SD, and 5 (7.9%) patients developed PD.

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TABLE 1 Baseline characteristics of the study participants.

Characteristic	Pembrolizumab (N=101)	Sintilimab (N=63)	P value
Median age (range), years	65 (55-74)	65 (57-72)	
Age, years			0.246
≥65	54(53.5%)	27(42.9%)	
<65	47(46.5%)	36(57.1%)	
Sex			0.484
Male	94 (93.1%)	61 (96.8%)	
Female	7 (6.9%)	2 (3.2%)	
Smoking history			0.089
Never	29 (28.7%)	9 (14.3%)	
Current	5 (5.0%)	3 (4.8%)	
Past	67 (66.3%)	51 (81.0%)	
Stage			0.744
IIIB/IIIC	16 (15.8%)	8 (12.7%)	
IV	85 (84.2%)	55 (87.3%)	
Family History			0.563
Yes	25 (24.7%)	19 (30.2%)	
No	76 (73.3%)	44 (69.8%)	
Metastasis			0.311
Brain	10 (9.9%)	3 (4.8%)	
Bone	16 (15.8%)	16 (25.4%)	
Liver	7 (6.9%)	5 (7.9%)	
Adrenal gland	9 (8.9%)	3 (4.8%)	
Pleural	6 (5.9%)	6 (9.5%)	
PD-L1 expression			0.455
Not examined	48 (47.5%)	37 (58.7%)	
<1%	17 (16.8%)	7 (11.1%)	
≥1%	36(35.6%)	19(30.1%)	
1%-49%	26 (25.7%)	12 (19.0%)	
≥50%	10 (9.9%)	7 (11.1%)	
Combination of chemotherapy			
Gemcitabine+Cisplatin	5(5.0%)	3(5.0%)	
Gemcitabine+Carboplatin	2(2.0%)	0	
Paclitaxel+Cisplatin	74(73.3%)	42(66.7%)	
Paclitaxel+Carboplatin	20(19.8%)	18(28.6%)	
Combination of radiotherapy			0.867
Yes	20 (19.8%)	11 (17.5%)	
No	81 (80.2%)	52 (82.5%)	

In the pembrolizumab group, 62 (61.3%) patients developed PR, 31 (30.7%) patients developed SD, and 8 (7.9%) patients developed PD. The ORRs in the sintilimab and pembrolizumab groups were 65.1% and 61.4% (P=0.634), and the DCRs were 92.0% and 92.0% (P=0.991), respectively (Table 2).

3.3 Long-term survival

There was a median PFS of 22.20 months in the sintilimab group and a median PFS of 16.50 months in the pembrolizumab group (HR = 0.734; 95% CI: 0.479-1.152; P = 0.599). In patients with negative PD-L1 expression, median PFS was not achieved after sintilimab treatment compared with a median PFS of 11.43 months after pembrolizumab treatment (HR = 5.837; 95% CI: 0.989-10.66; P=0.054). In patients with positive PD-L1 expression, the median PFS after sintilimab treatment was 12.83 months compared with a median PFS of 16.40 months with pembrolizumab treatment (HR = 0.765; 95% CI: 0.366-1.557; P=0.449). Subgroup analysis based on PD-L1 expression showed that patients with high PD-L1 expression did not achieve median PFS after treatment with sintilimab compared with a median PFS of 18.40 months for patients treated with pembrolizumab (HR = 0.914; 95% CI: 0.214-3.881; P = 0.901). In patients with low PD-L1 expression, the median PFS was 10.93 months after sintilimab treatment compared with a median PFS of 10.67 months after pembrolizumab treatment (HR = 0670; 95% CI: 0.278-1.514; P = 0.320; Figure 1). Subgroup analysis based on age, smoking status, tumor stage, PD-L1 expression level, and whether or not to combine chemotherapy revealed no significant difference in PFS between patients in the sintilimab and pembrolizumab groups (Figure 2A).

Overall survival analysis revealed a median OS of 30.70 months in the sintilimab group and a median OS not reached in the pembrolizumab group (HR = 1.045; 95% CI: 0.607-1.802; P=0.699). Subgroup analysis based on PD-L1 expression showed that median OS after sintilimab treatment was not achieved in patients with negative PD-L1 expression, while median OS in the pembrolizumab treatment group was 28.27 months (HR = 3.445; 95% CI: 0.609-9.729; P=0.210). In patients with positive PD-L1 expression, the median OS was not reached after pembrolizumab treatment, while the median OS in the sintilimab treatment group was 26.37 months (HR = 0.588; 95% CI: 0.208-1.506; P=0.253). Neither sintilimab nor pembrolizumab treatment-group patients achieved median OS if their levels of PD-L1 expression were high (HR = 0.211; 95% CI: 0.014-2.005; P=0.160). In patients with low PD-L1 expression, the median OS was 26.37 months after sintilimab treatment, while the median OS was not reached in the pembrolizumab treatment group (HR = 0.641; 95% CI: 0.203-1.852; P=0.390; Figure 3). Subgroup analysis based on age, smoking status, tumor stage, PD-L1 expression level, and whether or not to combine chemotherapy revealed no significant differences in OS between patients in the sintilimab and pembrolizumab groups (Figure 2B).

3.4 Adverse reactions

The incidence of treatment-related AEs of any grade was 92.1% and 90.1% in the sintilimab and pembrolizumab groups, respectively, while the incidence of grade 3-4 AEs was 42.9% and 37.6%, respectively. The most common adverse reactions in the sintilimab group were alopecia (50.8%), constipation (36.5%), anemia (34.9%), and nausea (33.3%), while in the pembrolizumab group, the most common adverse reactions were alopecia (44.6%), constipation (41.6%), anemia (38.6%), and nausea (35.6%). The most common grade 3-4 AE in the sintilimab group was alopecia (19.1%). The most common grade 3-4 AEs in the pembrolizumab group were alopecia (11.9%) and reduced white blood cell count (11.9%). No patient had a grade 5 AE (Table 3).

4 Discussions

Squamous lung cancer accounts for approximately 25%-30% of all lung cancers (9). Because of its unique clinical features, pathological manifestations and genetic mutation characteristics, squamous lung cancer is significantly different from lung adenocarcinoma in treatment and is often explored as a separate type in clinical studies. Patients with advanced squamous lung cancer are often unable to benefit from targeted therapy due to the lack of driver mutations (10, 11). Most patients with squamous lung cancer have a history of heavy smoking, resulting in complex genetic mutations and a high tumor mutational load (12). Complex mutations can cause neoantigen production, while a high tumor mutational load can drive effective antitumor immune responses and lead to a sustained clinical response to immunotherapy. These findings provide a rationale for lung squamous cancer patients to benefit from immunotherapy (13, 14).

TABLE 2 Comparison of short-term clinical outcomes between the two groups.

Best overall response	Pembrolizumab (N=101)	Sintilimab (N=63)	P value
CR	0	0	
PR	62	41	
SD	31	17	
PD	8	5	
ORR%	61.40%	65.10%	0.634
DCR%	92.10%	92.10%	0.991

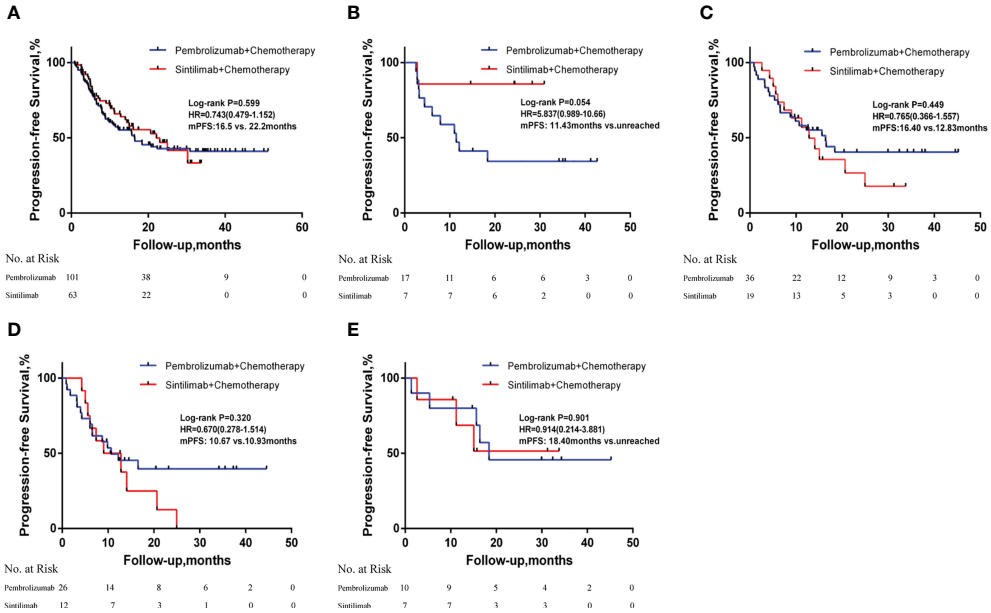

FIGURE 1
Kaplan-Meier curves of progression-free survival in **(A)** all patients; **(B)**patients with negative PD-L1 expression; **(C)** patients with positive PD-L1 expression; **(D)** patients with low PD-L1 expression and **(E)** patients with high PD-L1 expression.HR, hazard ratios; mPFS, median progression-free survival; PD-L1, programmed death-ligand 1.

In recent years, it has been shown that immunotherapy in combination with chemotherapy can lead to significant improvements in patient outcomes, possibly related to the immunological effects mediated by chemotherapeutic agents through direct and indirect stimulation of immune responses and increased tumor immunogenicity. Some clinical studies of immunotherapy combined with chemotherapy, such as KEYNOTE-407 (7), IMpower131 (15), and ORIENT-12 (16), compared the efficacy of immune-combination chemotherapy with chemotherapy alone. Treatment with a combination of paclitaxel/albumin paclitaxel + carboplatin and pembrolizumab significantly prolonged OS and PFS compared to chemotherapy

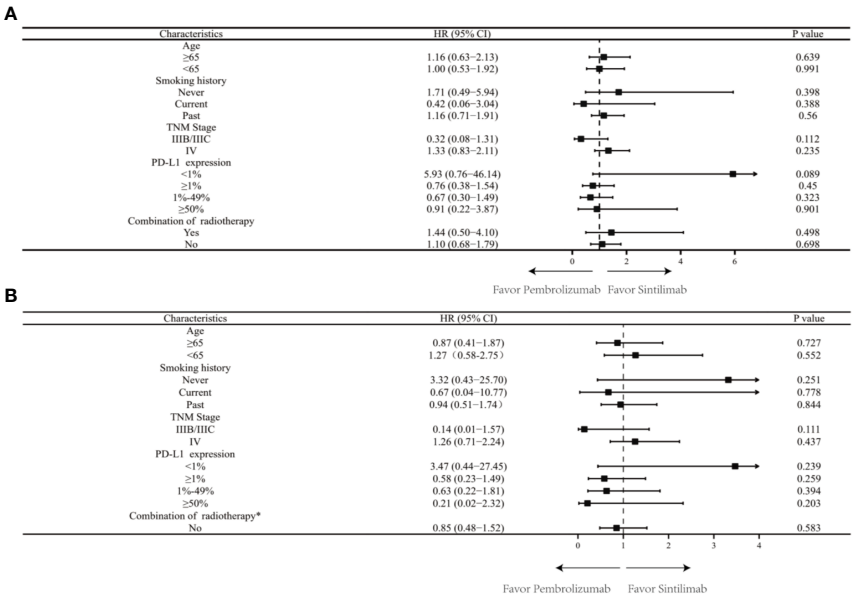

FIGURE 2
(A) Progression-free survival by subgroup in the full analysis set. TMN, tumor, node, metastasis; PD-L1, programmed death-ligand 1; HR, hazard ratios; CI, confidence interval; **(B)** Overall survival by subgroup in the full analysis set. TMN, tumor, node, metastasis; PD-L1, programmed death-ligand 1; HR, hazard ratios; CI, confidence interval.*Data not presented for subgroups of "Yes in Combination of radiotherapy" owing to very few patients which precludes any meaningful analysis.

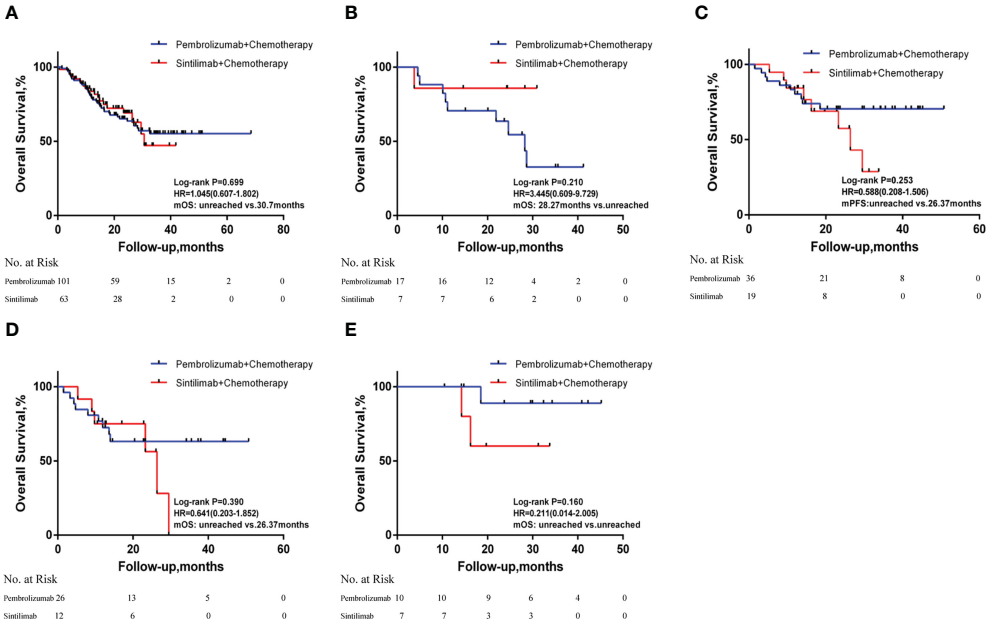

FIGURE 3
Kaplan-Meier curves of overall survival in **(A)** all patients; **(B)** patients with negative PD-L1 expression; **(C)** patients with positive PD-L1 expression; **(D)** patients with low PD-L1 expression and **(E)** patients with high PD-L1 expression. HR, hazard ratios; mOS, median overall survival; PD-L1, programmed death-ligand 1.

TABLE 3 Comparison of adverse events between the two groups.

Comparison of adverse drug reactions between the two groups (n, %)				
Adverse event	Pembrolizumab+chemotherapy (N=101)		Sintilimab+chemotherapy (N=63)	
	All grades	Grade III-IV	All grades	Grade III-IV
Any terms	91(90.1%)	38(37.6%)	58(92.1%)	27(42.9%)
Alopecia	45(44.6%)	12(11.9%)	32(50.8%)	12(19.1%)
Anemia	39(38.6%)	8(7.9%)	22(34.9%)	7(11.1%)
White blood cell count decreased	32(31.7%)	12(11.9%)	20(31.7%)	8(12.7%)
Neutrophil count decreased	31(30.7%)	9(8.9%)	19(30.2%)	8(12.7%)
Platelet count decreased	20(19.8%)	5(5%)	12(19%)	3(4.8%)
Nausea	36(35.6%)	2(2%)	21(33.3%)	3(4.8%)
Vomiting	9(8.9%)	1(1%)	8(12.7%)	2(3.2%)
Decreased appetite	24(23.8%)	5(5%)	18(28.6%)	3(4.8%)
Constipation	42(41.6%)	3(3%)	23(36.5%)	3(4.8%)
Diarrhea	9(8.9%)	2(2%)	8(12.7%)	1(1.6%)
Transaminases increased	21(20.8%)	4(4%)	16(25.4%)	2(3.2%)
Fatigue	30(29.7%)	4(4%)	18(28.6%)	2(3.2%)
Peripheral neuropathy	23(22.8%)	5(5%)	15(23.8%)	3(4.8%)
Rash	12(11.9%)	2(2%)	6(9.5%)	2(3.2%)
Weight decreased	20(19.8%)	2(2%)	10(15.9%)	3(4.8%)

alone (15.9 months vs. 11.3 months and 6.4 months vs. 4.8 months, respectively) (7). The IMpower131 study comparing atezolizumab combined with carboplatin and albumin-bound paclitaxel to chemotherapy alone in patients with stage IV squamous NSCLC revealed that the median OS in the ITT population was 14.2 months (95% CI: 12.3-16.8) vs. 13.5 months (95% CI: 12.2-15.1), HR=0.88 (95% CI: 0.73-1.05), p=0.158. However, in patients with high PD-L1 expression or in the TC3/IC3 subgroup, an OS advantage was seen with atezolizumab in combination with chemotherapy (23.4 months (95% CI: 17.8-NE) vs. 10.2 months (95% CI: 7.1-17.5), HR=0.48 (95% CI: 0.29-0.81) (15). In the ORIENT-12 study comparing the efficacy of the PD-1 inhibitor sintilimab in combination with gemcitabine and platinum versus chemotherapy alone, the median progression-free survival in the sintilimab in combination with gemcitabine and platinum group versus the chemotherapy alone group was 5.5 months and 4.9 months (HR=0.536, 95% CI: 0.422-0.681, p<0.00001), and the ORR in the sintilimab ORR in the combination chemotherapy group was 44.7% (16). Patients enrolled in a clinical trial (RCT) must meet the restrictions and criteria required by the trial, but as the availability of immunotherapeutic agents in oncology patients continues to increase, there are increasingly more patients in practice who do not meet the strict requirements of RCTs regarding treatment with these immunotherapeutic agents. The criteria in different clinical trials may not reflect the heterogeneity of real-world oncology populations. This study is based on real-world data and compares two agents with similar near-term efficacy, long-term survival benefit, and safety profile in the current Chinese clinical setting for treatment of patients with advanced squamous non-small cell lung cancer. To our knowledge, this study is the first real-world study to retrospectively compare treatment efficacy and safety of two PD-1 inhibitors in patients with advanced squamous lung cancer.

The values of ORR obtained in this study are similar to those obtained in previous clinical trials (4, 7, 16). In the pembrolizumab arm of this study, the ORR for squamous NSCLC was as high as 61.4%. The ORR for patients with squamous NSCLC in the sintilimab arm was 65.1%. In the pembrolizumab group, the DCR in squamous NSCLC patients was as high as 92.0%. The DCR in patients with squamous NSCLC in the sintilimab group was 92.0%. There was no statistically significant difference in ORR and DCR between the two drugs. Median OS data from the ORIENT-12 clinical trial are not yet available, and the median OS according to the KEYNOTE-407 Chinese population data was 30.1 months. The values of median OS in the two groups in our study were similar to the values arrived at in previous clinical trials. The median PFS in both groups in our study was longer than the PFS reported in the KEYNOTE-407 and ORIENT-12 clinical trials. In the pembrolizumab group, the median PFS for patients with squamous NSCLC was 16.5 months. In the sintilimab group, the median PFS for squamous NSCLC patients was 22.2 months, with no statistically significant difference in median PFS between the two groups. The following considerations may explain the phenomenon observed in the data of this study. First, the combined immune-drug chemotherapy regimen in the real world is different from the treatment regimen in clinical trials. Patients in the sintilimab group in this study received immune combination paclitaxel or albumin-bound paclitaxel and platinum regimens in approximately 92.1% of all patients, applied immune combination immune combination docetaxel and platinum in 3.2%, and applied immune combination gemcitabine and platinum in only 5%; this is different from the ORIENT-12 trial in which all patients used immune combination gemcitabine and platinum-based regimens and may have contributed to the differences in ORR and PFS in the sintilimab group in this study compared to the ORIENT-12 trial. It has been suggested that sintilimab combined with paclitaxel or albumin-bound paclitaxel chemotherapy may have similar clinical benefits compared to sintilimab combined with gemcitabine and platinum-based chemotherapy in patients with untreated advanced or metastatic squamous non-small cell lung cancer (17). In the pembrolizumab group in this study, immune therapy in combination with paclitaxel or albumin-bound paclitaxel and carboplatin regimens was received in a total of 17.8% of all patients, immune therapy in combination with paclitaxel or albumin-bound paclitaxel and cisplatin or loplatin in a total of 68.3%, immune therapy in combination with gemcitabine and platinum in 7%, and immune therapy in combination with docetaxel and platinum in tacitaxel and platinum in 7%, which is different from the KEYNOTE-407 trial in which all patients received immune therapy in combination with an albumin paclitaxel/paclitaxel + carboplatin regimen; this may have contributed to the differences in ORR and PFS values for patients in the pembrolizumab group in this study compared with the KEYNOTE-407 trial. Notably, there was no significant difference between ORR, PFS and OS in the two groups in this study. Second, the proportion of patients with high PD-L1 and positive PD-L1 expression in our study was much higher than that in the clinical trials (7, 16). Among patients who received PD-L1 expression assays prior to pembrolizumab treatment, 67.9% (36/53) were PD-L1 positive, exceeding the proportion reported in the KEYNOTE-407 clinical trial. Among patients who received PD-L1 expression assays prior to sintilimab treatment, 73.1% (19/26) were PD-L1 positive, exceeding the rate reported in the ORIENT-12 clinical trial study. PD-L1 has been found to be expressed at high levels in most NSCLC patients and appears to be a favorable prognostic factor for early-stage disease, and higher PD-L1 expression is associated with a survival benefit in NSCLC patients (18, 19); however, there remains a subset of patients with PD-L1 TPS < 1% who could benefit from immunotherapy alone, suggesting that PD-L1 is an imperfect predictive biomarker (20, 21).

It is worth noting that the binding targets and biological properties of the two drugs are not identical, which may account for the different ORR, PFS and OS values observed between the two groups of patients. Pembrolizumab is a humanized monoclonal antibody that binds to the programmed cell death protein 1 (PD-1) receptor on T cells and blocks its interaction with PD-L1 and PD-L2 ligands, which is a key immune checkpoint pathway. Pembrolizumab is composed of a human IgG4 kappa constant region and a murine anti-human PD-1 monoclonal antibody variable region (22). The predominant binding site for the

combination of pembrolizumab and PD-1 is the C'D loop structure, which currently stands as the most efficacious PD-1/PD-L1 inhibitor in terms of affinity. The unique structure of pembrolizumab provides high specificity and affinity for PD-1, leading to potent immune checkpoint inhibition. Sintilimab, on the other hand, is a fully human monoclonal antibody that also targets the PD-1 receptor on T cells, but it has a different antibody structure from pembrolizumab. Sintilimab is composed of a human IgG4 kappa constant region and a fully human anti-human PD-1 monoclonal antibody variable region (23). The fully human structure of sintilimab is thought to potentially reduce the risk of immunogenicity and infusion reactions compared to pembrolizumab. The primary binding site for the combination of sintilimab and PD-1 is the FG loop structure. Both pembrolizumab and sintilimab are effective immunotherapies that target the PD-1 receptor. However, the difference in efficacy between the two drugs due to the difference in drug structure and biological properties is unclear yet.

Our study showed no significant difference between the two groups in terms of median PFS and OS. In terms of PFS and OS in patients with advanced squamous lung cancer, the results of these subgroup analyses in different strata of PD-L1 as well as in different age strata suggest that, clinically, sintilimab is not inferior to pembrolizumab. Both sintilimab and pembrolizumab may have common adverse drug reactions such as fatigue, rash, diarrhea and nausea. It is worth noting that the mechanism of action of both drugs is to activate the killing function of T cells by inhibiting PD-1/PD-L1 pathway to achieve the purpose of killing tumor. However, in the process of activating T cells, the difference of the binding targets and biological properties of the two drugs may interfere with differences in the incidence of adverse reactions between the two drugs. In our study, the spectrum of adverse reactions in the two groups in this study was generally similar to the spectrum of adverse reactions observed in previous clinical trials for both drugs, while the incidence of AEs of any grade and grade 3-4 AEs in this study was relatively consistent between the sintilimab and pembrolizumab groups; there were no significant differences, and the safety profiles were good.

There are some limitations to this study. First, this was a single-center retrospective study with a relatively small sample size. Therefore, information bias cannot be avoided, and the study results need to be further confirmed by retrospective or prospective studies that involve large samples. Second, due to the limited follow-up period, the median OS of patients in both groups in some subgroup analyses was not reached. We will further extend the follow-up period to refine the study data. Third, the PD-L1 expression level is now expected to be the first potential predictive biomarker to predict the outcome and prognosis of patients with advanced NSCLC (24, 25). Patients representing a particular subset of squamous NSCLC cases in this study did not undergo immunohistochemical PD-L1 testing for various reasons, and it is necessary to retrospectively analyze pathological samples from this population to expand the sample size for further analysis. In addition, treatment selection bias was inevitable in the two groups of patients in this study. In the real world, dosing and chemotherapy regimens cannot be administered in exactly the same way as used in clinical trials due to various factors. Although these factors somewhat attenuate the validity and reliability of the conclusions, the findings of this study are still highly relevant to the selection of clinical treatment regimens.

5 Conclusions

In summary, this study demonstrates that in real-world patients with advanced squamous lung cancer, first-line treatment with sintilimab in combination with chemotherapy is similar in near-term efficacy, long-term survival benefit and safety to combined treatment with pembrolizumab and chemotherapy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

WY: study design, experiments, and manuscript writing. WY, MG, TW, and LW: analysis and interpretation of data.WY, TL, YB, and YL: collection and interpretation of data. FZ, HT, JM, FJ, LW, and YH: interpretation of data and manuscript revision. 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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Predictive factors and prognosis of immune checkpoint inhibitor-related pneumonitis in non-small cell lung cancer patients

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Objective: To investigate the influencing factors and prognosis of immune checkpoint inhibitor-related pneumonitis (CIP) in advanced non-small cell lung cancer (NSCLC) patients during or after receiving immune checkpoint inhibitors(ICIs).

Methods: The clinical and laboratory indicator data of 222 advanced NSCLC patients treated with PD-1/PD-L1 inhibitors at the First Affiliated Hospital of Zhengzhou University between December 2017 and November 2021 were collected retrospectively. The patients were divided into a CIP group (n=41) and a non-CIP group (n=181) according to whether they developed CIP or not before the end of follow-up. Logistic regression was used to evaluate risk factors of CIP, and Kaplan–Meier curves were used to describe the overall survival (OS) of different groups. The log-rank test was used to compare the survival of different groups.

Results: There were 41 patients who developed CIP, and the incidence rate of CIP was 18.5%. Univariate and multivariate logistic regression analyses showed that low pretreatment hemoglobin (HB) and albumin (ALB) levels were independent risk factors for CIP. Univariate analysis suggested that history of chest radiotherapy was related to the incidence of CIP. The median OS of the CIP group and non-CIP were 15.63 months and 30.50 months (HR:2.167; 95%CI: 1.355-3.463, $P<0.05$), respectively. Univariate and multivariate COX analyses suggested that a high neutrophil-to-lymphocyte ratio (NLR) level, a low ALB level and the development of CIP were independent prognostic factors for worse OS of advanced NSCLC patients treated with ICIs. Additionally, the early-onset and high-grade CIP were related to shorter OS in the subgroup.

Conclusion: Lower pretreatment HB and ALB levels were independent risk factors for CIP. A high NLR level, a low ALB level and the development of CIP were independent risk factors for the prognosis of advanced NSCLC patients treated with ICIs.

KEYWORDS

lung cancer, immune checkpoint inhibitor-related pneumonitis, hemoglobin, albumin, survival

1 Introduction

Lung cancer ranks first among all causes of cancer-related deaths around the world (1), while non-small cell lung cancer (NSCLC) accounts for more than 85% of lung cancers. Several clinical trials have confirmed that PD-1 inhibitors alone or combined with first-line chemotherapy for advanced NSCLC can bring significant survival benefits (2–4). However, the subsequent adverse reactions can not be ignored. Immune checkpoint inhibitors (ICIs) may cause immune-related adverse events(irAEs) such as rash, pruritus, pneumonitis, diarrhea, immune-mediated colitis, hepatitis and endocrine system problems (5–7). Among them, immune checkpoint inhibitor-related pneumonitis (CIP), which is a rare but fatal immune-related adverse reaction, has an incidence of 2% to 5%, with a mortality rate of 20% for grade 3 or higher CIP (8). The occurrence of CIP may also be associated with the tumor type, with a meta-analysis showing that, compared with patients suffering other cancers, patients with lung cancer are more likely to experience all-grade or high-grade CIP (9).

Previous studies have suggested that age, smoking history, preexisting lung diseases, history of chest radiotherapy, and the combination of two or more ICIs may be associated with the development of CIP (10–13). However, the sample size of CIP patients in these studies was small, and more influencing factors of CIP warrant further investigation. Hematologic inflammatory parameters can reflect the inflammatory status of the body; they have the advantages of being easily available, economical and convenient and play an important predictive role in the prognosis of tumors. The most explored parameters are the neutrophil-to-lymphocyte ratio (NLR) (14) and platelet-to-lymphocyte ratio (PLR) (15). Studies on the hematological inflammatory parameters of CIP are rarely reported. Thus, this study aimed to explore the risk factors for CIP, the relationship between hematological inflammatory parameters and the occurrence of CIP, and the survival of CIP patients.

2 Materials and methods

2.1 Study population

The included population was 222 advanced NSCLC patients treated with ICIs at the First Affiliated Hospital of Zhengzhou University between December 2017 and November 2021.

There were 145 patients who were treated with ICIs as first-line treatment, and 77 patients received immunotherapy as second- or further-line treatment. According to the ASCO guideline, CIP was diagnosed on the basis of computed tomography scans and clinical manifestations, excluding the diagnosis of disease progression, lung infection, and radiation pneumonitis (16). The treating investigators graded the severity of the pneumonitis using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The study was conducted following the guidelines of Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (2022-KY-1316-001).

2.2 Data collection

Age, sex, smoking status, primary tumor type, clinical stage, underlying lung disease, whether targeted therapy was used, therapeutic regimen, hematological indexes within 1 week prior to immunotherapy, and history of prior radiotherapy were all obtained from medical records for all patients. The NLR was calculated as the neutrophil count/lymphocyte count. Baseline was defined as the moment to initiate ICIs; overall survival (OS) was defined as the interval between the start of immunotherapy and the date of death owing to any reason, or the last follow-up. We conducted the last follow-up up to June 29, 2022, by telephone and medical records.

2.3 Statistical analysis

All statistical analyses were conducted using SPSS version 21.0, and the results were then plotted by GraphPad prism version 8.0. The Kolmogorov–Smirnov test was used to determine whether continuous data had a normal distribution. Continuous data with a normally distributed distribution are reported as the mean ± standard deviation, and were compared by Student's *t* test. Categorical variables are summarized as the number of patients and percentages and were compared by the chi-square test or Fisher's exact test. Logistic regression was used to evaluate risk factors of CIP, and the Kaplan–Meier curve was used to describe the OS of different groups. The log-rank test was performed to compare the survival of different groups. The Cox regression method was used to evaluate the correlation of CIP and clinical characteristics with OS. $P<0.05$ was considered a statistically significant difference.

3 Results

3.1 Characteristics of CIP

According to whether they developed CIP or not before the end of follow-up, 222 patients were divided into a CIP group (n=41) and a non-CIP group (n=181). The numbers of patients with grade 1, 2, 3, 4 and 5 CIP were 10(24.39%),18(43.90%), 7 (17.07%), 6(14.63%) and 0 (0.00%) respectively. The median time from ICI initiation to the occurrence of CIP was 109 days [(interquartile range(IQR) :30-221] after ICI treatment, and the incidence of CIP was approximately 18.5%. The most common symptoms were cough (78.05%), fever (43.90%), dyspnea (53.66%) and chest tightness (34.15%). Early-onset CIP was defined as occurring within 6 weeks after commencement of ICI treatment, and late-onset CIP was defined as occurring beyond 6 weeks after starting ICI treatment (17). There were 12 patients who developed early-onset CIP, while 29 patients developed late-onset CIP. The results are shown in Table 1.

TABLE 1 Details of CIP.

Characteristics	Number
Grade of CIP	
1	10 (24.39%)
2	18 (43.90%)
3	7 (17.07%)
4	6 (14.63%)
5	0(0.00%)
Time from ICIs initiation to the occurrence of CIP(days)	109 (IQR: 30-221)
Occurrence within 6weeks	12 (29.27%)
Common symptoms	
Cough	32 (78.05%)
Fever	18 (43.90%)
Dyspnea	22 (53.66%)
Chest tightness	14 (34.15%)

3.2 The relationship between the occurrence of CIP in advanced NSCLC patients treated with ICIs and hematological parameters and clinical characteristics

The results of univariate analysis showed that there were no statistically significant differences between the two groups in terms of age, pathological type, preexisting lung disease, smoking status, immunotherapy combined with chemotherapy, brain metastases, the type of ICIs, NLR or monocytes (P>0.05). There was a difference in the history of chest radiotherapy between the two groups; 22.0% of the patients in the CIP group and 8.8% of the patients in the non-CIP group had a history of radiotherapy. The mean baseline hemoglobin (HB) level was lower in the CIP group (113.34 ± 15.20 g/L) than in the non-CIP group (124.37 ± 17.92 g/L) (P<0.001, t=3.653). The pretreatment albumin (ALB) level was lower in the CIP group (37.55g/L, IQR: 34.38-40.35) than in the non-CIP group (39.65g/L, IQR: 36.75-42.30, P<0.05) (Table 2).

3.3 Results of multivariate analysis

Multivariate logistic regression analysis showed that pretreatment HB and pretreatment ALB levels were independent predictive factors for CIP (Table 3). The best cutoff value was obtained by plotting the receiver operating characteristic (ROC) curve (Figure 1) with the occurrence of CIP before the end of follow-up as the status variable and pretreatment HB and ALB as the test variables. The area under the curve (AUC) of pretreatment HB value was 0.678 (95%CI: 0.596-0.759, P<0.001), with the highest predictive value at a pretreatment HB value of 120.9 g/L, resulting in a sensitivity of 68.3% and a specificity of 61.3% for predicting the occurrence of CIP. The AUC of pretreatment ALB value was 0.641 (95% CI:0.549-0.734, P<0.05), with the highest predictive value at a pretreatment ALB value of 38.75 g/L, resulting in a sensitivity of 65.8% and a specificity of 57.3% for predicting the occurrence of CIP (P<0.05; Figure 1).

3.4 Prognosis

The median OS of the CIP group (15.63 months, 95% CI: 6.33-24.94) was shorter than that of the non-CIP group (30.50 months, 95% CI: 21.67-39.33), and there was a statistically significant difference (P< 0.05; Figure 2A). The median OS of the grade 3-4 CIP group (4.07months, 95%CI:2.32-5.82) was shorter than that of the grade 1-2 CIP group (24.87months, 95%CI:11.66-38.08), and there was a statistically significant difference (P<0.05, Figure 2B). The median OS of early-onset CIP patients (4.07 months, 95% CI: 1.57-6.56) was shorter than that of the late-onset CIP patients (24.73months, 95% CI: 14.66-34.81, P<0.05, Figure 2C). The Cox multivariate regression analysis results showed that a lower pretreatment ALB level, the occurrence of CIP and a high baseline NLR value were negative predictors for the OS of NSCLC patients treated with ICIs (Table 4).

4 Discussion

At present, there are still a few reports about CIP, some from clinical trials and some from the real-world, but there are no clear conclusions about its incidence and risk factors. Some clinical trials and reports have shown that the incidence of CIP is approximately 5% (18, 19). A multi-institutional cohort study recently has found that the risk of pneumonitis associated with PD-1/PD-L1 inhibitors compared with non-immunotherapy was 2.49% (95% CI: 1.50%-3.47%), and the median time to the onset of CIP was 3.9 months (IQR: 2.1-7.3) (20). In our study, the median time to the occurrence of CIP was 109 days (IQR: 30-221) after ICI treatment, and the incidence of CIP was approximately 18.5%. Some reports have shown that the incidence of CIP is higher than in those clinical trials (21, 22), which is consistent with our results. The rising occurrence of CIP in the real world may be due to the increased vigilance of clinicians toward CIP in recent years. The incidence rate of CIP requires more real-world data for feedback and verification in studies with larger sample size.

Our study demonstrated that the occurrence of CIP was increased in patients who had undergone thoracic radiotherapy. Although the results of our multivariate logistic regression analysis showed that previous chest radiotherapy was not an independent risk factor for CIP, the Keynote-001 trial demonstrated that patients who received thoracic radiotherapy before pembrolizumab were more likely to develop CIP of any grade than those who did not (23). This may be due to the damage to lung function caused by a certain dose of radiation to the lung, the continuous low-level release of inflammatory factors caused by radiotherapy, and ICIs promoting an increase in the level of inflammatory factors. This also suggests that

TABLE 2 Basic information of patients in the two groups.

Variables	The occurrence of CIP			*P*
		YES (n=41)	NO (n=181)	
Age	<65	25 (61.0%)	115 (63.5%)	0.759
	≥65	16 (39.0%)	66 (36.5%)	
Gender	Male	28 (68.3%)	150 (82.9%)	0.034
	Female	13 (31.7%)	31 (17.1%)	
Smoking history	Yes	17 (41.5%)	93 (51.4%)	0.251
	Never	24 (58.5%)	88 (48.6%)	
Clinical Stage	IIIb-IIIc	16 (39.0%)	64 (35.4%)	0.659
	IV	25 (61.0%)	117 (64.6%)	
Preexisting Lung Disease	Yes	6 (14.6)	27 (14.9)	0.963
	No	35 (85.4)	154 (85.1)	
Histology	Squamous cell	18 (43.9%)	92 (50.8%)	0.537
	Adenocarcinoma	21 (51.2%)	76 (42.0%)	
	Others	2 (4.9%)	13 (7.2%)	
PD-L1 Expression	TPS≥50%	7 (17.1%)	26 (14.4%)	0.594
	1%≤TPS<50%	8 (19.5%)	50 (27.6%)	
	TPS<1%	10 (14.4%)	50 (27.6%)	
	Unknown	16 (39.0%)	55 (30.4%)	
Brain Metastases	Yes	8 (19.5%)	30 (16.6%)	0.652
	No	33 (80.5%)	151 (83.4%)	
Chest Radiotherapy History	Yes	9 (22.0%)	16 (8.8%)	0.034
	No	32 (78.0%)	165 (91.2%)	
ALK/EGFR-TKIs History	Yes	12 (29.3%)	26 (14.4%)	0.022
	No	29 (70.7%)	155 (85.6%)	
Therapy Protocols	Immune monotherapy	2 (4.9%)	20 (11.0%)	0.366
	ICIs± chemotherapy	39 (95.1%)	161 (89.0%)	
Treatment Line	1st line	24 (58.5%)	121 (66.9%)	0.313
	≥2nd line	17 (41.5%)	60 (33.1%)	
ICIS	PD-1	39 (95.1%)	177 (97.8%)	0.676
	PD-L1	2 (4.9%)	4 (2.2%)	
Laboratory Findings	ALB (g/L)	37.6 (34.4, 40.4)	39.7 (36.8, 42.3)	0.006
	HB (g/L)	113.3 ± 15.2	124.4 ± 17.9	<0.001
	NLR	4.0 (2.3,5.4)	3.0 (2.1,5.4)	0.329
	Monocyte (10^9/L)	0.6 (0.4,0.8)	0.6 (0.4,0.7)	0.824

radiation has an immunomodulatory effect. Radiation-induced cell death generates molecular signals and inflammatory cytokines that facilitate the ability of dendritic cells to deliver antigens to T cells (24). Therefore, radiotherapy is often used in combination with ICIs for NSCLC because of their synergistic effects, but we should be wary of the increase in toxicity during application.

One study found that a low serum ALB level with pembrolizumab was an independent predictor of CIP (25), consistent with the finding of our study. In addition, Hu et al. found that increased ALB concentration was associated with improved lung function (26). ALB is an acute phase reactant that can show the inflammatory state of the body; a decrease in ALB may

TABLE 3 Analyses of risk factors for the occurrence of CIP.

Variables	Univariate analysis			Multivariate analysis		
	P	OR	95% CI	*P*	OR	95% CI
Male	0.038	0.445	0.208-0.955	0.112	0.481	0.195-1.186
EGFR/ALK-TKI history	0.025	2.467	1.119-5.349	0.085	2.293	0.893-5.889
Chest radiotherapy history	0.020	2.900	1.179-7.135	0.067	2.711	0.934-7.871
ICI treatment ≥2nd line	0.314	0.700	0.350-1.401			
Smoking history	0.253	1.492	0.751-2.964			
HB (g/L)	0.001	0.965	0.946-0.985	0.029	0.974	0.951-0.997
NLR	0.904	1.006	0.910-1.113			
ALB (g/L)	0.015	0.912	0.847-0.982	0.045	0.919	0.846-0.998

be related to the inflammatory state of the body, and these mechanisms lead to the occurrence of CIP. In addition, to our knowledge, we are the first to find that a lower pretreatment HB level is associated with the occurrence of CIP. Although no study has reported that HB values can predict the occurrence of CIP, He et al. found that low HB was independently associated with the occurrence of community-acquired pneumonia in pregnant women (27). HB plays the role of transporting oxygen, and its deficiency is related to hypoxia, which may promote the deficiency of lung function, making patients susceptible to pneumonitis. On the other hand, decreased HB levels are related to weakened immunity (28), leading to insufficient cellular immunity, which also promotes the development of pneumonitis to some extent. Zhao et al. found that anemia was also correlated with T-cell deficiency in mice (29), so the decline in HB may also predispose people to CIP through immunosuppression. As we know, previous anticancer treatment can have an impact on HB and ALB levels. Bone marrow suppression induced by chemotherapy or radiotherapy can make HB decrease, and gastrointestinal adverse effects cause patients to lack appetite, malnutrition, and ALB decline. In our study, there was no difference in HB and ALB levels between patients treated with ICIs in the first-line and second-line and beyond (Supplementary Table 1). In addition, we found that the treatment lines of immunotherapy did not correlate with CIP. However, a different result has been reported. Khunger et al. have conducted a Meta-analysis showing that the incidence of all grades of CIP was significantly higher in treatment naive patients than in previously treated ones (30). Whether the treatment lines of immunotherapy are related to the incidence of CIP by affecting levels of HB and ALB still needs to be explored in the future.

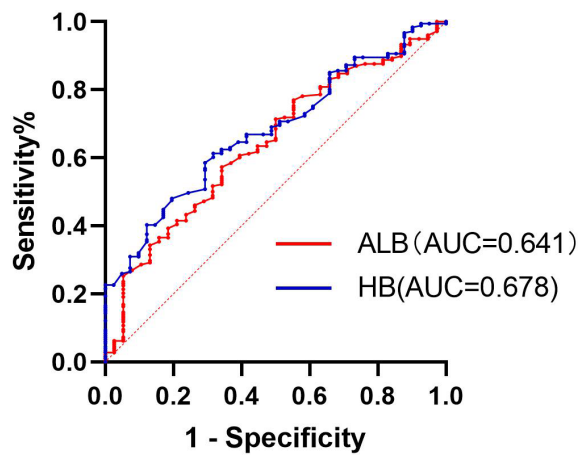

FIGURE 1
The ROC curve of ALB and HB for predicting the occurrence of CIP.

In this study, the OS of patients in the CIP group was shorter than that of patients in the non-CIP group. We also found that patients who developed early-onset and high-grade CIP had shorter OS than those who developed late-onset and low-grade CIP. Previous research showed that compared with patients without irAEs, the OS of patients with irAEs was substantially prolonged (31), suggesting that the presence of irAEs may be related to prognosis. A recent study by Haratani et al. showed that the occurrence of any irAE was related to longer PFS and OS in advanced NSCLC patients (10), and other studies have reported similar results (17, 31–33). In light of these findings, irAEs are generally regarded as indicators of NSCLC patients' improved response to PD-1 inhibitors and longer survival. Nevertheless, the number of patients with CIP included in these reports was quite small, and some studies reported different findings. One study showed that grade 1-2 CIP was linked to good OS; however, grade 3-4 CIP was not (34). Fukihara et al. revealed that CIP patients had considerably shorter OS than non-CIP patients (25). This may be in part because patients with CIP frequently need to stop using PD-1 inhibitors since they can induce deadly respiratory failure, unlike those with skin responses or thyroid problems. What's more, CIP directly affects the patient's respiratory function and thus survival. In addition, some studies have shown that the use of glucocorticoids may shorten patient's OS (35, 36), which a proportion of CIP patients usually have difficulty avoiding using. These factors may together contribute to the shortened survival of CIP patients.

In addition, this study found that pretreatment ALB was associated with OS in patients with advanced NSCLC receiving immunotherapy. ALB reflects nutritional status and response to inflammation and is related to the treatment outcome of NSCLC.

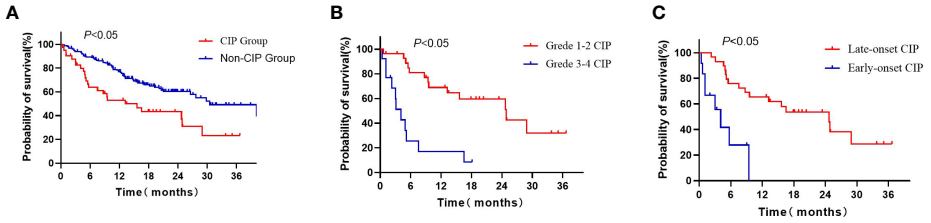

FIGURE 2
Kaplan–Meier curves for OS of advanced NSCLC patients: **(A)** Kaplan–Meier curves for OS of CIP patients and non-CIP patients; **(B)** Kaplan–Meier curves for OS of grade 1-2 CIP and grade 3-4 CIP patients; **(C)** Kaplan–Meier curves for OS of late-onset CIP and early-onset CIP patients. CIP, imune checkpoint inhibitor-related pneumonitis; OS, overall survival.

TABLE 4 Analyses of factors potentially associated with overall survival of the advanced NSCLC patients treated with ICIs.

Variables	Univariate analysis			Multivariate analysis		
	P	HR	95% CI	*P*	HR	95% CI
Male	0.617	1.147	0.671-1.960			
Age(y)	0.047	1.026	1.000-1.052	0.678	1.005	0.980-1.031
Smoking history	0.139	0.729	0.480-1.107			
Preexisting lung disease	0.448	1.257	0.697-2.265			
Chest radiotherapy history	0.412	0.759	0.392-1.467			
ICI treatment ≥2nd line	0.528	0.871	0.568-1.337			
Occurrence of CIP	0.001	2.167	1.355-3.463	0.006	2.019	1.219-3.345
NLR	0.001	1.083	1.032-1.137	0.009	1.080	1.019-1.145
ALB (g/L)	<0.001	0.909	0.870-0.950	0.011	0.943	0.901-0.987
HB (g/L)	<0.001	0.976	0.965-0.987	0.093	0.988	0.975-1.002

Hypoalbuminemia has been reported to be associated with low survival rates in tumor patients (37). Our study also found that a high NLR before treatment was associated with a worse prognosis after immunotherapy. The NLR is an effective index to reflect the degree of the inflammatory response and immune status. The systemic inflammatory response is considered to be closely related to the occurrence and progression of tumors. Some studies have shown that high levels of the NLR are closely related to the poor prognosis of lung cancer (38), which was in agreement with our results.

The findings of this study on CIP are valuable for clinicians to better understand the risk factors and prognosis for the development of CIP, and help us to recognize populations with these characteristics. In this way, we can take full account of possible toxicity risks when immunotherapy is administered to these patients, and be alert to the incidence of CIP and make appropriate clinical decisions to obtain the maximum benefit of immunotherapy. Compared to the current published studies facing the same topic, our work had several strengths: First, we were the first to find that a lower pretreatment HB level was associated with the occurrence of CIP. In addition, we have found that patients who developed CIP had a worse prognosis than those who did not; Third, we found that early-onset and high-grade CIP were associated with a worse prognosis than late-onset and low-grade ones. However, there were two limitations in our study: First, it was a retrospective study, and we could not determine the patient's treatment strategy. In addition, there was no preassessment of the patient's lung function, and the sample size was not large enough. Treatment modalities still need to be explored in studies with larger sample sizes.

5 Conclusion

Lower pretreatment HB and ALB levels were independent predictors of CIP. The occurrence of CIP, a lower pretreatment ALB level, and a high pretreatment NLR value were negative predictors for the prognosis of NSCLC patients treated with ICIs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study design was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

XL: study design, data collection and analysis, writing-original draft, and writing – review & editing. NH: study design, data collection, and writing – review & editing. SY: study design, data collection, and writing – review & editing. JL: study design, data collection, and writing– review & editing. LW: formal analysis and writing – review & editing. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1145143/full#supplementary-material

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Preoperative diagnosis of solitary pulmonary nodules with a novel hematological index model based on circulating tumor cells

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Objective: Preoperative noninvasive diagnosis of the benign or malignant solitary pulmonary nodule (SPN) is still important and difficult for clinical decisions and treatment. This study aimed to assist in the preoperative diagnosis of benign or malignant SPN using blood biomarkers.

Methods: A total of 286 patients were recruited for this study. The serum FR^+CTC, TK1, TP, TPS, ALB, Pre-ALB, ProGRP, CYFRA21-1, NSE, CA50, CA199, and CA242 were detected and analyzed.

Results: In the univariate analysis, age, FR^+CTC, TK1, CA50, CA19.9, CA242, ProGRP, NSE, CYFRA21-1, and TPS showed the statistical significance of a correlation with malignant SPNs (P <0.05). The highest performing biomarker is FR^+CTC (odd ratio [OR], 4.47; 95% CI: 2.57–7.89; P <0.001). The multivariate analysis identified that age (OR, 2.69; 95% CI: 1.34–5.59, P = 0.006), FR^+CTC (OR, 6.26; 95% CI: 3.09–13.37, P <0.001), TK1 (OR, 4.82; 95% CI: 2.4–10.27, P <0.001), and NSE (OR, 2.06; 95% CI: 1.07–4.06, P = 0.033) are independent predictors. A prediction model based on age, FR^+CTC, TK1, CA50, CA242, ProGRP, NSE, and TPS was developed and presented as a nomogram, with a sensitivity of 71.1% and a specificity of 81.3%, and the AUC was 0.826 (95% CI: 0.768–0.884).

Conclusions: The novel prediction model based on FR^+CTC showed much stronger performance than any single biomarker, and it can assist in predicting benign or malignant SPNs.

KEYWORDS

pulmonary nodules, diagnosis, biomarkers, hematological index model, nomogram

Introduction

A solitary pulmonary nodule (SPN) is a single intraparenchymal lung lesion with a diameter of less than 3 cm. Most SPNs are benign nodules, such as pulmonary hamartoma and tuberculoma (1). The incidence of malignancies for SPNs ranged from 0.5% to 3.5%, mostly primary lung cancer (2). It depends on the age of patients, smoking status, history of cancer, nodule diameter, nodule volume, spiculated margins, and nodule location (3). The most common pathological types of malignant SPNs are adenocarcinoma and squamous cell carcinoma (4, 5). However, both nodules share similar imaging features, such as spiculated margins and lobulated structures (6, 7). The imaging diagnostics of lung cancer patients include morphological imaging modalities such as chest X-ray (CXR) and computed tomography (CT) and nuclear medicine procedures such as positron emission tomography (PET). Most of the pulmonary nodules smaller than 1 cm will not be visible on chest radiographs (8). Additionally, at least 95% of the nodules identified by computed tomography (CT) are benign (9). In clinical practice, differentiating malignant from benign nodules by conventional imaging alone has been challenging, with false positive and false negative rates up to 75% and 48%, respectively (10). Functional abnormalities can be found using PET before they appear morphologically on traditional imaging, and some studies have shown good diagnostic performance in SPN (11, 12). However, their performance is affected by the patient's stratification. A meta-analysis comparing the diagnostic value of ^{18}F-FDG-PET/CT versus CT observed no significant differences in sensitivity, specificity, PLR, NLR, DOR, and AUC (10). Serum biomarkers have many advantages over tissue-based detection, such as being non-invasive and easily repeatable. Nevertheless, they have low sensitivity in diagnosing malignancies yet high false-positive rates in benign tumors or infections (13). The utility of single serum biomarkers in SPN diagnosis is thus limited, and clinical guidelines generally recommend that combinations of serum biomarkers be used to improve detection efficiency (14). Though many prediction models have been developed, few are widely used in clinical practice (15, 16). It is, therefore, imperative to identify novel biomarkers and prediction models supporting the early diagnosis of non-small cell lung cancer (NSCLC).

Folate receptor alpha (FRα) is a glycoprotein that is anchored to the cell membrane of normal epithelial cells and highly expressed in a variety of tumor cells of epithelial origin, including lung, colorectal, ovarian, etc. (17–19). An FR-based CTC detection has been developed, and the related FR-positive CTC (FR$^+$CTC) detection kit has been approved by the CFDA for clinical application. FR$^+$CTCs have high sensitivity (73.2%–81.8%) and specificity (84.1%–93.2%) for the diagnosis of lung cancer (20, 21). FR$^+$CTCs combined with common cancer biomarkers have been proven to improve diagnostic efficiency significantly in patients with NSCLC (20, 22). Xue et al. reported that FR$^+$CTCs are reliable biomarkers that have a better performance than serum carcinoembryonic antigen (CEA), neuron–specific enolase (NSE), cytokeratin 19 fragments (CYFRA21–1), squamous cell carcinoma antigen (SCC), progastrin–releasing peptide (Pro-GRP), and heat shock protein 90–α (Hsp90α) in patients with small-sized nodules (23). FR$^+$CTCs for the diagnosis of SPNs have been examined in a small prospective study (24). However, the utility of FR$^+$CTC levels in combination with serum and tumor biomarkers to build a diagnostic model in NSCLC patients with SPNs was not reported.

In this study, we aimed to explore the expression of peripheral blood FR$^+$CTCs, establish a diagnostic model based on FR$^+$CTCs, and combine serum biomarkers in patients with SPNs. Furthermore, the study helps guide the clinical treatment strategies for pulmonary nodules.

Methods

Patients and data collection

A total of 1,627 patients diagnosed with lung cancer or pulmonary nodules at the Sichuan Cancer Hospital & Institute from November 2016 to December 2020 were analyzed retrospectively. Finally, 271 patients were included in this study (Figure 1) based on the inclusion and exclusion criteria as follows: (1) Patients with chest CT images indicated pulmonary nodules; (2) pulmonary nodules were less than 3 cm; and (3) pretreatment hematological detection, including folate receptor-positive circulating tumor cell (FR$^+$CTC) level, thymidine kinase 1 (TK1), total protein (TP), albumin (ALB), pre-albumin (PALB), pro-gastrin-releasing peptide (ProGRP), recombinant cytokeratin fragment antigen 21-1 (CYFRA21-1), tissue polypeptide specific antigen (TPS), neuron-specific enolase (NSE), carbohydrate antigen 50 (CA50), carbohydrate antigen 199 (CA19.9), and carbohydrate antigen 242 (CA242) were available. Exclusion criteria: (1) patients had a history of malignancy or any other serious chronic diseases; and (2) patients underwent surgery, chemotherapy, anti-infection, anti-tuberculosis, or targeted therapy.

This study was approved by the Ethics Committee of Sichuan Cancer Hospital (No. SCCHEC-02-2017-042). All samples for hematological detection were collected from each patient before the initiation of treatment. Demographic characteristics were collected through the hospital information system (HIS). We present the following article in accordance with the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) reporting guideline.

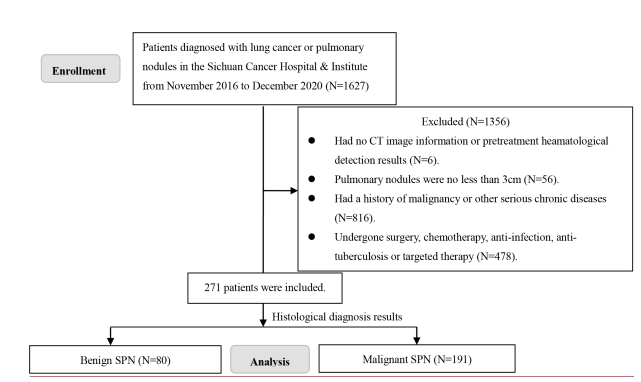

FIGURE 1
Screening flowchart of the participants.

FR^+CTC extraction and quantification

After collection, whole blood samples for Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis FR^+CTC detection were conducted within 24 h according to the manufacturer's protocol for the CytoploRare Kit (Genosaber Biotech, Shanghai, China). At first, the erythrocytes were lysed with lysing buffer, and then leukocytes and macrophages were removed with anti-CD45 and anti-CD14, respectively. Secondly, the enriched samples were labeled with detection probes that contained conjugates of a tumor-specific ligand folic acid and a synthesized oligonucleotide. The oligonucleotide (5'-CTCAA CTGGT GTCGT GGAGT CGGCA ATTCA GTTGA GGGTT CTAA-3') was used for subsequent PCR amplification.

Folate receptor-expressing cells were eluted and quantified by the ABI 7500 Real-Time PCR System (ThermoFisher, MA, USA) after washing out free conjugates. The primer sequences were as follows: forward primer 5'-TATGA TTATG AGGCA TGA-3'; reverse primer 5'-GGTGT CGTGG AGTCG-3'; and TaqMan probe 5'-FAM-CAGTT GAGGG TTC-MGB-3'. The quantitative analysis of FR-positive CTC was calculated through the amplification curve of the sample and standard reference.

Detection of serum biomarkers

TK1 was detected by enhanced chemiluminescence dot blot assay (Sino-Swed Tong Kang Bio-Tech, Shenzhen, China). The serum TP, ALB, and pre-ALB were determined with a Clinical Laboratory Beckman Coulter AU5800. CA50 and CA242 were measured with the electrochemiluminescence immunoassay system CL-6000i (Mindray, China). In addition, the electrochemiluminescence immunoassay system LIAISON® XL (Nanjing Tao Ze Bio-Technology, China) was also used to detect TPS and NSE. Moreover, ProGRP, CYFRA21-1, and CA199 were detected by the electrochemiluminescence immunoassay system Cobas E411 (Roche, Germany), respectively.

Statistical analyses

At first, numerical data was applied to the normality test. Normally distributed data were shown as mean ± standard deviation (SD). Alternatively, other data were shown as medians (interquartile range, IQR). Student's t-tests were used to analyze normally distributed data between groups. Also, non-normally distributed data were analyzed by the Mann–Whitney U test. Categorical data were presented as numbers (percentages) and compared using the Chi-square test. The clinical data and hematological biomarkers were used to construct a univariate logistic regression model and a multivariate logistic regression model for the whole cohort. The final multivariate logistic model was developed by stepwise regression to obtain the best result with the smallest Akaike information criterion (AIC) (25). A nomogram was drawn based on the multivariate logistic regression model. The validity of the nomogram was evaluated by the calibration curve and the Hosmer–Lemeshow goodness of fit test. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of hematological biomarkers based on the area under the curve (AUC). We define the maximum Youden Index as the optimal cutoff value. Statistical analysis was conducted using R software version 4.1.0 (The Free Software Foundation, Boston, MA, USA). The "pROC" and "ggplot2" packages were used to draw the ROC and calibration curves. The "generalhoslem" package was used to conduct the Hosmer–Lemeshow test. A two-sided P <0.05 was considered significant.

Results

Characteristics of malignant and benign SPNs

In total, 191 malignant solitary pulmonary nodules (SPNs) and 80 benign SPN patients with pretreatment hematological biomarkers were included in this study (Figure 1). The mean age of the malignant and benign SPN groups was 59.24 ± 10.91 years old and 52.48 ± 9.51 years old, respectively. The median FR^+CTC level in the malignant SPN group was 10.69 (95% CI: 9.16, 13.59), which was higher than that of the benign SPN group at 8.91 (95% CI: 6.68, 13.36) (P = 0.0014) (Figure 2A) (Table 1). CA19.9, ProGRP, CYFRA21.1, and TPS were significantly different between the malignant and benign groups (all P <0.05) (Figures 2B–E) (Table 1). The detailed information on the clinical characteristics and pretreatment hematological biomarkers of the patients is summarized in Table 1.

Univariate and multivariate analysis of hematological biomarkers in distinguishing malignant SNPs

In the univariate analysis, sex, TP, ALB, and PALB were not significantly correlated with malignant SPNs (all P >0.05, Table 2). Age, FR^+CTC, TK1, CA50, CA19.9, CA242, ProGRP, NSE, Cyfra21.1, and TPS showed statistical significance of correlation with malignant SPNs (all P <0.05, Table 2). The highest performing hematological biomarker is FR^+CTC (odd ratio [OR], 3.44; 95% CI: 2.57–7.89; P <0.001) (Table 2). These factors, which showed significant results in the univariate analysis, were prepared for multivariate analysis. AIC was applied to variate selection, and age, FR^+CTC, TK1, CA50, CA242, ProGRP, NSE, and TPS were included in the final multivariate prediction model. The formula of the prediction model was: (logit(p) = −3.09 + 0.99 ∗ Age + 1.83 ∗ CTC + 1.57 ∗ TK1 + 0.56 ∗ CA50 + 0.84 ∗ CA242 + 0.52 ∗ ProGRP + 0.72 ∗ NSE + 0.56 ∗ TPS). The multivariate analysis identified that age (OR, 2.69; 95% CI: 1.34–5.59; P = 0.006), FR^+CTC (OR, 6.26; 95% CI: 3.09–13.37; P <0.001), TK1 (OR, 4.82; 95% CI: 2.40–10.27; P <0.001) and NSE (OR, 2.06; 95% CI: 1.07–4.06; P <0.001) are independent predictors (Table 2).

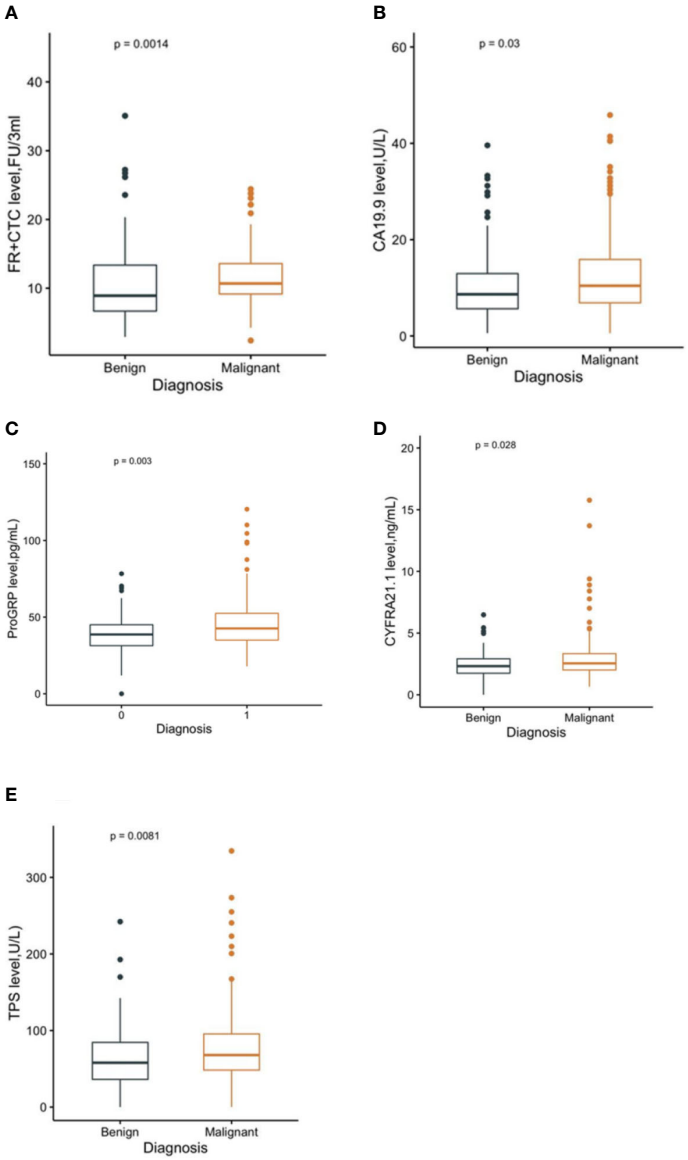

FIGURE 2
The level of FR$^+$CTC **(A)**, CA19.9 **(B)**, ProGRP **(C)**, CYFRA21.1 **(D)**, and TPS **(E)** in malignant and benign groups, respectively.

Diagnostic value of hematological biomarkers in distinguishing malignant SNPs

The ROC curve was used to further analyze the diagnostic value of pretreatment hematological biomarkers in distinguishing malignant SNPs (Figure 3). The optimal diagnostic cutoff values for FR$^+$CTC, TK1, CA50, CA242, ProGRP, NSE, and TPS were 9.005 FU/3 ml, 1.965 pM, 5.24 U/L, 1.705 U/L, 41.085 pg/ml, 10.515 ng/ml, and 67.155 U/L, respectively. A single marker did not perform well in distinguishing between malignant and benign SNPs (all AUC <0.70) (Table 3). The multivariate prediction model, based on stepwise logistic regression and combined age, FR$^+$CTC, TK1, CA50, CA242, ProGRP, NSE, and TPS, showed much stronger performance, with a sensitivity of 71.1% and specificity of 81.3%, and the AUC was 0.826 (95% CI: 0.768–0.884) (Table 3; Figure 3).

Nomogram development and validation

The prediction model containing age, FR$^+$CTC, TK1, CA50, CA242, ProGRP, NSE, and TPS was presented as a nomogram (Figure 4A). The Hosmer–Lemeshow test yielded significant goodness of fit (P = 0.04) (Figure 4B), and the C-index of the nomogram was 0.826 (Table 3).

TABLE 1 Baseline characteristics of benign and malignant SPNs.

Characteristics	Overall (N = 271)	Benign SPN (N = 80)	Malignant SPN (N = 191)	P-value
Age (mean (SD), years)	57.24 (10.94)	52.48 (9.51)	59.24 (10.91)	<0.001
Sex (n, %)				
Female	140 (51.7)	44 (55.0)	96 (50.3)	0.563
Male	131 (48.3)	36 (45.0)	95 (49.7)	
FR^{+}CTC (median [IQR], FU/3 ml)	10.36 [8.49, 13.52]	8.91 [6.68, 13.36]	10.69 [9.16, 13.59]	0.001*
TK1 (median [IQR], pM)	2.03 [1.46, 2.80]	1.82 [1.36, 2.66]	2.15 [1.50, 2.84]	0.073
TP (median [IQR], g/L)	64.30 [60.55, 67.25]	64.30 [61.25, 67.70]	64.30 [60.40, 67.20]	0.614
ALB (median [IQR], g/L)	39.10 [37.30, 41.70]	39.80 [37.00, 42.30]	39.10 [37.30, 41.60]	0.755
PALB (median [IQR], mg/L)	227.60 [202.90, 260.85]	229.10 [203.87, 262.45]	226.90 [202.70, 259.80]	0.968
CA50 (median [IQR], U/L)	5.89 [3.96, 8.64]	5.03 [3.47, 7.60]	6.19 [4.15, 8.95]	0.063
CA19.9 (median [IQR], U/L)	9.83 [6.46, 15.14]	8.66 [5.65, 12.95]	10.44 [6.90, 15.96]	0.026*
CA242 (median [IQR], U/L)	3.49 [2.05, 5.89]	3.38 [1.65, 5.49]	3.53 [2.24, 5.94]	0.14
ProGRP (median [IQR], pg/ml)	41.03 [34.22, 48.30]	38.67 [31.34, 44.99]	42.60 [34.99, 52.46]	0.003*
NSE (median [IQR], ng/ml)	9.99 [8.75, 11.49]	9.89 [9.15, 10.60]	10.09 [8.61, 11.70]	0.585
CYFRA21.1 (median [IQR], ng/ml)	2.46 [1.91, 3.20]	2.32 [1.75, 2.92]	2.56 [2.01, 3.34]	0.024*
TPS (median [IQR], U/L)	63.47 [45.85, 92.80]	57.92 [36.19, 84.44]	68.14 [48.30, 96.30]	0.007*

*indicates that it is statistically significant.

Discussion

The popularization of computed tomography (CT) increases the detection rate of pulmonary nodules. However, at least 95% of all identified pulmonary nodules are benign (9). Currently, the differentiation between benign and malignant SNPs smaller than 3 cm is still a major clinical challenge. A study by Laura et al. on ^{18}F-FDG-PET/CT showed good diagnostic performance in SPN, reporting a sensitivity and specificity of 85.6% and 85.7%, respectively (12). However, they excluded indeterminate SPN

TABLE 2 Univariate and multivariate analysis of distinguishing malignant SNPs.

	Univariate analysis			Multivariate analysis		
Characteristic	HR	95% CI	P-value	HR	95% CI	P-value
Sex (male)	1.21	0.72–2.05	0.477	–	–	–
Age (≥60 years old)	3.44	1.93–6.4	<0.001*	2.69	1.34–5.59	0.006*
FR^{+}CTC (FU/3 ml)	4.47	2.57–7.89	<0.001*	6.26	3.09–13.37	<0.001*
TP (g/L)	1.93	0.48–12.84	0.408	–	–	–
TK1 (pM)	2.41	1.42–4.16	0.001*	4.82	2.4–10.27	<0.001*
ALB (g/L)	0.69	0.41–1.17	0.166	–	–	–
PALB (mg/L)	0.74	0.44–1.26	0.263	–	–	–
CA50 (U/L)	2.26	1.33–3.87	0.003*	1.75	0.87–3.55	0.118
CA19.9 (U/L)	2.19	1.28–3.82	0.005*	–	–	–
CA242 (U/L)	2.56	1.35–4.85	0.004*	2.31	0.99–5.53	0.055
ProGRP (pg/ml)	2.37	1.39–4.1	0.002*	1.68	0.87–3.27	0.121
NSE (ng/ml)	2.11	1.21–3.79	0.01*	2.06	1.07–4.06	0.033*
CYFRA21.1 (ng/ml)	1.98	1.13–3.58	0.02*	–	–	–
TPS (U/L)	2.23	1.3–3.91	0.004*	1.74	0.92–3.36	0.093

*indicates that it is statistically significant.

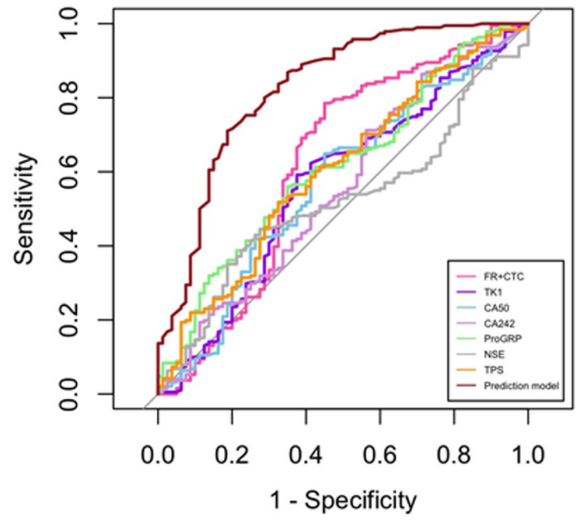

FIGURE 3
Receiver operating characteristic (ROC) curve-single marker and prediction model.

patients. Moreover, in many infectious and inflammatory disorders with active macrophages, especially granulomatous diseases, FDG-PET may produce false positive results (10%–25%) (26). A review published in JAMA reveals that most of the detected benign nodules are granulomas or intrapulmonary lymph nodes (9), impacting the accuracy of PET imaging results. To improve the diagnostic accuracy, we detected the hematologic biomarkers of these patients and found that a single biomarker was poor at predicting the benign and malignant SNPs. Univariate and multivariate analyses were used to establish the first liquid biopsy model to predict benign and malignant SNPs. The novel predicting liquid biopsy model combined age, FR$^+$CTC, TK1, CA50, CA242, ProGRP, NSE, and TPS, with a sensitivity of 71.1% and a specificity of 81.3%. It has excellent predictive value. In addition, the nomogram was generated from the predicting liquid biopsy model, which is more convenient for daily use by clinicians.

In a previous study, FR$^+$CTC displayed the highest AUC compared with NSE, CEA, CA125, Cyfra21-1, and SCC Ag and could satisfactorily discriminate patients with NSCLC from controls, even in early-stage NSCLC (20). The results of our study are consistent with the previous study; the AUC of FR$^+$CTC was higher than that of NSE and Cyfra21-1. In the study by Wang et al., FR$^+$CTC showed the highest diagnostic efficiency in the diagnosis of lung cancer when compared with CEA, CYFRA21-1, and NSE. Notably, the combination of FR$^+$CTC, NSE, CEA, and CYFRA21-1 could significantly improve diagnostic efficacy in differentiating patients with lung cancer from those with benign lung disease (27). Xue et al. reported that FR$^+$CTC showed the highest AUC value among CEA, NSE, CYFRA21−1, SCC, ProGRP, and Hsp90α in the whole cohort and for participants with nodule sizes of ≤3 cm, the AUC, sensitivity, and specificity were 0.8063 (95% CI: 0.6769–0.9356), 80.00%, and 75.00%, respectively, which were lower than in the whole cohort (23). While in our study, all participants had a nodule size of ≤3 cm, however, the AUC, sensitivity, and specificity were lower in the above study. This difference may be caused by the small sample size. Recently, Zhou et al. found that the AUC of FR$^+$CTC was the highest compared with CEA, CYFRA21-1, NSE, and SCC. The sensitivity and specificity for differentiating malignant from benign nodules were 78.6%–82.7% and 68.8%–78.4%, respectively (28). In our study, the prediction model was not developed based on significant factors in the results of multivariate analysis, while it was based on significant variables selected by AIC. In this way, enough variables were included in the model to avoid errors caused by the inclusion of variables only based on multivariate regression statistical differences. Our study found that the prediction model combined age, FR$^+$CTC, TK1, CA50, CA242, ProGRP, NSE, and TPS had the best performance.

TABLE 3 The diagnosis values of hematological biomarkers.

Biomarker	Cutoff	Specificity	Sensitivity	AUC	95% CI	P-value(CTC reference)
FR$^+$CTC	9.005	0.550	0.785	0.623	0.540–0.705	–
TP	74.45	0.975	0.047	0.481	0.405–0.556	0.012$^{\#}$
TK1	1.965	0.625	0.592	0.569	0.492–0.646	0.377
ALB	39.85	0.500	0.591	0.512	0.434–0.590	0.067
PALB	239.25	0.450	0.623	0.502	0.425–0.579	0.037$^{\#}$
CA50	5.24	0.550	0.649	0.572	0.495–0.649	0.382
CA19-9	10.375	0.675	0.513	0.586	0.51–0.661	0.527
CA242	1.705	0.288	0.864	0.557	0.479–0.634	0.262
ProGRP	41.085	0.650	0.560	0.614	0.542–0.687	0.873
NSE	10.515	0.725	0.445	0.521	0.449–0.593	0.075
CYFRA21-1	2.83	0.738	0.414	0.587	0.513–0.660	0.504
TPS	67.155	0.675	0.518	0.604	0.530–0.678	0.725
Prediction model$^{\#}$	–	0.813	0.711	0.826	0.768–0.884	<0.001

$^{\#}$Age + FR$^+$CTC +T K1 + CA50 + CA242 + ProGRP + NSE + TPS.

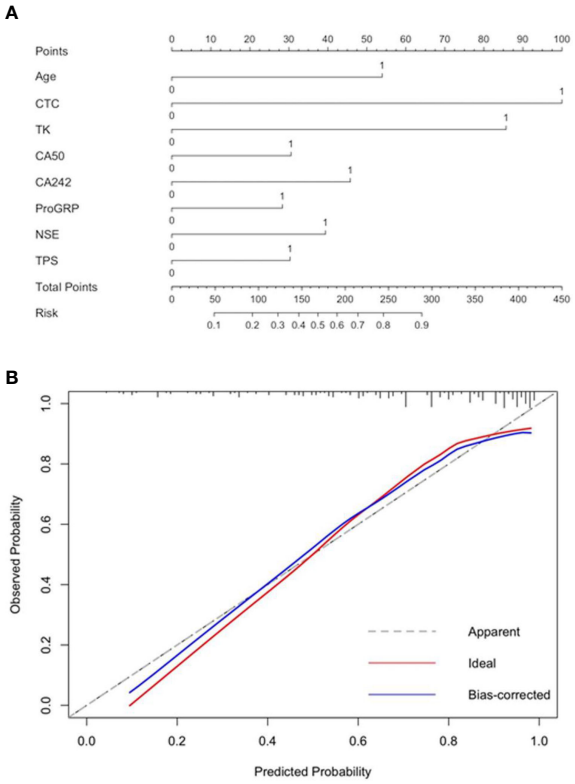

FIGURE 4
Nomogram **(A)** and the result of Hosmer–Lemeshow test for the model **(B)**.

However, multivariate analysis revealed that only older, higher FR^{+}CTC levels, higher TK1 levels, and higher NSE are significant independent risk factors for malignant SPNs. By contrast, CA50, CA242, ProGRP, and TPS have been ignored. Among them, ProGRP was proven to be a novel biomarker in lung cancer (23). CA50, CA242, and TPS were proven to be novel biomarkers in lung cancer patients, although with a relatively lower AUC value (<0.7). In the previous study, CA50 and CA242 showed poor diagnostic efficacy for lung cancer screening with low AUC values. However, when combined with the other carbohydrate antigen (CA) biomarkers (CA125, CA15-3, CA19-9, and CA724), the AUC value was up to 0.776. Moreover, when coupled with CYFR21, CEA, NSE, and SCC, the AUC value was up to 0.884 (29). TPS is a specific fragment of keratin 18, which belongs to type I intermediate filaments found in epithelia. The TPS level significantly differed between the control and NSCLC groups, but multivariate analyses showed it was not an independent prognostic factor for advanced NSCLC (30). In the metastatic lung adenocarcinoma group, the TPS level is higher than in the non-metastatic group. However, it cannot predict the metastatic status because of the low AUC value (31). TK1 is strongly associated with DNA synthesis and cell proliferation and has demonstrated high diagnostic value in NSCLC. The serum levels of TK1 in NSCLC patients were higher than those of healthy individuals, and the AUC value was 0.667 (32), which is a promising biomarker for lung cancer.

Some limitations must be considered in our study. Firstly, this is a retrospective single-center study. A multicenter cohort study is warranted. Secondly, the sample size is not large enough. Therefore, it cannot represent the situation of all populations. Finally, the application value of this novel model is limited, and it is only suitable for the diagnosis of small pulmonary nodules. We will initiate a study on its relationship with prognosis after surgery.

Conclusions

We established a preoperative prediction model with age and hematological indicators to improve the diagnostic workflow for small pulmonary nodules. In the meantime, we provide a nomogram that can be used for preoperative screening of early NSCLC patients and helps thoracic surgeons make a clinical decision.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Sichuan Cancer Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception and design: QZ and QH. Administrative support: WH and LP. Provision of study materials or patients: DL, JZ, and YH. Collection and assembly of data: KS, AL, KXL, and KL. Data analysis and interpretation: QZ and WH. Manuscript writing: QZ, QH, and WH. 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.

Publisher's note

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Etoposide/platinum plus anlotinib for patients with transformed small-cell lung cancer from EGFR-mutant lung adenocarcinoma after EGFR-TKI resistance: a retrospective and observational study

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Objective: The histological conversion of lung adenocarcinoma (LUAD) into small-cell lung cancer (SCLC) is an important resistance mechanism for epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI)-resistant LUAD. Anlotinib has been recommended as the third-line treatment for SCLC patients. The efficacy of etoposide/platinum (EP) as the main treatment is very limited for patients with transformed SCLC. However, little is known about EP plus anlotinib for transformed SCLC. The present study retrospectively explored the clinical response to EP combined with anlotinib in patients with transformed SCLC from LUAD after EGFR-TKI failure.

Methods: A total of 10 patients who underwent SCLC transformation from EGFR-TKI-resistant LUAD were retrospectively reviewed from September 1, 2019, to December 31, 2022, in three regional hospitals. All of the patients were treated with the combination regimen of EP and anlotinib for four to six cycles, followed by anlotinib maintenance therapy. The clinical efficacy indices including objective response rate (ORR), disease control rate (DCR), median progression-free survival (mPFS), median overall survival (mOS), and toxicities were evaluated.

Results: The median time from EGFR-TKI treatment to SCLC conversion was 20.1 ± 2.76 months (17–24 months). Genetic examination after transformation showed that 90% of the patients retained their original EGFR gene mutations. Additional driver genes were found, including BRAF mutation (10%), PIK3CA mutation (20%), RB1 loss (50%), and TP53 mutation (60%). The ORR and DCR were 80% and 100%, respectively. The mPFS was 9.0 months (95% CI, 7.9–10.1 months), and the mOS was 14.0 months (95% CI, 12.0–15.9 months). Less than

10% of grade 3 toxicities were observed, and no grade 4 toxicity and death events were reported.

Conclusion: The EP plus anlotinib regimen appears to be a promising and safe strategy in transformed SCLC patients after EGFR-TKI resistance, which warrants further investigation.

KEYWORDS

etoposide/platinum (EP), anlotinib, transformation, small-cell lung cancer (SCLC), lung adenocarcinoma (LUAD), epidermal growth factor receptor (EGFR)

1 Introduction

Epidermal growth factor receptor (EGFR) is the most prominent driving gene in non-small-cell lung cancer (NSCLC), mainly including EGFR exon 19 deletion and L858R mutation. EGFR-tyrosine kinase inhibitors (EGFR-TKIs) have been listed as the preferable standard of care in EGFR-mutant NSCLC patients, in particular for lung adenocarcinoma (LUAD). However, nearly all patients inevitably experience acquired resistance to EGFR-TKI. Among these patients, 5–15% display histological transformation from NSCLC to small-cell lung cancer (SCLC) (1). The underlying mechanisms are very complicated, and most of them remain unclear. For transformed SCLC, chemotherapy with etoposide/platinum (EP) is the most common regimen, but the clinical prognosis is dismal, with a median overall survival (mOS) of merely 6–10.9 months (2, 3).

As the principal angiogenic growth factor, vascular endothelial growth factor (VEGF) modulates the process of angiogenesis during the growth, invasion, and metastasis of tumors (4). In SCLC, antiangiogenic agents targeting VEGF have not become an important therapeutic strategy until the advent of anlotinib. As an oral antiangiogenic tyrosine kinase inhibitor (TKI), anlotinib has been recommended by Chinese Society of Clinical Oncology (CSCO) as a third-line treatment for SCLC and NSCLC (5, 6). Majority of patients with transformed SCLC after EGFR-TKI resistance of LUAD have received more than one systemic treatment. Furthermore, the combination of anlotinib with EP regimen has been administered as the first-line treatment of extensive-stage SCLC with an objective response rate (ORR) of 87.2% and a median progression-free survival (mPFS) of 9.0 months (7). However, little is known about the efficacy of the combination regimens in transformed SCLC from EGFR-TKI-resistant LUAD. Therefore, this study retrospectively analyzed the clinical efficacy and safety of the EP regimen plus anlotinib for patients with the histological conversion from EGFR-TKI-resistant LUAD to SCLC.

2 Materials and methods

2.1 Study design and patients

This was a multicenter retrospective observational study. All transformed SCLC patients who received anlotinib combined with EP chemotherapy were from three regional hospitals, namely the Affiliated Hospital of Jiujiang University, Ruichang People Hospital, and Lushan People Hospital, between September 1, 2019, and December 31, 2022. The clinical data of the patients were collected, including age, sex, ECOG PS, histopathology, molecular examination, TNM stage, anlotinib dose, and adverse reaction.

2.2 Inclusion and exclusion criteria

The inclusion criteria for patients were as follows: (1) 18–75 years of age; (2) pathologically confirmed histological transformation from EGFR-mutant LUAD to SCLC; (3) more than one systemic treatment of EGFR-TKI before transformation; (4) ECOG PS ≤ 2; and (5) TNM stage: IIIB–IV; (6) no obvious abnormality in liver and kidney function; (7) no obvious hematological abnormality; (7) no active bleeding and coagulation abnormalities; (8) no clinically significant electrocardiograph abnormality.

The exclusion criteria for patients were as follows: (1) age >75 years; (2) initial histopathological diagnosis of SCLC; (3) ECOG PS >2; and (4) presentation of active bleeding; (5) presence of contraindications to chemotherapy.

2.3 Next-generation sequencing

DNA was extracted from tumor tissue and matched pleural fluid samples. Next-generation sequencing (NGS) was performed *via* a panel of at least 73 genes in Daan Gene Co., Ltd. (Guangzhou, China), covering all exons of EGFR gene with a mean coverage depth of >800×.

2.4 Therapeutic methods

All of the patients were treated with a combination regimen of EP and anlotinib, i.e., 80 mg/m^2 etoposide (Qilu Phar., Jinan, China) for days 1–3, carboplatin (Qilu Phar., Jinan, China) (AUC = 5) on day 1, and anlotinib (10 mg/day) (Chia-Tai Tianqing Phar., Nanjing, China) orally for days 1–14. The cycle was repeated every 3 weeks for four to six cycles, and then anlotinib was maintained every 21 days. Dose adjustment was made according to the patients' actual situation. The treatment was terminated if disease progression, death, or unacceptable toxicity occurred.

2.5 Efficacy and safety evaluation

The clinical efficacy was evaluated according to the RECIST standard (ver. 1.1). The objective responses were classified as complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). The primary end points were ORR (CR + PR) and mPFS, and the secondary end points were DCR (CR + PR + SD) and median overall survival (mOS).

The adverse reaction grades were classified following the Common Terminology Criteria for Adverse Events (CTCAE) (ver. 5.0).

2.6 Follow-up and statistical analysis

PFS was defined as the period from the initial date of chemotherapy plus anlotinib to disease progression or death. OS was determined from the start of chemotherapy with anlotinib to death or the date of last follow-up evaluation. Time to SCLC transformation was calculated from the initial date of EGFR-TKI treatment to confirmation of transformed SCLC.

The cutoff date for follow-up was December 31, 2022. The Kaplan–Meier method was used to analyze the median PFS, OS, and 95% confidence interval (CI). All of the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, ver. 20.0, Chicago, Illinois, U.S.A) and GraphPad Prism (ver. 7.0, San Diego, California, U.S.A).

3 Results

3.1 Baseline clinical features of patients

Out of 152 patients with EGFR mutations, a total of 10 patients (6.57%) with transformed SCLC were enrolled in the present study. Their baseline clinical features are given in Tables 1, 2. All of the included patients were in IIIB–IVB stage. The initial mutation

TABLE 1 Baseline clinical features of 10 patients with transformed SCLC from EGFR-mutant LUAD.

Case No.	Age (Years)	Smoking status	Gender	Stage	Initial mutation status (specimen type)	Primary tumor lesion	TKI therapy before transformation	Time to SCLC transformation (months)	Comorbidities	Specimen type (2nd NGS)
1	62	Yes	Male	IIIB	EGFR exon 19 del (tissue)	left lower lung	Osimertinib	19	chronic bronchitis	tissue
2	54	Never	Female	IVA	EGFR exon 19 del (tissue)	right lower lung	Osimertinib	22	none	tissue
3	46	Never	Male	IVB	EGFR exon 21 L858R, (tissue)	right middle lung	Osimertinib	17	none	pleural fluid
4	59	Yes	Male	IIIB	EGFR exon 19 del, (tissue)	left upper lung	Aumolertinib	23.5	chronic bronchitis	lymph node
5	67	Yes	Female	IIIB	EGFR exon 19 del, T790M (+) (tissue)	right lower lung	Aumolertinib	21	obstructive emphysema	tissue
6	38	Yes	Male	IVB	EGFR exon 21 L858R, (tissue)	right lower lung	Osimertinib	16	chronic bronchitis	pleural fluid
7	46	Yes	Male	IIIB	EGFR exon 19 del, (tissue)	left lower lung	Osimertinib	22.5	chronic bronchial asthma	tissue
8	61	Yes	Female	IVA	EGFR exon 21 L858R, (tissue)	right upper lung	Osimertinib	19.5	chronic bronchial asthma	lymph node
9	57	Never	Male	IVA	EGFR exon 19, (tissue)	right lower lung	Aumolertinib	20.5	diabetes mellitus	lymph node
10	59	Never	Male	IVB	EGFR exon 21 L858R, T790M (+) (tissue)	left lower lung	Osimertinib	25.5	hypertension	tissue

TABLE 2 The post-transformation gene mutations of NGS in the 10 patients.

Gene (Mutation point)	Patients (Mutation abundance (%))									
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
EGFR	Exon 19 del	Exon 19 del	Exon 21 L858R	✘	Exon 19 del	Exon 21 L858R	Exon 19 del	Exon 21 L858R	Exon 19 del	Exon 21 L858R
T790M	✘	✘	✘	✘	✓(12.4%)	✘	✘	✘	✘	✓(17.6%)
RB1 loss	✓(9.2%)	✘	✓(6.5%)	✓(13.4%)	✓(15.2%)	✘	✓(5.7%)	✓(7.4%)	✘	✓(13.7%)
TP53	✓ (p.A159D) (8.4%)	✓ (p.R273H) (13.7%)	✓ (p.R273H) (20.3%)	✓ (p.A159D) (17.8%)	✓ (p.A159D) (5.9%)	✘	✓ (p.A159D) (7.6%)	✓ (p.R273H) (13.4%)	✓ (p.R273H) (22.8%)	✓ (p.A159D) (14.6%)
MYC amplification	✘	✘	✘	✘	✘	✘	✘	✘	✓(28.4%)	✘
NF1 (p.R461T)	✘	✘	✘	✘	✘	✓(22.5%)	✘	✘	✘	✘
PIK3CA (p.E545K)	✘	✘	✘	✘	✘	✘	✓(4.6%)	✘	✘	✘
PTEN loss	✓(16.8%)	✘	✘	✘	✘	✘	✘	✘	✘	✘
CCNE1 (Exon7, c.476A > G)	✘	✘	✘	✓(8.9%)	✘	✘	✘	✘	✘	✘
CDK6 amplification	✘	✓(14.2%)	✘	✘	✘	✘	✘	✘	✘	✘
BRAF (p.D594G)	✘	✘	✓ (5.6%)	✘	✘	✘	✘	✘	✘	✘

✓ indicating the presence of gene mutation, ✘ indicating the absence of gene mutation.

status included EGFR exon 19 Del (60%, 6/10) and EGFR exon 21 L858R mutation (40%, 4/10). One patient (no. 10) had a concurrent T790M mutation. 80% (8/10) of the patients received osimertinib (AstraZeneca Phar., London, UK) as the first-line treatment, while only 20% (2/10) of the patients received aumolertinib (HanSoh Phar., Lianyungang, China) treatment. The median interval from initial treatment to transformation was 20.1 ± 2.76 months (17–24 months).

All of the patients underwent the second genetic testing, and the specimens included tissue, pleural fluid, and lymph node. Compared with the initial gene mutation, the second mutation status of transformed SCLC was very complicated. Except for patient no. 4, who lost the initial EGFR exon 19 deletion, the nine remaining patients retained their original EGFR gene mutations. These mutations were accompanied by additional driver gene mutations, including TP53 mutation (60%), RB1 loss (50%), PIK3CA mutation (20%), BRAF mutation (10%), PTEN (10%), CDK6 (10%), CCNE(10%), NF1 (10%) and MYC (10%). Of note, patient no. 3 carried TP53 mutation but did not experience RB1 loss, while patient no. 10 harbored RB1 loss and TP53 mutation but lost T790M mutation.

3.2 Clinical efficacy

All the patients discontinued osimertinib or aumolertinib treatment after disease progression, and then receive the combination treatment of EC plus anlotinib. In the present study, four patients were in stage IIIB, but they exhibited poor performance status (PS=2) due to the comorbidities including as chronic bronchitis (no. 1 and 4), obstructive emphysema (no.5), and chronic bronchial asthma (no.7), respectively. So, they only received EGFR-TKI therapy alone without thoracic radiotherapy.

Except for one patient (no. 8), who only received four cycles of the combination treatment, the remaining nine patients received six cycles of EP and anlotinib treatment. All of the patients received anlotinib as maintenance therapy after the completion of the combination treatment. One patient achieved CR, seven patients achieved PR, and two patients had SD (Figure 1). The ORR was 80%, and the DCR was 100% (Table 3). The median PFS was 9.0 months (95% CI, 7.9–10.1 months), and the median OS was 14.0 months (95% CI, 12.0–15.9 months) (Figure 2). The median follow-up time was 15.2 months (95% CI, 13.4-16.8 months).

3.3 Safety

All of the patients were included in the safety assessment. Adverse reactions were assessed from the start of the combination treatment until disease progression or the last follow-up date. The treatment-related adverse effects included vomiting and nausea, granulocytopenia, leukopenia, thrombocytopenia, hypertension, proteinuria, fatigue, hand–foot syndrome, and leukopenia (Table 4). The grade 3 toxicities were granulocytopenia (10%), leukopenia (5%), and hypertension (10%). No grade 4 toxicities were recorded, and no deaths were observed.

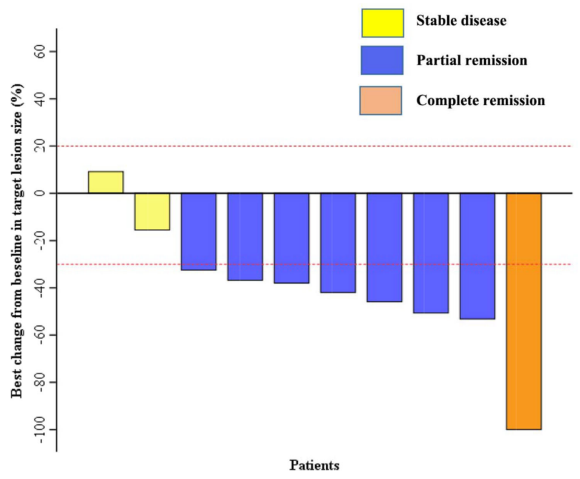

FIGURE 1
Changes from baseline in target lesions size (%).

4 Discussion

In 2006, a female NSCLC patient carrying EGFR exon 19 deletion was first reported to transform to SCLC (8). Since then, cases of LUAD conversion to SCLC have been continually presented (9–11). Statistically, 4–14% of EGFR-mutant NSCLC patients experience histological transformation to SCLC after EGFR-TKI failure. The histological conversion to SCLC represents one of the important mechanisms governing EGFR-TKI resistance. Three prevailing mechanisms may be proposed to explain the histological conversion from LUAD to SCLC. First, twin clones (i.e., both LUAD and SCLC clones) coexist in the tumor sites in the initial stages of tumorigenesis. LUAD cells are the dominant clones during the early stage, which become constrained under the pressure of EGFR-TKI treatment. Accordingly, the new clones of SCLC emerge and replace the previously predominant clones. Second, both SCLC and LUAD originate from the common precursor, i.e., alveolar type II cells. With long exposure to EGFR-TKI, the resistant clones survive and then convert to SCLC type. Finally, secondary gene alterations appear in the process of transformation, including RB1 loss, TP53 mutation, and PIK3CA and BRAF mutation. Recent studies have revealed some novel gene alterations that are associated with the course of transformation, which include WNK1 mutation (12), SPP1 upregulation (13), REST inactivation (14), and ETV1 mutation (15). In addition, Xie et al. reported that the conversion of LUAD to SCLC may result from somatic copy number variation (CNV) events rather than from mutational events. The burden of CNV is closely associated with the interval time to transformed SCLC and OS after SCLC conversion (16). The definitive mechanisms behind the histological transformation are very complicated and remain to be fully clarified.

Currently, an increasing number of researchers prefer the shared-origin theory. Logistically, if the theory of twin clones is true, it is difficult to explain PR or even CR response to first-line EGFR-TKI treatment. In the present study, 80% of the patients achieved more than PR response (including CR in one patient). Importantly, 90% of the patients retained their prior EGFR gene mutations. These results strongly support the common-precursor doctrine of LUAD and SCLC. Furthermore, RB1 loss was found in 50% of the patients, and TP53 mutation occurred in 60% of the patients. One patient (no. 3) harbored only gene alterations of TP53 but without RB1 loss. These findings indicate that RB1 loss and TP53 mutation are not universally present in transformed SCLC patients, which has also been confirmed by others (12, 17). Additionally, PIK3CA mutation was found in 20% of the patients, and BRAF mutation occurred in 10% of the patients, suggesting the molecular heterogeneity of transformation from LUAD to SCLC. Finally, the interval time from EGFR-TKI treatment to histological transformation was 20.1 months, which is consistent with previous reports of 17.8–22.7 months (2, 12, 18).

Currently, antiangiogenesis therapy targeting VEGF has become an indispensable strategy for cancer treatment. VEGF overexpression has been found in almost 80% of SCLC patients, indicating highly vascularized tumor of SCLC. The anti-VEGF agents, such as thalidomide, sorafenib, and sunitinib, showed disappointing clinical efficacy but increased treatment-related toxicity (19–21). In extensive-stage SCLC patients, bevacizumab plus EP regimen prolonged the PFS (6.7 vs. 5.7 months, P=0.03) but didn't translate into the benefit of OS (8.9 vs. 9.8 months, P=0.113) compared with EP regimen (22). Obviously, the role of antiangiogenic drugs remains controversial in the treatment of SCLC until the advent of anlotinib.

In ALTER 1202 study, the novel antiangiogenic agent anlotinib as a third- or further-line treatment achieved better mPFS (4.1 vs. 0.7 months, P < 0.0001) and mOS (7.3 vs. 4.9 months, P = 0.0029) than the placebo group for patients with extensive-stage SCLC (ES-SCLC) (23). Consequently, the Chinese Society of Clinical Oncology (CSCO) recommended anlotinib as the only antiangiogenic agent for refractory ES-SCLC in China on August 30, 2019. Furthermore, a prospective study of ACTION-2 reported that EP plus anlotinib regimen as the first-line treatment for ES-SCLC achieved an ORR of 87.2%, a DCR of 97.7%, an mPFS of 9.0 months, and an mOS of 19.0 months (7). A single-arm trial showed that anlotinib plus EP as the first-line treatment for ES-SCLC achieved an ORR of 85.71%, a DCR of 94.29%, an mPFS of 8.02

TABLE 3 Clinical outcome of transformed SCLC from LUAD with EP plus anlotinib.

Clinical efficacy	Number (%)
CR	1 (10%)
PR	7 (70%)
SD	2 (20%)
PD	0 (0%)
ORR (CR+PR)	8 (80%)
DCR (CR+PR+SD)	10 (100%)

CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; ORR, overall response rate; and DCR, disease control rate.

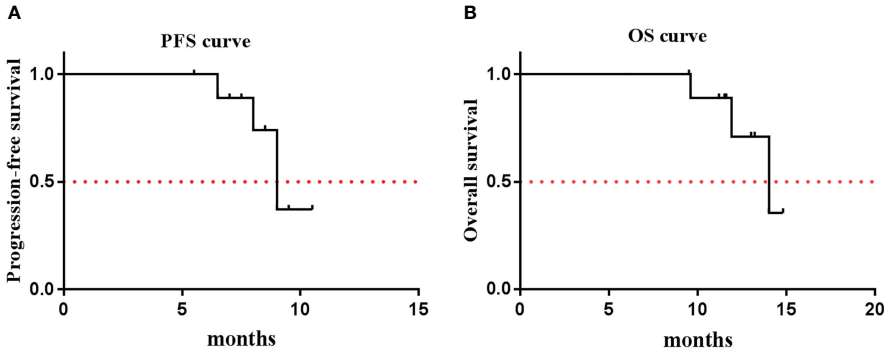

FIGURE 2
Kaplan-Meier Estiamtes of survival. **(A)** PFS: 9.0 months (95% CI: 7.9~10.1); **(B)** OS: 14.0 months (95% CI: 12.0~15.9).

TABLE 4 Treatment-related adverse effects (n (%)).

Adverse effects	No. of patients				Total
	Grade 1	Grade 2	Grade 3	Grade 4	
Vomiting and nausea	3 (30%)	1 (10%)	0	0	4 (40%)
Granulocytopenia	4 (40%)	1 (10%)	1 (10%)	0	6 (60%)
Leukopenia	5 (50%)	2 (20%)	1 (10%)	0	8 (80%)
Thrombocytopenia	4 (40%)	1 (10%)	0	0	5 (50%)
Hypertension	6 (60%)	1 (10%)	1 (10%)	0	8 (80%)
Proteinuria	2 (20%)	1 (10%)	0	0	3 (30%)
Fatigue	5 (50%)	2 (20%)	0	0	7 (70%)
Oral mucositis	4 (40%)	1 (10%)	0	0	5 (50%)
Hand-foot syndrome	3 (30%)	1 (10%)	0	0	4 (40%)

months, and an mOS of 15.87 months (24). Inspired by this, we attempted to explore the combination of EP with an anlotinib regimen in transformed SCLC patients.

In *de novo* extensive SCLC, immune-combination therapy has been recommended as the first-line treatment with mOS reaching 13–15.4 months (25–27). Conversely, no objective responses were observed in 17 transformed SCLC cases who received immunotherapy (2). The EP regimen is the most common therapy for the transformed SCLC but with limited efficacy (only 3.2–4.0 months of mPFS and 8.0–10.9 months of mOS) (2, 12, 18, 28, 29). Wang et al. reported that the ORR and mPFS of EP chemotherapy for transformed SCLC were 44.4% and 3.5 months, respectively, but the ORR and mPFS of anlotinib alone were 66.7% and 6.2 months, respectively, indicating anlotinib as an optional choice in this population (29). In the present study, the combination of EP with an anlotinib regimen was used to treat transformed SCLC patients. The mPFS and mOS were 9.0 months (95% CI, 7.9–10.1 months) and 14.0 months (95% CI, 12.0–15.9 months), respectively. Additionally, the combination regimen was associated with a favorable safety profile, with less than 10% of grade 3 toxicities and no grade 4 toxicities and deaths. Therefore, EP plus anlotinib regimen in our study seems to achieve higher clinical efficacy than EP chemotherapy or anlotinib alone in previous studies.

5 Conclusions

Heretofore, no treatment guidelines have been established for the transformed SCLC. Our study revealed that EP combined with anlotinib may be a better choice for transformed SCLC originating from EGFR-TKI-resistant LUAD compared with EP or anlotinib alone treatment. However, this study also has some shortcomings. On the one hand, the sample size was small. On the other hand, the present study was retrospective in nature, and the results were observational. Due to the study limitations, further well-designed prospective studies with large sample sizes should be performed to confirm these findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Jiujiang University Affiliated Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Conceptualization and writing: JD. Patients' data collection: ZL, HG and XJ. Data processing: YS. All of the authors have read and agreed to the published version of 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.

Publisher's note

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Prediction of distant organ metastasis and overall survival of lung cancer patients: a SEER population–based cohort study

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Background: Distant organ metastasis is a common event in lung cancer (LC). However, the preferential metastatic pattern of different pathological types of LC and its effect on prognosis have not been comprehensively elucidated. This study aimed to explore the distant metastasis pattern and construct nomograms predicting the metastasis and survival of LC patients using the Surveillance, Epidemiology, and End Results (SEER) database.

Methods: LC data were downloaded from the SEER database to conduct logistic regression and investigate risk factors for developing organ metastasis. A Cox regression analysis was conducted to investigate prognostic factors of LC. A Kaplan–Meier analysis was used to estimate overall survival outcomes. Nomograms were constructed to predict the probability of organ metastasis and the 1-, 3- and 5-year survival probability of LC patients. Receiver operating characteristic curves were used to evaluate the diagnostic accuracy of the nomograms. All statistical analyses were conducted within R software.

Results: The liver is the most common metastatic organ of small cell carcinoma. The brain is the most likely metastasis site of large cell carcinoma, and bone is the most likely metastasis site for squamous cell carcinoma and adenocarcinoma. Patients with triple metastases (brain-bone-liver) have the worst prognosis, and for nonsquamous carcinoma with single organ metastasis, liver metastases conferred the worst prognosis. Our nomograms based on clinical factors could predict the metastasis and prognosis of LC patients.

Conclusion: Different pathological types of LC have different preferential metastatic sites. Our nomograms showed good performance in predicting distant metastasis and overall survival. These results will provide a reference for clinicians and contribute to clinical evaluations and individualized therapeutic strategies.

KEYWORDS

lung cancer, distant metastasis, risk factor, prognosis, SEER

1 Introduction

According to the estimation, there will be 236740 new cases of lung cancer in the USA in 2022, with 130180 deaths. LC is the second most common cancer in both men and women, less than prostate cancer (in males) and breast cancer (in females), and LC is the leading cause of death among cancer patients, with a low 5-year survival rate (1). Metastasis is a characteristic of cancer and is responsible for the greatest number of cancer-related deaths (2). Approximately 20% of cancer patients will develop brain metastases (BMs) (3), and brain metastases from LC account for approximately 45% of the total cases of BMs (4, 5). Approximately 10% of SCLC patients have brain metastases at the time of the initial diagnosis (6). In addition to the brain, the liver and bone are also common metastasis sites of lung cancer (7, 8). Despite the rapid development of multiple therapies, such as targeted therapies and immunotherapy (9), the prognosis of patients with advanced lung cancer remains poor (3). The median survival time of BM patients is approximately 10.6 months (9). It is very important to identify risk and prognostic factors, evaluate individual metastatic risk and make accurate diagnoses to improve the survival outcome. Providing individualized treatment for different patients to maximize personal survival benefits is a research direction (10).

The survival rates for LC patients with different distant organ metastases are not the same. Understanding the epidemiology of the most common distant organ metastasis patterns in different pathological types of LC, as well as their overall survival, will help the process for clinical decisions. A previous study suggested that for SCLC patients with brain metastasis, male sex, older age, liver metastasis, and insurance status were associated with increased death risk (11). For NSCLC patients, age, race, sex, pathology, T stage and N stage were associated with the occurrence of brain metastasis and overall survival (12–14). However, few studies have compared the survival risk among LC patients with different distant metastases and focused on the prediction of distant metastases. The purpose of our study was to describe a detailed landscape of distant organ metastasis status and explore the effects of distant organ metastasis status on overall survival in different pathological types of lung cancer. We also analyzed the risk factors for organ metastasis and prognostic factors in LC patients based on data from the Surveillance, Epidemiology, and End Results (SEER) database (15). Moreover, we tried to construct nomograms predicting the organ metastasis and overall survival of LC patients.

Abbreviations: LC, lung cancer; BM, brain metastases; SEER, the Surveillance, Epidemiology, and End Results database; KM, Kaplan–Meier; ROC, receiver operating characteristic; AUC, area under the curve; SCC, squamous cell carcinoma; AD, adenocarcinoma; SCLC, small cell lung carcinoma; NSCLC-NOS, non-small cell lung carcinoma(Not otherwise specified); LCC, large cell carcinoma; LLL, left lower lobe; LMB, left main bronchus; LUL, left upper lobe; RLL, center lower lobe; RMB, center main bronchus; RMB, center middle lobe; RUL, center upper lobe.

2 Methods

2.1 Population

In this population-based study the LC patient data were downloaded from the SEER*Stat Database: Incidence - SEER Research Plus Data. SEER*Stat version 8.4.0 (https://seer.cancer.gov/seerstat/) was used to obtain the patient information (15). The extraction condition was "the site of the tumor: lung". The following variables were extracted: Age recode; Race recode;patient ID, Sex, Year of diagnosis, Primary Site, ICD-O-3 Hist/behav, Laterality, Separate Tumor Nodules Ipsilateral Lung Recode; SEER Combined Mets at DX-bone; SEER Combined Mets at DX-brain; SEER Combined Mets at DX-liver; Mets at DX-Distant LN; Survival months; Vital status recode; 8th edition AJCC classification; Sequence number. LC patients who were diagnosed between 2010 and 2017 were included in this study. Patient information was excluded when the lung was not the first primary site. Patient information with survival time was used to explore the metastasis pattern of the LC patients. The data with a definite metastasis status were used to evaluate the prognostic effect of metastasis pattern. After excluding the data without a tumor stage, we investigated the risk factors for developing organ metastasis and the prognostic factors for LC patients and thus constructed the prediction nomogram. The inclusion and exclusion process is shown in Figure 1.

2.2 Statistical analysis

This study included the following variables: age (<50, 50–59, 60–69, 70–79,>=80); sex (male and female); race (white, black, other (American Indian/AK Native, Asian/Pacific Islander), unknown); pathology (adenocarcinoma, non-small cell lung carcinoma, small cell carcinoma, large cell carcinoma, squamous cell carcinoma, other); site of primary tumor (left main bronchus, left upper lobe, left lower lobe; center main bronchus; center upper lobe; center middle lobe; center lower lobe; other); separate tumor nodule (Yes, No, unknown);T stage (T0, T1, T2, T3, T4, unknown); N stage (N0, N1, N2,N3, unknown); liver metastasis (Yes, No, unknown); bone metastasis

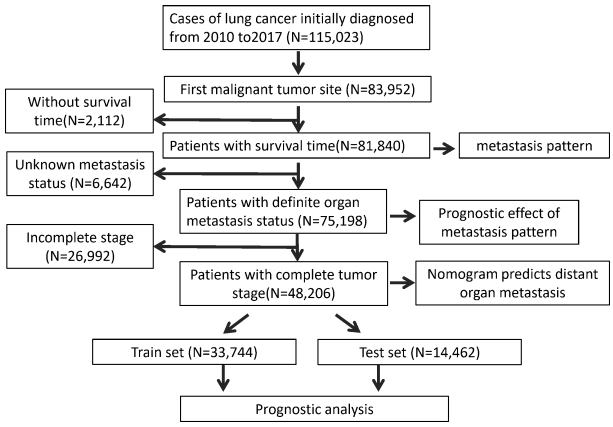

FIGURE 1
Flowchart of the process of data selection.

(Yes, No, unknown); brain metastasis (Yes, No, unknown). The site of the primary tumor was determined according to "laterality" and "primary site". Pathology with ICD-O-3 including 8070/8071/8072/8073/8074/8075/8084 was classified as SCC, ICD-O-3 8012/8013/8014 was classified as LCC, ICD-O-3 8040 was classified as AD, ICD-O-3 8046 was classified as NSCLC-NOS, and ICD-O-3 8002/8041/8042/8043/8044/8045 was classified as SCLC. Other variables are directly obtained from the SEER database.

All statistical analyses were conducted within R software (version 4.1.0). Univariate and multivariate logistic regression were used to identify the risk factors for distant organ metastasis. The factors that significantly associated with organ metastasis in univariate logistic regression were included in the following multivariate logistic regression. The Kaplan–Meier (KM) method was used to investigate overall survival outcomes using the log-rank test. Univariate and multivariate Cox regression analyses were used to identify potential prognostic factors for LC patients. The "rms" package was used to construct nomograms that predict the probability of organ metastasis and the 1-, 3- and 5-year survival probability of LC patients. The concordance index (c-index) was calculated to assess the prognostic performance of the nomogram based on the "survival" package. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic accuracy of the nomograms, which was achieved by the "pROC" package (16). The area under the curve (AUC) was related to the accuracy of the nomogram. $P<0.05$ was considered to be statistically significant.

3 Results

3.1 Effects of distant metastasis on patient survival

The brain, bone, and liver are common organs involved in distant metastasis of lung cancer. Therefore, we conducted a subgroup analysis based on pathological types to explore the effect of different distant metastasis modes on LC patient survival. There were a total of 81,840 patients with survival data, consisting of 15,489 SCC patients, 31,100 AD, 10,037 SCLC patients, 5,170 NSCLC-NOS patients, 1,041 LCC patients and 19,003 patients with other types of LC. A total of 6642 patients had unknown organ metastases. The results showed that the probability of distant metastasis was highest in SCLC and lowest in SCC. The liver (14.4%) is the most common single metastatic organ of SCLC, and approximately 10.04% of SCLC patients develop both liver and bone metastases. On the other hand, the brain is the most likely metastasis site for LCC. Moreover, bone is the most common metastatic organ for SCC and AD. The number of patients included in each subgroup is shown in Table 1.

Then, we performed a Cox analysis to explore the impact of different metastasis statuses on the prognosis of patients. The subgroup analysis suggested that for all pathologic types, the patients without distant metastasis had a better prognosis than the patients with brain metastasis alone, and the patients with brain plus liver metastasis had a worse prognosis than the patients with brain metastasis alone. For SCC, the prognosis of the patients with a single brain metastasis was similar to that of the patients with a single liver metastasis, while the prognosis of the patients with a single bone metastasis was worse. For AD, the survival time of the patients with single bone or single liver metastasis was shorter than that of the patients with single brain metastasis. Among the other pathologic types of lung cancer, single liver metastases were associated with the worst prognosis compared with single brain or single bone metastases, and there was no significant difference in survival between single bone and single brain metastases. Among lung SCC, the patients with triple metastases (brain-bone-liver) had the worst prognosis. For the patients with more than one organ metastasis, bone-liver metastasis in AD and LCC and brain-liver metastasis in NSCLC-NOS and SCLC are associated with the shortest survival (Table 2).

3.2 Predictive factors and nomogram for distant organ metastases in patients with LC

Since distant organ metastasis of LC patients is closely related to prognosis, we tried to screen the clinical factors that can predict organ metastasis and to establish a prediction model. After

TABLE 1 Number of patients included in subgroup analysis.

Pathology	Number of patients									
	total	No (%)	brain (%)	bone (%)	liver (%)	brain +bone (%)	brain +liver (%)	bone +liver (%)	brain+bone +liver (%)	excluded (%)
Squamous cell caicinoma	15489	12094 78.08%	532 3.43%	1096 7.08%	400 2.58%	167 1.08%	71 0.46%	359 2.32%	97 0.63%	673 4.35%
Adenocarcinoma	31100	18218 58.58%	2827 9.09%	3995 12.85%	778 2.50%	1486 4.78%	282 0.91%	1133 3.64%	677 2.18%	1704 5.48%
Non-small cell carcinoma-NOS	5170	2828 54.70%	539 10.43%	665 12.86%	210 4.06%	189 3.66%	67 1.30%	227 4.39%	88 1.70%	357 6.91%
Small cell carcinoma	10037	4397 43.81%	888 8.85%	821 8.18%	1449 14.44%	221 2.20%	245 2.44%	1008 10.04%	282 2.81%	726 7.23%
Large cell carcinoma	1041	603 57.93%	106 10.18%	81 7.78%	65 6.24%	31 2.98%	19 1.83%	58 5.57%	21 2.02%	57 5.48%
Other	19003	11915 62.70%	923 4.86%	1179 6.20%	790 4.16%	266 1.40%	138 0.73%	506 2.66%	161 0.85%	3125 16.44%

excluding patients with incomplete stage information, a total of 48,206 patients were enrolled in the following analysis. Of these patients, 8,391 (17.4%) patients were older than 80 years old. A total of 24,956 (51.8%) patients were male. Over half of the patients were white (79.4%, N= 38264). For the lesion site, the center upper lobe was the most common, at approximately 30.2% (N=14548). A total of 24.5% of the patients were diagnosed with separate tumor nodules. Most patients did not have bone metastases (N=39234, 81.4%), liver metastases (N=42875, 88.9%), or brain metastases (N=41858, 86.8%); 68.5% of the patients had no distant organ metastases; and 21.8% of the patients had one single organ metastasis. The cohort of 48,206 patients was divided into a train set (N=33,744) and a test set (N=14,462), with a ratio of 7:3. More details about the clinical characteristics are shown in Table 3.

We conducted univariate and multivariate logistic regression analyses to analyze the risk factors for distant organ metastasis in patients with LC. The results showed that age, race, sex, pathology, site, separate tumor nodule, T stage, and N stage were related to bone metastasis (Supplementary Table 1). Age, race, pathology, site, separate tumor nodule, T stage, and N stage were related to brain metastasis (Supplementary Table 2). Race, sex, pathology, site, separate tumor nodule, T stage, and N stage were related to liver metastasis (Supplementary Table 3). Then, these predictive clinical factors were used to construct nomograms to predict distant metastasis of the bone, brain and liver. The predictive model was constructed based on the train set and was verified in the test set. The nomograms are shown in Figure 2. The total points, based on the calculation of each variable point, were associated with the probability of organ metastasis.

Then, an ROC curve was constructed using the test set to assess the accuracy of the nomogram in predicting the development of distant organ metastasis. The results are shown in Figure 3. The AUC of bone metastasis was 0.724 (see Figure 3A), the AUC of brain metastasis was 0.717 (see Figure 3B), and the AUC of liver metastasis was 0.754 (see Figure 3C). These results suggested that the nomograms we constructed could accurately predict organ metastasis.

3.3 Nomogram predicting the survival probability of LC patients

Next, we investigated the clinical factors affecting the prognosis of LC patients and attempted to construct a prognostic nomogram based on these clinical characteristics. KM analysis was used to show the survival of LC patients among the different subgroups

TABLE 2 Cox analysis revealed the prognosis of patients with different organ metastases.

Pathology	Distant metastases sites								
Squamous cell carcinoma	metastases HR (95% CI) P value	Brain 1 (reference) 1 (reference)	No 0.336(0.3073-0.3675) <0.001	bone 1.113 (1.0023-1.237) 0.045238	liver 0.9116-1.1866) 0.55909	brain+bone 1.406(1.1804-1.675) <0.001	brain+liver 1.439 (1.1225-1.8437) 0.004074	bone+liver 1.35(1.1789-1.5461) <0.001	brain+bone +liver 1.785(1.4351-2.2195) <0.001
Adenocarcinoma	metastases HR (95% CI) P value	brain 1 (reference) 1 (reference)	No 0.4567 (0.4373-0.477) <0.001	bone 1.1839 (1.1253-1.246) <0.001	liver 1.5116 (1.392-1.641) <0.001	brain+bone 1.2096 (1.1327-1.292) <0.001	brain+liver 1.587 (1.3988-1.8801) <0.001	bone+liver 1.7215 (1.6034-1.848) <0.001	brain+bone +liver 1.5787 (1.4481-1.721) <0.001
Non-small cell carcinoma-NOS	metastases HR (95% CI) P value	brain 1 (reference) 1 (reference)	No 0.5306 (0.4819-0.5841) <0.001	bone 1.1016 (0.9811-1.237) 0. 10156	liver 1.2563 (1.0673-1.4788) 0.00608	brain+bone 1.1698 (0.9876-1.3858) 0.06952	brain+liver 1.8444 (1.4269-2.384) <0.001	bone+liver 1.3962 (1.1922-1.635) <0.001	brain+bone +liver 1.357(1.0774-1.7091) 0.0095
Small cell carcinoma	metastases HR (95% CI) P value	brain 1 (reference) 1 (reference)	No 0.5918(0.549-0.6379) <0.001	bone 1.0371 (0.9409-1.1431) 0. 46344	liver 1.5683 (1.4402-1.7078) <0.001	brain+bone 1.2634 (1.0874-1.468) 0.00226	brain+liver 1.7331 (1.5022-1.9994) <0.001	bone+liver 1.6116 (1.4699-1.7669) <0.001	brain+bone +liver 1.6131 (1.4088-1.8471) <0.001
Large cell carcinoma	metastases HR (95% CI) P value	brain 1 (reference) 1 (reference)	No 0.374 (0.2999-0.4664) <0.001	bone 1.188 (0.8862-1.5923) 0. 249288	liver 1.559 (1.1404-2.1319) 0.005383	brain+bone 1.145 (0.7575-1.73) 0.520945	brain+liver 1.694 (1.0368-2.7671) 0.035355	bone+liver 1.861(1.344-2.5773) <0.001	brain+bone +liver 1.442(0.9006-2.3086) 0.127512
Other	metastases HR (95% CI) P value	brain 1 (reference) 1 (reference)	No 0.2809 (0.2614-0.3019) <0.001	bone 1.0948 (1.0017-1.1965) 0.0459	liver 1.5279 (1.3861-1.6843) <0.001	brain+bone 1.0516 (0.9143-1.2096) 0.481	brain+liver 1.4595 (1.2165-1.751) <0.001	bone+liver 46(1.3067-1.6312) <0.001	brain+bone +liver 1.4675 (1.2378-1.7398) <0.001

TABLE 3 Clinical characteristics of lung cancer patients.

	Clinical characteristics of LC patients			
	Overall	train set	test set	p value
N	48206	33744	14462	
Age (%)				0.46
<50	2020 (4. 2)	1395(4.1)	625(4.3)	
50-59	7988 (16. 6)	5587(16.6)	2401(16. 6)	
60-69	15130 (31.4)	10573(31.3)	4557(31.5)	
70-79	14677 (30. 4)	10351 30. 7)	4326(29.9)	
>=80	8391 (17. 4)	5838 (17.3)	2553(17.7)	
Race (%)				0.648
Black	4405 (9. 1)	3092 9. 2)	1313(9.1)	
White	38264 (79. 4)	26817(79.5)	11447 (79. 2)	
Other	5463(11.3)	3784(11.2)	1679(11.6)	
Unknown	74(0. 2)	51(0.2)	23(0.2)	
Sex (%)				0.085
Female	23250 (48. 2)	16362(48. 5)	6888(47.6)	
Male	24956 (51. 8)	17382(51.5)	7574(52.4)	
Pathol ogy (%)				0.534
AD	18800 (39. 0)	13239(39.2 2)	5561(38.5)	
SCC	10044 (20. 8)	6980 (20. 7)	3064 (21. 2)	
LCC	668 1. 4)	476(1.4)	192(1. 3)	
NSCLC-NOS	3351 (7. 0)	2351 (7.0)	1000(6.9)	
SCLC	5772(12. 0)	4012(11.9)	1760(12.2)	
other	9571 (19. 9)	6686(19.8)	2885(19.9)	
Site of the primary tumor	(%)			0.561
LLL	5730(11. 9)	4054(12.0)	1676(11. 6)	
LMB	886(1. 8)	616(1. 8)	270(1. 9)	
LUL	11343(23.5)	7989(23.7)	3354(23. 2)	
RLL	7337 (15.2)	5125(15.2)	2212(15. 3)	
RMB	1196 (2.5)	846(2.5)	350 2. 4)	
RML	2289 (4.7)	1598(4.7)	691 (4. 8)	
RUL	14548 (30. 2)	10152(30.1)	4396(30.4)	
other	4877 (10. 1)	364(10. 0)	1513 (10. 5)	
Separate tumor nodule (%)				0.399
NO	34437 (71. 4)	24164(71.6)	10273(71.0)	
YES	11789 (24. 5)	8194(24.1 3)	3595(24. 9)	
Other	1980(4.1)	1386(4. 1)	594(4.) 1)	
Bone metastasis (%)				1
NO	39234 (81. 4)	27464(81.4)	11770(81.4)	
Yes	8972(18.6 6)	6280(18.6)	2692(18.6)	

(Continued)

TABLE 3 Continued

	Clinical characteristics of LC patients			
	Overall	train set	test set	p value
N	48206	33744	14462	
Liver metastasis (%)				0.523
NO	42875 (88. 9)	30033 (89. 0)	12842(88.8)	
Yes	5331 (11. 1)	3711 (11. 0)	1620(11. 2)	
Brain metastasis (%)				0.346
NO	41858 (86. 8)	29333 (86. 9)	12525(86.6)	
Yes	6348(13.2)	4411 (13. 1)	1937(13. 4)	
T stage (%)				0.364
TO	309 (0. 6)	207 (0. 6)	102(0. 7)	
T1	11170 (23. 2)	7883(23. 4)	3287(22. 7)	
T2	14753 (30. 6)	10283 (30. 5)	4470(30.9)	
T3	10513 (21. 8)	7380(21.9)	3133(21.7)	
T4	11461 (23. 8)	7991 (23. 7)	3470(24.0)	
N stage (%)				0.159
NO	19456 (40. 4)	13730 (40. 7)	5726(39.6) 6)	
N1	4468 9. 3)	3120(9. 2)	1348(9. 3)	
N2	17062 (35. 4)	11866 (35. 2)	5196(35.1 9)	
N3	7220 (15. 0)	5028 (14. 9)	2192(15.2)	
Number of organ etastases				0.682
0	33021 (68. 5)	23125(5)	9896(68. 4)	
1	10529 (21. 8)	7393(21.9)	3136(21.7)	
2	3846 (8. 0)	2669(7.9)	1177(8.1 1)	
3	810(1. 7)	557(1 7)	253 (1. 7)	

SCC, squamous cell carcinoma; AD, adenocarcinoma; SCLC, small cell lung carcinoma; NSCLC-NOS, non-small cell lung carcinoma-not otherwise specified; LCC, large cell carcinoma; LLL, left lower lobe; LMB, left main bronchus; LUL, left upper lobe; RLL, center lower lobe; RMB, center main bronchus; RMB, center middle lobe; RUL, center upper lobe.

(Figure 4). The median OS for all the patients in the whole cohort was 12 months. The median OS times of the patients with bone, liver and liver metastasis were 4, 5 and 3 months, respectively.

Then, univariate and multivariate Cox analyses were conducted to explore the potential prognostic factors. The results showed that age, sex, race, pathology, primary lesion site, separate tumor nodule, T stage, N stage, and number of organ metastases were all associated with the development of brain metastasis. The Cox analysis results are shown in Table 4. All prognostic factors were used to construct a nomogram predicting the survival of LC patients at 1, 3, and 5 years based on the training set (see Figure 5). The c-index of this nomogram was 0.719 (95% CI, 0.715-0.723) for the training set and 0.718 (95% CI, 0.714-0.722) for the test set. Then, we constructed ROC curves to evaluate the accuracy of the nomogram in predicting the 1-, 3- and 5-year survival probabilities in the test set (Figure 6). The AUC of 1-year survival was 0.798, 3-year survival was 0.833 and 5-year survival was 0.842. These results suggested that the nomogram had good predictive performance for LC patient survival.

3.4 Prognostic value of the nomogram score

Based on the nomogram we constructed, we scored the patients in the test set and divided them into high- and low-risk groups according to the median nomogram score. The median survival time of the high-risk group was 6 months, while the median survival time of the low-risk group was 34 months. The KM analysis suggested that the survival difference between the high- and low-risk groups was significant (Figure 7). The patients in the high-risk group had shorter survival times. This result indicated that the predicted score of our model is closely associated with patient prognosis.

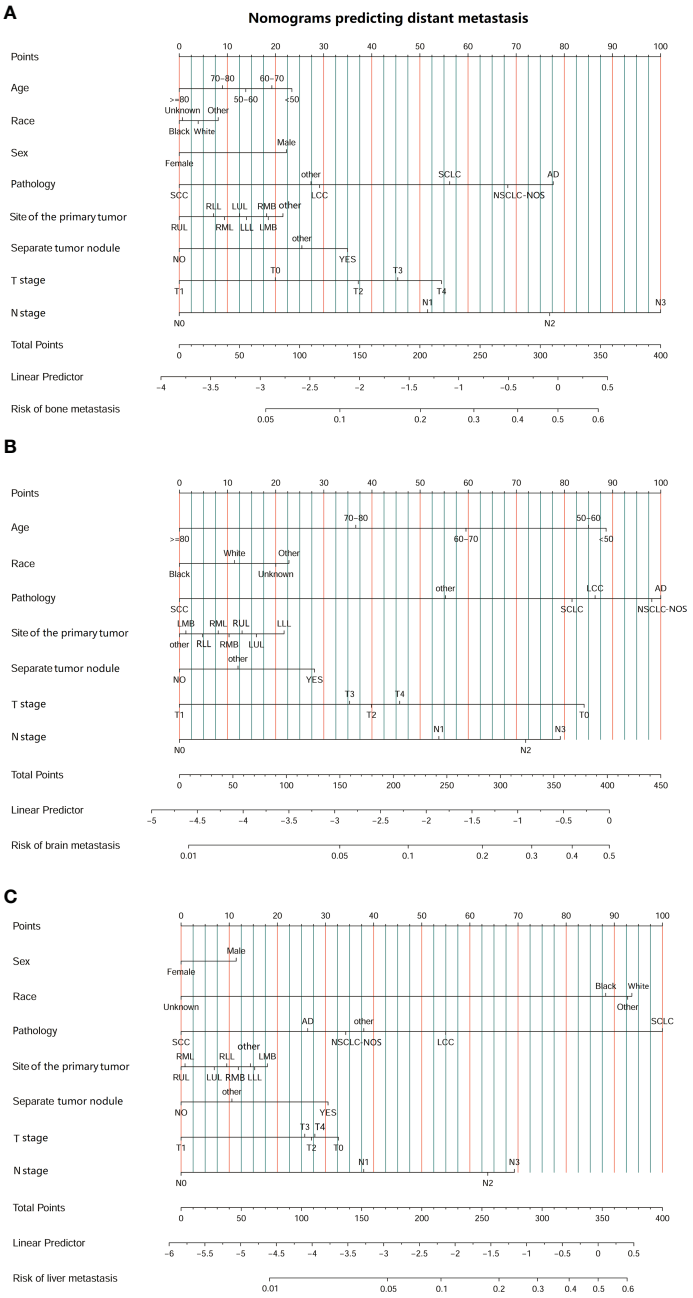

FIGURE 2
Nomograms predicting the risk of organ metastasis in patients with lung cancer. **(A)** Nomogram predicting the risk of bone metastasis in patients with LC. **(B)** Nomogram predicting the risk of brain metastasis in patients with LC. **(C)** Nomogram predicting the risk of liver metastasis in patients with LC. (SCC, squamous cell carcinoma; AD, adenocarcinoma; SCLC, small cell lung carcinoma; NSCLC-NOS, non-small cell lung carcinoma-not otherwise specified; LCC, large cell carcinoma; LLL, left lower lobe; LMB, left main bronchus; LUL, left upper lobe; RLL, right lower lobe; RMB, right main bronchus; RMB, right middle lobe; RUL, right upper lobe.).

4 Discussion

As the second most common tumor, LC is a serious threat to human health. In recent years, with the development of comprehensive cancer treatments, including surgery, radiotherapy, traditional chemotherapy, targeted therapy and immunotherapy, the survival time of LC patients has been prolonged. However, distant metastasis of LC is still an obstacle to treatment and affects the survival of patients. Evaluating the possibility of developing distant metastasis based on clinical characteristics and examining patients at high risk to detect distant organ metastasis earlier could help physicians adjust treatment plans and improve the prognosis of patients.

Previous studies have suggested that SCLC usually metastasizes to the liver, bone, brain and other organs. Genetic changes may affect its metastatic site (17). In addition, the injection of SCLC cells into the middle vein of mice in one study specifically led to the occurrence of liver metastasis rather than lung metastasis (18). This

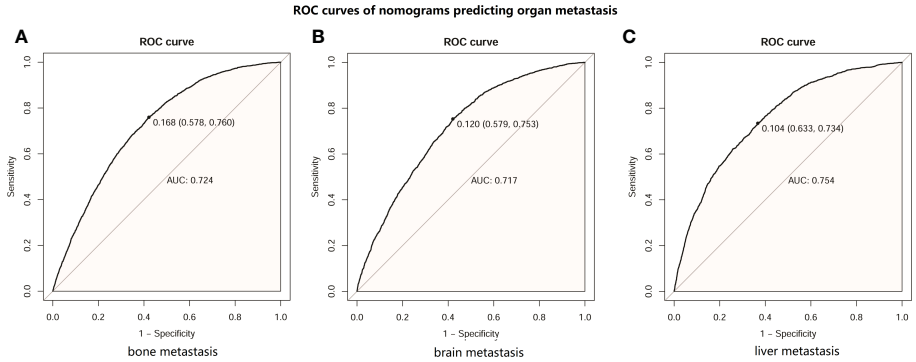

FIGURE 3
ROC curves of distant metastasis prediction nomograms in patients with lung cancer (LC). **(A)** ROC curve of nomogram predicting bone metastasis in patients with LC. **(B)** ROC curve of nomogram predicting brain metastasis in patients with LC. **(C)** ROC curve of nomogram predicting liver metastasis in patients with LC.

suggests that small cell lung cancer cells may be more likely to metastasize to the liver through the blood, and the potential mechanism of its metastasis remains to be studied. For NSCLC, the AD and LCC pathological types are associated with a higher risk of brain metastasis than SCC (12, 19). Few studies have focused on the survival risk comparison among LC with different distant metastases and the prediction of distant metastases. In our study, our results showed that various pathologic types of LC show a strong correlation with site-specific metastasis patterns. Our results revealed that the most common metastatic organs are bone for SCC and AD, liver for SCLC and brain for LCC. SCLC is the most prone to distant organ metastasis, as well as multiple organ metastases, especially bone+liver metastasis, while SCC is the least prone. This is consistent with the results of a previous study (20). Regarding patient survival, a study focusing on NSCLC showed that adenocarcinoma is the most common variant for NSCLC, and the mortality risk is highest in multiple metastasis and liver metastasis groups (21). Similarly, in our study, the patients with triple metastases (brain-bone-liver) had the worst prognosis, and for nonsquamous carcinoma with single organ metastasis, liver metastases conferred the worst prognosis, which is consistent with the results of another study (22, 23). However, for SCC patients, there was no significant difference between the survival of SCC patients with single liver and single brain metastasis.

Approximately 30-40% of NSCLC patients will have bone metastasis (22, 24). Research has found that bone metastasis of LC is associated with the cytokines TGF-β and PTHrP, which can promote osteolysis (25–27). SCC can produce a large amount of MMP9 *via* stimulation by collagen I, thus establishing bone metastasis and releasing tumor cell chemokines during osteolysis (28). Our results suggest that SCC patients with bone metastasis alone have the worst prognosis compared with those with brain metastasis and liver metastasis alone. Two previous retrospective studies (29, 30) on esophageal and gallbladder cancer also reached similar conclusions; that is, among patients with single-organ metastasis, patients with bone metastasis had the worst prognosis (compared with patients with liver metastasis and lung metastasis). In addition, in a real-world study on patients with lung squamous cell carcinoma in 2022, the author found that only bone metastasis was found in distant organ metastasis, which was significantly related to the shorter PFS of these patients (31). This suggests that bone metastasis may be the special metastatic site of these tumors. Because few studies have compared the prognosis of different metastatic modes/sites of lung squamous cell carcinoma, the reason for this phenomenon is not clear. Its potential mechanism needs to be elaborated in future research.

We also investigated the risk factors for developing different organ metastases. LC patients of other races (American Indian/Alaska Native race) were more prone to brain and bone metastasis but not liver metastasis. A study exploring the risk factors for BM from esophageal cancer revealed that other races (American Indian/Alaska Native race) were positively associated with the occurrence of brain metastasis (32). All these results suggest that race is an important factor influencing tumor metastasis with unknown

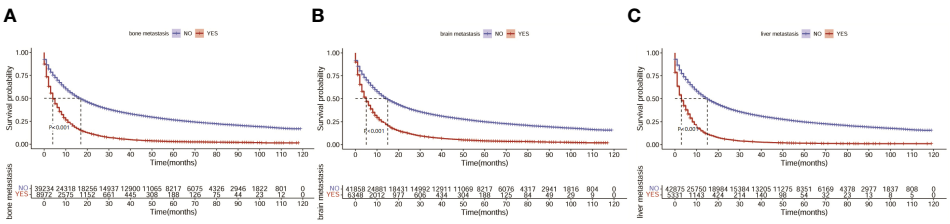

FIGURE 4
Survival of LC patients with distant metastasis. **(A)** Survival of LC patients with bone metastasis. **(B)** Survival of LC patients with brain metastasis. **(C)** Survival of LC patients with liver metastasis.

TABLE 4 Univariate and multivariate cox analysis results of the prognostic factors LC patients.

	Univariate cox analysis		Multivariate cox analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age				
<50	1 (reference)	1 (reference)	1 (reference)	1 (reference)
50-59	1.29(1.22-1.37)	<0.001	1.36(1.28-1.44)	<0.001
60-69	9(1.31-1.47)	<0.001	3(1.54-1.73)	<0.001
70-79	61(1.52-1.70)	<0.001	2.15(2.03-2.28)	<0.001
>=80	2.25(2.12-2.39)	<0.001	3.46(3.27-3.67)	<0.001
Race				
Black	1 (reference)	1 (reference)	1 (reference)	1 (reference)
White	0.98 (0.94-1.01)	0.1910	0.94(0.90-0.97)	<0.001
Other	0.91 (0.87-0.95)	<0.001	0.77(0.74-0.81)	<0.001
Unknown	0. 35(0.24-0.50)	<0.001	0.45(0.31-0.65)	<0.001
Sex				
Female	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Male	1.3(1.27-1.33)	<0.001	1.23(1.21-1.26)	<0.001
Pathology				
AD	1 (reference)	1(reference)	1 (reference)	1 (reference)
SCC	(1.05-1.11)	<0.001	1.13(1.10-1.16)	<0.001
LCC	(1.11-1.31)	<0.001	1.29(1.19-1.41	<0.001
NSCLC-NOS	(1.48-1.60)	<0.001	38(1.33-1.44)	<0.001
SCLC	1.72(1.67-1.77)	<0.001	1.22(1.18-1.26)	<0.001
Other	0.75(0.73-0.77)	<0.001	1.02(0.99-1.06)	0.1268
Site of the primary tumor				
LLL	1 (reference)	1 (reference)	1 (reference)	1 (reference)
LMB	65 (1.53-1.78)	<0.001	1.18(1.10-1.28)	<0.001
LUL	1.04 (1.00-1.07)	0.0551	0.99(0.95-1.03)	0.5721
RLL	1.04(1.00-1.08)	0.0580	1.03(0.99-1.07)	0.1550
RMB	1.88(1.76-2.01)	<0.001	1.31(1.22-1.40)	<0.001
RML	0.90(0.86-0.96)	<0.001	0.94(0.89-0.99)	0.0268
RUL	1.03(0.99-1.07)	0.1060	0.99(0.96-1.03)	0.6923
Other	1.85(1.77-1.93)	<0.001	1.31(1.26-1.37)	<0.001
Separate tumor nodule				
NO	1 (reference)	1 (reference)	1 (reference)	1 (reference)
YES	1.74(1.70-1.78)	<0.001	0.94(0.92-0.97)	<0.001
other	97(1.88-2.07)	<0.001	1.33(1.26-1.39)	<0.001
T stage				
T1	1 (reference)	1 (reference)	(reference)	1 (reference)
T2	1.80(1.75-1.86)	<0.001	1.45(1.40-1.49)	<0.001

(Continued)

TABLE 4 Continued

	Univariate cox analysis		Multivariate cox analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
T3	2.56(2.49-2.65)	<0.001	1.86(1.79-1.93)	<0.001
T4	18(3.08-3.28)	<0.001	1.99(1.92-2.06)	<0.001
TO	53(2.24-2.86)	<0.001	1.14(1.01-1.29)	0.0406
N stage				
NO	1 (reference)	1 (reference)	1 (reference)	1 (reference)
N1	66(1.60-1.72)	<0.001	41(1.36-1.46)	<0.001
N2	57(2.51-2.64)	<0.001	1.88(1.83-1.93)	<0.001
N3	2.90(2.81-2.99)	<0.001	1.99(1.93-2.06)	<0.001
Number of metastases	1.87(1.85-1.89)	<0.001	1.67(1.64-1.69)	<0.001

mechanisms. A previous study suggested that male sex is associated with a higher risk of brain metastasis in SCLC (33). According to our results, in addition to brain metastasis, male patients are also more likely to develop liver and bone metastasis. The logistic regression analysis showed that the characteristics of the primary tumor, including the location of the primary tumor, pathological type, T stage, N stage, and separate nodules, were closely related to the occurrence of distant metastasis. Compared with the left lower lobe, tumors with a primary focus in the center lung are less prone to organ metastasis, especially tumors with a primary focus in the upper and middle lobes of the center lung. Previous studies have reported that lung adenocarcinoma with a primary focus within the left side has a higher risk of skull metastasis than lung adenocarcinoma with a primary focus within the center side.

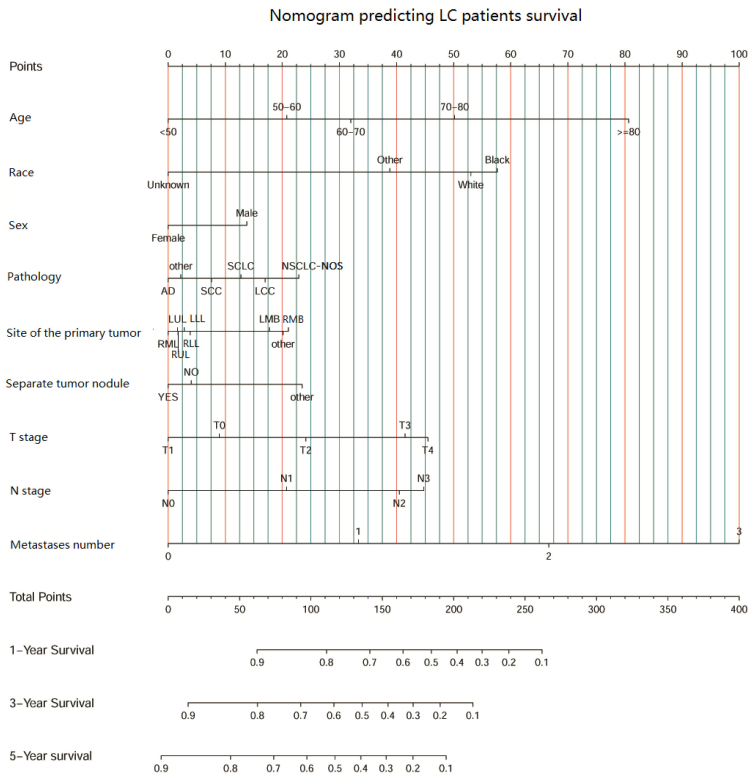

FIGURE 5
The nomogram predicting LC patient survival based on clinical factors.(SCC, squamous cell carcinoma; AD, adenocarcinoma; SCLC, small cell lung carcinoma; NSCLC-NOS, non-small cell lung carcinoma-not otherwise specified; LCC, large cell carcinoma; LLL, left lower lobe; LMB, left main bronchus; LUL, left upper lobe; RLL, right lower lobe; RMB, right main bronchus; RMB, right middle lobe; RUL, right upper lobe.).

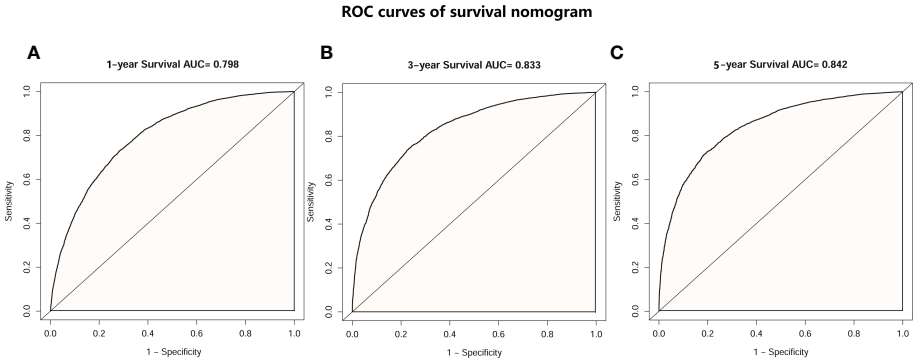

FIGURE 6
ROC curves of the survival nomogram. **(A)** ROC curve of 1-year survival prediction nomogram in patients with LC. **(B)** ROC curve of 3-year survival prediction nomogram in patients with LC. **(C)** ROC curve of 5-year survival prediction nomogram in patients with LC.

Peripheral lung cancer was associated with brain metastasis (34), while central lung cancer was associated with bone metastasis (35). However, the research results of Mujoomdar et al. suggested that the risk of brain metastasis of NSCLC was not related to the location of the primary tumor (36). The relationship between the location of the primary lesion and distant metastasis has not been agreed upon. In addition, our results suggest that among various pathological types, patients with SCC are the least likely to have organ metastasis, patients with AD have a higher risk of bone metastasis and brain metastasis, and patients with SCLC have a higher risk of liver metastasis than patients with other pathological types. Patients with higher T stage, N stage and independent tumor nodules have a higher risk of bone, brain and liver metastasis, which is consistent with several previous studies (9, 13). Our nomograms based on these clinical factors showed good performance in predicting the occurrence of bone (AUC=0.724), brain (AUC=0.754) and liver (AUC=0.717) metastasis.

In this study, based on the multivariate Cox analysis, it was suggested that a primary lesion located in the main bronchus was a poor prognostic factor for LC patients, which caught our attention. Similar results were achieved in previous studies focusing on AD and LCC (37–39). The researchers found that the SUV value of tumors located in the center of PET was significantly higher, which may indicate that there was more active tumor metabolism (40). Main bronchial tumors are closely related to a higher risk of lymph node metastasis and organ metastasis (38, 41). In addition, patients with central tumors are more likely to develop obstructive pneumonia, which also leads to poor prognosis (42). These are all possible factors leading to poor prognosis of main bronchial tumors. In the future, more multicenter clinical studies are needed to reveal the potential mechanisms.

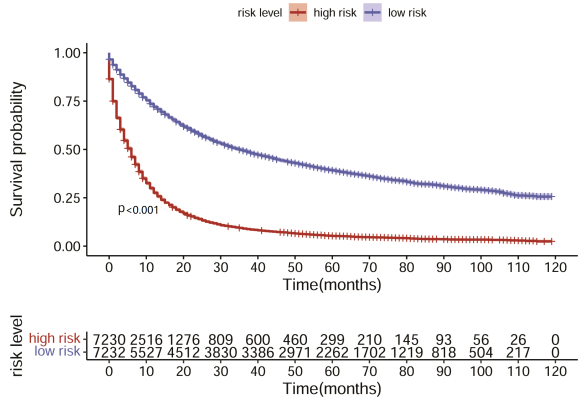

FIGURE 7
KM analysis showed survival outcome of high- and low- risk patients.

Based on the results of the Cox analysis, we established a survival nomogram to predict the survival of LC patients. The nomogram included age, race, sex, pathology type, primary site, whether there was a separate tumor nodule, T stage, N stage and the number of organ metastases. Although several prognostic models were established for patients with LC or NSCLC with brain metastasis in previous studies (13, 14, 43), separate tumor nodules, primary lesion sites and the number of organ metastases were included together as prognostic factors for the first time. Our model gives individual scores according to the clinical characteristics of each patient and predicts the 1-year, 3-year and 5-year survival rates. The model was independently verified in the test set, and the results showed a high concordance index of 0.718 (95% CI, 0.714-0.722) and an AUC score of 0.842. This suggests that our nomogram has a satisfactory predictive ability and shows better performance than the previous prediction models (37, 44, 45). The combination of the separate nodule status, the location of the primary lesion and the number of organ metastases makes this model more comprehensive and personalized to predict the prognosis and survival of different LC patients.

This study has some limitations. Not all the metastasis statuses of LC patients in the SEER database are clearly described. Therefore, the incidence of brain metastasis, liver metastasis and bone metastasis may be inaccurate. The SEER database does not provide detailed information about the follow-up treatment (including chemotherapy, immunotherapy, targeted treatment, etc.) that the patients received, which may contribute to potential bias and may influence the prognosis of patients, and follow up treatment was not included in this study. In addition, previous

studies suggested that extrathoracic lymph node metastasis (46–48) was also a key factor affecting the prognosis of LC patients, but this variable is missing for the patients including in this cohort in the SEER database. This will be a key clinical factor for optimizing our predictive model in the following studies. In future research, more clinical data of stage IV LC patients with distant metastasis could be collected prospectively, as well as the therapy and survival time of these patients, so as to verify the results in this study in clinical data and test the accuracy and predictive performance of the model we constructed. What's more, the molecular mechanisms underlying the preferential metastatic sites of different types of LC need to be elucidated in future studies.

5 Conclusion

In this study, we described a detailed landscape of distant organ metastasis statuses and their effects on overall survival in different pathological types of lung cancer. We found that the pathology of LC showed a strong correlation with site-specific metastasis patterns. Moreover, we investigated the risk factors for developing distant organ metastases in LC patients using data downloaded from the SEER database and constructed nomograms that predict distant metastasis and OS with good performance. These results are helpful for clinicians to conduct clinical evaluations and develop individualized therapeutic strategies.

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

SEER database is publicly accessible worldwide. The authors signed the SEER database agreement and got the license to access SEER data.

Author contributions

YH: research design, data collection, interpretation and analysis, manuscript drafting. GL: research design, critical manuscript revision. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1075385/full#supplementary-material

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Factors associated with outcomes of second-line treatment for *EGFR*-mutant non-small-cell lung cancer patients after progression on first- or second-generation EGFR-tyrosine kinase inhibitor treatment

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Purpose: Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are standard first-line treatments for advanced *EGFR*-mutant non-small-cell lung cancer (NSCLC) patients. However, factors associated with outcomes after progression on first-line therapy are seldom investigated.

Materials and methods: From January 2016 to December 2020, we enrolled 242 EGFR-mutant stage IIIB–IV NSCLC patients who progressed on first- or second-generation EGFR-TKI treatments, and 206 of them receive second-line treatments after disease progression. The factors that predict the survival outcomes of different second-line treatments after disease progression were evaluated. Clinical and demographic characteristics, including metastatic sites, neutrophil-to-lymphocyte ratio (NLR) at first-line progression, and second-line treatment regimens, and whether re-biopsied after disease progression or not, were reviewed for outcome analysis.

Results: The univariate analysis showed that the PFS was shorted in male patients ($p = 0.049$), patients with ECOG performance state ≥ 2 ($p = 0.014$), former smokers ($p = 0.003$), patients with brain metastasis ($p = 0.04$), second-line

chemotherapy or EGFR-TKIs other than osimertinib (p =0.002), and NLR ≥5.0 (p=0.024). In addition, second-line osimertinib was associated with longer OS compared to chemotherapy and other EGFR-TKI treatment (p =0.001). In the multivariate analysis, only second-line osimertinib was an independent predictor of PFS (p =0.023). Re-biopsy after first-line treatment was associated with a trend of better OS. Patients with NLR ≥5.0 at disease progression had shorter OS than patients with NLR <5.0 (p = 0.008).

Conclusion: The benefits of osimertinib necessitate that aggressive re-biopsy after progression on first- or second-generation EGFR-TKI treatment is merited for appropriate second-line treatments to provide better outcomes for these patients.

KEYWORDS

epidermal growth factor receptor-tyrosine kinase inhibitor, neutrophil-to-lymphocyte ratio, non-small-cell lung cancer, osimertinib, re-biopsy, second-line

Highlights

- Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are standard first-line treatments for advanced *EGFR*-mutant non-small-cell lung cancer (NSCLC) patients. Factors associated with the outcomes in NSCLC patients with disease progression on first- or second-generation EGFR-TKI like gefitinib, erlotinib, or afatinib, have rarely been investigated.
- We enrolled 242 patients treated with gefitinib, erlotinib, or afatinib as first-line treatment. Upon disease progression, only 70 (28.9%) patients underwent re-biopsy and 206 (85.1%) received second-line treatment. Outcome analysis indicated a better outcome in patients who underwent re-biopsy or received osimertinib as the second-line treatment or whose neutrophil-to-lymphocyte ratio was < 5 at disease progression on first-line treatment.
- Aggressive re-biopsy after progression on gefitinib, erlotinib, or afatinib treatment is merited for appropriate second-line treatment such as osimertinib to provide better outcomes for patients.

Introduction

Lung cancer is the leading cause of mortality due to cancer in the world, with an estimated 1.8 million deaths in 2020.(1) Non-small-cell lung cancer (NSCLC) accounts for 80–90% of all lung cancer cases, and more than 70% of NSCLC patients present with locally advanced or metastatic disease (Stage III or IV) at initial diagnosis.(2) Epidermal growth factor receptor (*EGFR)* mutations are observed in 40–60% and 10–20% of NSCLC patients in Asian and non-Asian populations, respectively.(3) For patients with advanced *EGFR*-mutant NSCLC, EGFR-tyrosine kinase inhibitors (TKIs) are the standard first-line treatment. TKIs have been reported to show a higher response rate and longer progression-free survival (PFS) compared to conventional chemotherapy.(4–6) Osimertinib, a third-generation ECGR-TKI, is the preferred treatment recommended in NCCN guidelines.(7) However, in the FLAURA study, osimertinib did not demonstrate an overall survival (OS) benefit in Asian population subgroups and patients with the *L858R* mutation compared to first-generation EGFR-TKI.(8, 9) In addition, osimertinib may not be available or affordable in some countries due to its relatively high cost.

In patients treated with first- or second-generation EGFR-TKIs, such as gefitinib, erlotinib, and afatinib, disease progression inevitably occurred after a median time of 10 to 14 months. Of these patients, approximately 50% developed EGFR T790M as the resistance mechanism, which could be effectively treated with osimertinib as a subsequent-line treatment.(10) Nevertheless, the detection of EGFR T790M or other resistance mechanisms for the next line of treatment relies on re-biopsy of the tissue or liquid biopsy. The ESMO guidelines recommend switching to platinum-based doublet chemotherapy for patients who cannot undergo tissue biopsy or for those in whom the T790M mutation is not detected.(11)

Previous studies reported that approximately 70% of patients received subsequent treatment after progressing on first- or second-generation EGFR-TKI treatment. Although the re-biopsy rate (tissue or liquid) was 85–87% after disease progression, only 30–46% of patients tested positive for the T790M mutation and received osimertinib treatment.(12, 13) A retrospective, real-world study from Greece showed a T790M positivity rate of 21.9% (based on cobas® molecular testing of plasma and/or tissue biopsy), which may compromise the clinical outcome due to the lack of subsequent osimertinib therapy.(14) However, in patients with the T790M mutation, no statistically significant OS benefit was observed for osimertinib compared to platinum–pemetrexed chemotherapy.(10)

Data on factors related to the safety and efficacy of different second-line treatments and their impacts on the prognosis of *EGFR*-mutant NSCLC are limited. The aim of this study was to investigate the factors associated with the efficacy and prognosis in *EGFR*-mutant NSCLC patients who received second-line treatment after progression on first- or second-generation EGFR-TKIs.

Materials and methods

Patient selection and data collection

This was a multicenter, retrospective study that included a medical center and three regional hospitals in Taiwan. Between January 2016 and December 2020, patients fulfilled the following criteria were enrolled in the study: **1)** a diagnosis of locally advanced or metastatic (Stage IIIb–IV) *EGFR*-mutant NSCLC; **2)** first- or second-generation EGFR-TKI including gefitinib, erlotinib, or afatinib administered as the first-line treatment; and **3)** confirmed disease progression on first-line EGFR-TKI treatment. Patients who switched to another anti-cancer drug or regimen due to intolerance or reasons other than disease progression were excluded. This study was approved by the Institutional Review Boards of all participating institutions.

Demographic and clinical data related to lung cancer were collected, including age, sex, smoking status, cancer staging at diagnosis, initial metastatic sites, *EGFR* mutation subtype, the type of EGFR-TKI therapy, Eastern Cooperative Oncology Group- Performance Status (ECOG PS) score, neutrophil-to-lymphocyte ratio (NLR) at disease progression on first-line treatment, whether or not re-biopsied after disease progression on first-line and subsequent treatment regimens (including osimertinib, other EGFR-TKIs, and different chemotherapeutic drugs). Other EGFR-TKIs include those patients with treatment beyond progression or switched to TKIs other than osimertinib, or those who have declined or are contraindicated to chemotherapy. NLR was obtained after disease progression on the first-line treatment, which was calculated by dividing the number of neutrophils by number of lymphocytes from peripheral blood sample. PFS was defined as the period calculated from the initiation of a single treatment to disease progression or death. The OS was defined as the period calculated from the initiation of the first-line EGFR-TKI treatment to date of death.

Statistical analysis

Efficacy and prognosis were analysed for all patients who received afatinib, erlotinib, or gefitinib as first-line treatment and osimertinib, other EGFR-TKIs, or chemotherapeutic drugs as second-line therapy. The medians (ranges) of continuous variables with non-normal distributions are reported, whereas the frequencies (percentages) of categorical variables are reported. The Kruskal–Wallis test was used to compare continuous variables between different groups. The chi-square and Fisher's exact tests were used to compare the efficacy between different subgroups. The median time to PFS or OS was calculated using the Kaplan–Meier method. Univariate and multivariate Cox regression analyses were used to evaluate the effects of clinical factors, including different first-line and second-line treatments, on PFS and OS of the patients. Hazard ratios (HR) with 95% confidence interval (95% CI) were calculated. All statistical analyses were performed using SPSS 25.0 and R 3.6.0 software. The level of statistical significance was set at $p < 0.05$.

Results

A total of 242 patients were enrolled in this study. Table 1 shows the demographic characteristics of the enrolled population. The median age was 66 years (range = 36 to 90 years). A higher proportion of the patients were female (51.5%), had never smoked (72.0%), and had a ECOG PS score of 0–1 (81.0%). Most patients had stage IV (88.4%) disease at diagnosis with < 3 metastatic sites (87.6%). In the case of metastases, 28.1% of the patients showed brain metastases and 12.8% liver metastases. An NLR of ≥5 was observed in 27.3% of all patients at disease progression on first-line treatment.

The median PFS and OS of the whole cohort were 19.1 (95% confidence interval [CI] = 17.7–20.5) months and 29.6 (95% CI = 27.1–32.1) months, respectively (Supplementary Figure 1). PFS and OS were better in patients that received afatinib as the first-line treatment (14.3 months and 34.1 months, respectively) than in those that received gefitinib (11.9 months and 25.8 months, respectively) or erlotinib (10.8 months and 26.7 months, respectively; Supplementary Figure 2).

Upon disease progress, 206 (85.1%) out of the 242 patients received second-line treatment, including 18 (8.7%) received osimertinib, 26 (12.6%) received EGFR-TKIs other than osimertinib, and 162 (78.6%) received chemotherapy. The survival outcomes of the second-line treatment are shown in Table 2. The median PFS and OS of the whole cohort were 5.03 (95% confidence interval [CI] = 4.47–5.60) months and 14.4 (95% CI = 12.80–16.0) months, respectively. A better PFS and OS were observed in patients who received second-line therapy than in those that did not (PFS 17.1 versus 12.4 months, $p = 0.015$; Figure 1A, and OS 30.4 versus 21.6 months, $p = 0.032$ for OS; Figure 1B).

The univariate analysis (Table 3) showed that the PFS was shorter in male patients (1.3, 95% CI 1.0 - 1.78, p =0.049), patients with ECOG performance state ≥ 2 (HR 1.59, 95% CI 1.1 - 2.33, p =0.014), former smokers (1.79, 95% CI 1.23 - 2.63, p =0.003), patients with brain metastasis (HR 1.4, 95% CI 1.02-1.93, = 0.04), second-line with other EGFR-TKI or chemotherapy (HR 1.54, 95% CI 1.06-2.24, p =0.021). A NLR ≥5.0 was also associated with a shorter PFS (HR 1.45, 95% CI 1.05-2.01, p=0.024, Figure 2A), but not for OS (Figure 2B). Compared to second-line osimertinib, as shown in Figure 3, other EGFR-TKIs or chemotherapy was associated with a lower PFS (HR 1.54, 95% CI 1.06-2.24, p =0.021) and OS (HR 3.47, 95% CI 1.61-7.50, p =0.023). In the multivariate analysis, only second-line osimertinib was an independent predictor of better PFS (HR 2.74, 95% CI 1.32-5.69, p =0.007). OS was marginally longer in patients who underwent re-biopsy than in those that did not (19.33 versus 16.42 months, hazard ratio [HR] = 0.69, 95% CI = 0.48–1.0, p = 0.059; Figure 4B), but no significant difference for PFS (Figure 4A).

TABLE 1 Baseline characteristics of patients enrolled in this study.

Characteristic	Population (N=242)	Characteristic	Population (N=242)
Age at diagnosis, median (range)	66 (36, 90)		
Gender		**Brain metastasis at diagnosis**	68 (28.1%)
Male	117 (48.5%)	**Liver metastasis at diagnosis**	31 (12.8%)
Female	125 (51.5%)	**Metastatic sites**	
Smoking status		0	38 (15.7%)
Current smoker	34 (14.0%)	1	96 (39.7%)
Former smoker	34 (14.0%)	2	78 (32.2%)
Never smoked	174 (72.0%)	≥3	30 (12.4%)
Staging at diagnosis		**Neutrophil-to-lymphocyte ratio (NLR) at progression**	(n =188)
Stage III	28 (11.6%)	<5	133 (70.7%)
Stage IV	214 (88.4%)	≥5	55 (29.3%)
ECOG Performance Status		**First-line EGFR-TKI**	
0–1	196 (81.0%)	Gefitinib	70 (28.9%)
2–4	46 (19.0%)	Erlotinib	50 (20.7%)
EGFR Mutation		Afatinib	122 (50.4%)
Exon 19 deletion	109 (45.0%)	**Second-line regimen**	(n =206)
L858R mutation	120 (49.6%)	Osimertinib	18 (8.7%)
Uncommon mutations	13 (5.4%)	Other EGFR-TKIs	26 (12.6%)
Re-biopsy after 1st-line progression	70 (28.9%)	Chemotherapy	162 (78.6%)
Re-biopsy after 2nd-line progression	30 (11.5%)		

Table 4 shows the univariate and multivariate analyses for the predictors of OS. Patients with NLR ≥5.0 had shorter OS than patients with NLR <5.0 (12.3 versus 14.5 months, HR =1.66, 95% CI 1.14-2.42, p = 0.008; Figure 2A). No significant associations were found between survival outcomes (PFS and OS) with ECOG PS, no metastasis sites, mutation type, and time of diagnosis.

Discussion

In this multicenter retrospective study, we investigated the factors associated with survival outcomes of second-line treatments for *EGFR*-mutant NSCLC in patients with disease progression on first- or second-generation EGFR-TKI in Taiwan. Our study showed that PFS was shorter in male gender, ECOG status ≥2, former smoking, brain metastasis, second-line chemotherapy or EGFR-TKIs other than osimertinib, and NLR ≥5.0. The multivariate analysis indicated osimertinib as the second-line treatment was an independent predictor of PFS. These results suggest that the benefits of osimertinib necessitates that aggressive re-biopsy after progression on first- or second-generation EGFR-TKI treatment is merited for appropriate second-line treatments to provide better outcomes for these patients.

EGFR-TKI therapy is the standard treatment for patients with advanced *EGFR*-mutated NSCLC; it has a higher response rate and provides better symptom control and quality of life improvements compared to conventional chemotherapy or immunotherapy.(15, 16) However, disease progression with acquired resistance is inevitable and tissue re-biopsy or liquid biopsy is recommended for the guide of second-line treatment. Nevertheless, the re-biopsy rate after disease progression on first-line EGFR-TKI is variable in real-world studies, ranging from 60% to 90%.(17–20) The low re-biopsy rate (28.9%) in this study is likely due to osimertinib not been reimbursed by the National Institutes of Health (NIH) of Taiwan until early 2020. This might have led to fewer patients consenting to re-biopsy and reduced clinicians' willingness to perform it, since most patients may not be able to afford osimertinib as a subsequent treatment even when the outcome is positive for the t790M mutation. However, PFS and OS were significantly longer in those patients receiving osimertinib as a second-line treatment than in those who received other treatments or did not receive any subsequent treatment. This result is comparable to or even better than the results of previous retrospective real-world studies, which showed a PFS of 9.4 to 10.1 months and OS of 24 to 47.3 months for osimertinib as a second-line or subsequent treatment.(21–23) The better PFS and OS in this study may be due to the relatively small number of patients and the focus on only those treated with osimertinib as the second-line therapy.

For NSCLC patients without the T790M mutation, platinum-based chemotherapy is the recommended second-line therapy after progression on first- and second-generation EGFR-TKIs.(24)

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TABLE 2 Analysis of lines of treatment and outcomes of patients on second-line treatment (n =206).

Subgroups	PFS						OS					
	N	No. of events	Median	0.95 LCL	0.95 UCL	*P-value*	N	No. of events	Median	0.95 LCL	0.95 UCL	*P-value*
Whole cohort	206	195	5.03	4.47	5.60		205	132	14.40	12.80	16.00	
Age												
<65 years	68	63	5.03	3.58	6.48	0.510	96	53	22.70	14.68	30.72	0.052
≥65 years	138	132	5.00	4.44	5.56		109	79	13.53	12.37	14.70	
Sex												
Female	96	88	5.13	3.73	6.54	0.047	96	53	15.87	11.94	19.79	0.098
Male	110	107	5.00	4.44	5.56		109	79	13.86	12.47	15.27	
ECOG												
0-1	171	161	5.07	4.36	5.78	0.013	170	111	14.23	12.51	15.96	0.957
≥2	35	34	4.33	2.88	5.79		35	21	15.03	9.04	21.03	
Smoking status												
Never smoker	139	128	5.13	4.58	5.69	0.009	138	88	14.40	12.61	16.19	0.407
Current	32	32	4.20	2.98	5.42		32	20	13.23	11.75	14.71	
Former	35	35	3.77	1.68	5.85		35	24	15.77	10.60	20.93	
Metastatic site												
<3	177	166	4.93	4.37	5.50	0.817	177	118	13.93	12.70	15.17	0.329
≥3	29	29	5.60	4.28	6.92		28	14	17.23	15.90	18.57	
Re-biopsy after first-line progression												
No	136	129	6.20	3.73	13.20	0.187	135	92	16.42	12.47	17.67	0.054
Yes	70	66	8.13	4.40	16.30		70	40	19.33	12.74	22.46	
Brain Metastasis												
No	150	142	5.10	4.31	5.89	0.038	150	96	14.40	12.38	16.42	0.527
Yes	56	53	4.20	2.94	5.46		55	36	14.10	10.20	18.00	
Liver Metastasis												
No	177	167	5.03	4.39	5.68	0.849	176	118	14.23	12.42	16.04	0.390
Yes	29	28	5.00	3.36	6.64		29	14	22.70	7.32	38.08	
Neutrophil-to-lymphocyte ratio (5.0)												
< 5.0	133	124	5.10	4.14	6.06	0.023	132	83	14.53	13.30	15.78	0.112
≥ 5.0	55	53	4.87	3.80	5.93		55	38	12.30	9.94	14.66	
Second-line treatment												
EGFR-TKI (others)	26	24	2.67	0.00	4.70	0.053	25	15	13.27	1.00	21.30	<0.001
Chemotherapy	162	156	5.00	4.70	6.60	0.123	159	108	14.27	27.90	35.30	0.021
EGFR-TKI (Osimertinib)	18	12	11.50	4.00	13.00		18	7	37.50	48.00	82.50	
Second-line treatment												
EGFR-TKI (others) + chemotherapy	188	180	4.87	4.30	5.50	0.002	184	123	14.27	13.30	32.50	0.001
EGFR-TKI (Osimertinib)	18	12	11.50	3.90	19.10		18	7	37.50	38.70	82.50	

*p < 0.05; PFS, progression-free survival; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitors; BSC, best supportive care.

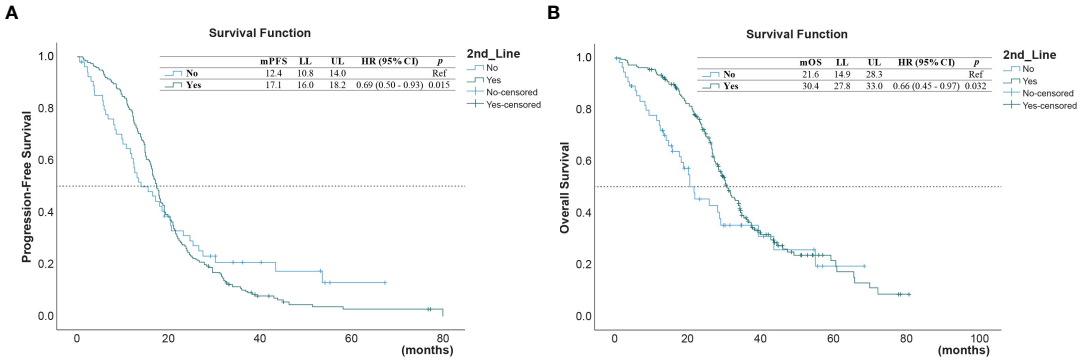

FIGURE 1
(A) Progression-free survival (PFS) and **(B)** overall survival (OS) in those who received second-line treatment after first-line treatment, and in those who did not*. (* reference).

TABLE 3 Uni and multivariate Cox regression analysis for predictor of PFS on second-line treatment (n =206).

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P value*	Adjusted HR (95% CI)	*P value*
Age				
<65 years old	Ref		Ref	
≥65 years old	1.11 (0.82 - 1.49)	0.512		
Sex				
Female	Ref		Ref	
Male	1.3 (1.0 - 1.78)	0.049*	3.37 (0.68 - 16.61)	0.136
ECOG PS				
0-1	Ref		Ref	
≥2	1.59 (1.1 - 2.33)	0.014*	0.46 (0.09 - 2.36)	0.352
Smoking status				
Never smoker	Ref		Ref	
Current smoker	1.19 (081 - 1.77)	0.369	—	
Former smoker	1.79 (1.23 - 2.63)	0.003*	2.8 (.36 - 20.97)	0.329
Metastatic sites				
<3	Ref		Ref	
≥3	0.97 (0.65 - 1.45)	0.87	—	
Re-biopsy after 1st-line progression				
No	Ref		Ref	
Yes	0.82 (0.61 - 1.1)	0.190	—	
Brain Metastasis				
No	Ref		Ref	
Yes	1.4 (1.02 -1.93)	0.04*	1.52 (0.42 - 5.58)	0.529
Liver Metastasis				
No	Ref		Ref	
Yes	1.04 (0.69 - 1.6)	0.850		

(Continued)

TABLE 3 Continued

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P value*	Adjusted HR (95% CI)	*P value*
Second-line treatment				
EGFR-TKI (osimertinib)	Ref		Ref	
EGFR-TKI (others)	2.64 (1.28 - 5.42)	0.008	2.45 (0.99 - 6.09)	0.053
Chemotherapy	2.68 (1.45 - 4.97)	0.002	1.86 (0.85 - 4.07)	0.123
Second-line treatment				
EGFR-TKI (osimertinib)	Ref		Ref	
EGFR-TKI (others) + chemotherapy	1.54 (1.06 - 2.24)	0.021	2.74 (1.32 - 5.69)	0.007*
NLR				
<5	Ref		Ref	
≥5	1.45 (1.05 - 2.01)	0.024*	2.95 (0.61 - 14.37)	0.180

*p < 0.05; PFS, progression-free survival; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitors.

Nevertheless, a randomised phase 2 trial of 96 patients in Korea concluded that the outcomes of pemetrexed therapy for NSCLC patients with disease progression after first-line EGFR-TKI were not improved by adding cisplatin.(25) Thus, which chemotherapeutic regimen was optimal as the treatment standard was unclear. Our study found that different chemotherapeutic regimens resulted in similar PFS and OS. A previous meta-analysis, which included one randomised controlled trial and three retrospective studies, showed that second-line treatment with pemetrexed chemotherapeutic regimens provided significantly longer PFS and OS than non-pemetrexed chemotherapeutic regimens.(26) However, this meta-analysis was greatly limited by the small sample size; therefore, its results should be interpreted cautiously. Additional well-designed prospective studies are warranted to resolve this controversial issue.

There is increasing evidence that NLR, a surrogate of inflammatory and immunologic indicators, is an independent predictor of poor prognosis in cancer patients, including those with NSCLC.(27, 28) A retrospective study of 190 metastatic NSCLC patients receiving EGFR-TKIs indicated that a higher NLR was associated with poor prognosis.(27) Recently, a pooled analysis of two phase III NSCLC clinical trial datasets also indicated that a higher baseline NLR (≥3.8) was associated with worse PFS (HR = 1.37, $p = 0.0004$) and OS (HR = 1.65, $p < 0.0001$).(28) Our results are in agreement with these previous findings. NLR is an easily accessible and effective prognostic biomarker for NSCLC patients. However, the specific mechanism of its prognostic value is not clear. Further well-designed studies are required to modify the prognostic role of NLR in lung cancer patients.

One of the limitations of this study is that the retrospective nature of real-world, population-based settings tend to generate selection bias when the study population is examined using different patient characteristics. For example, patients with good performance status and an easily accessible tumor site are more likely to receive re-biopsy or second-line treatment, whereas those with rapid disease progression or an unavailable tumor site (such as progression with brain metastases) are less likely to undergo re-biopsy or subsequent treatment. In addition, liquid biopsy is not covered by Taiwan's NIH; therefore, only some patients being able to afford the test and further treatment could affect survival

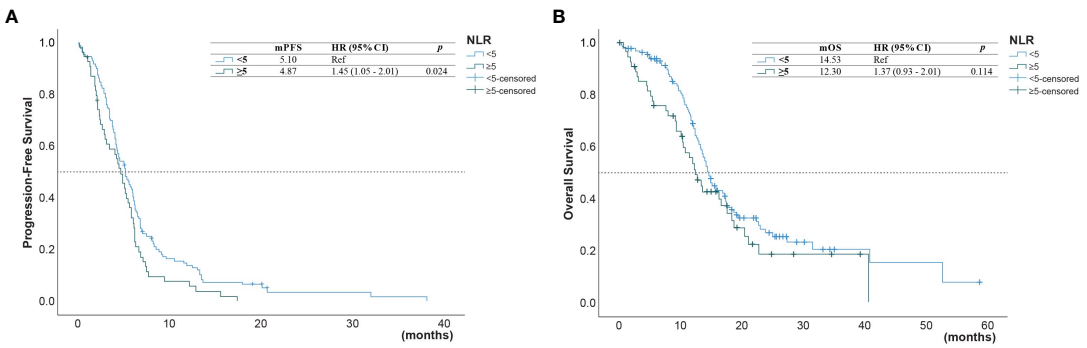

FIGURE 2
(A) Progression-free survival (PFS) and **(B)** overall survival (OS) of patients with a neutrophil-to-lymphocyte ratio (NLR) below 5.0 and those with an NLR above 5.0.

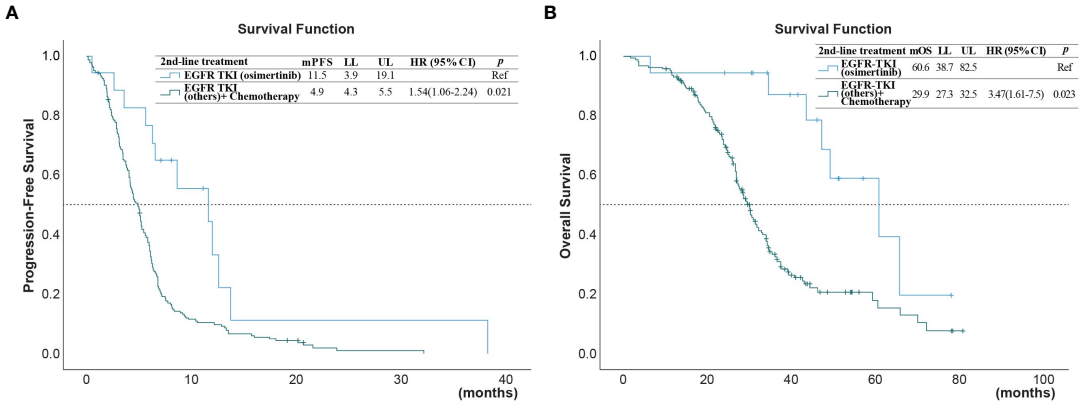

FIGURE 3
(A) Progression-free survival (PFS) and **(B)** overall survival (OS) of patients after second-line treatment with EGFR-TKI (osimertinib) versus treatments with chemotherapy or other EGFR-TKI (*reference group).

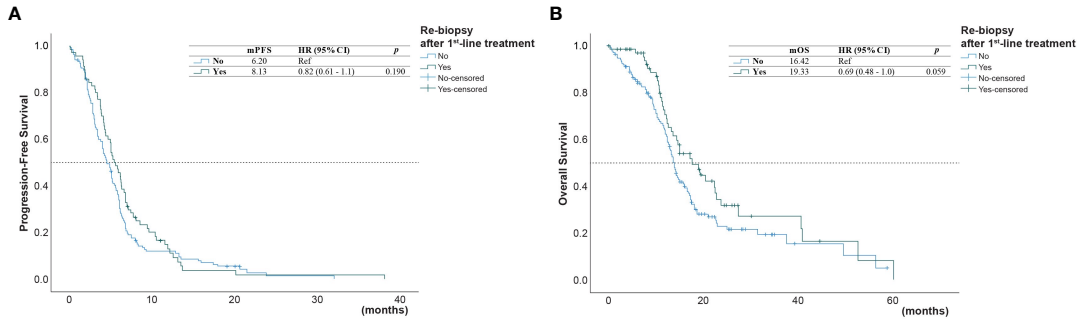

FIGURE 4
(A) Progression-free survival (PFS) and **(B)** overall survival (OS) of patients who underwent or did not undergo* re-biopsy after first-line progression (*reference group).

TABLE 4 Uni and multivariate Cox regression analysis for predictor of OS on second-line treatment (n =206).

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P value*	Adjusted HR (95% CI)	*P value*
Age				
<65 years old	Ref		Ref	
≥65 years old	1.46 (0.99 - 2.14)	0.054	—	
Sex				
Female	Ref		Ref	
Male	0.86 (0.72 - 1.03)	0.10	—	
ECOG PS				
0-1	Ref		Ref	
≥2	1.01 (0.63 - 1.63)	0.957	—	
Smoking status				
Never smoker	Ref		Ref	
Current smoker	0.86 (0.52 - 1.43)	0.586	—	
Former smoker	1.29 (0.82 - 2.02)	0.279	—	

(Continued)

TABLE 4 Continued

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P value*	Adjusted HR (95% CI)	*P value*
Metastatic sites				
<3	Ref		Ref	
≥3	0.76 (0.44 - 1.32)	0.331	—	
Re-biopsy after 1st-line progression				
No	Ref		Ref	
Yes	0.69 (0.48 - 1.0)	0.059	—	
Brain Metastasis				
No	Ref		Ref	
Yes	1.13 (0.77 - 1.67)	0.528	—	
Liver Metastasis				
No	Ref		Ref	
Yes	0.78 (0.45 - 1.37)	0.394	—	
Second-line treatment				
EGFR-TKI (osimertinib)	Ref		Ref	
EGFR-TKI (others)	3.9 (1.59 - 9.6)	0.003	—	
Chemotherapy	3.47 (1.61 - 7.5)	0.002	—	
Second-line treatment				
EGFR-TKI (osimertinib)	Ref		Ref	
EGFR-TKI (others) + chemotherapy	3.47 (1.61 – 7.5)	0.023	—	
NLR				
<5	Ref		Ref	
≥5	1.37 (0.93 - 2.01)	0.114	—	

*p < 0.05; PFS, progression-free survival; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitors.

outcomes. Furthermore, the sample size in this study was relatively small, which may also introduce bias and limit the possibility of highlighting general implications. Finally, we did not include patients with acquired T790M mutation after re-biopsy as an endpoint, so the information on the treatment for patients with acquired T790M mutation after re-biopsy and the reasons for patients not receiving re-biopsy were not available in the present study. However, it is reasonable according to the statement from American Society of Clinical Oncology (29), which indicated that the key elements of framework are the clinical benefits (e.g., hazard ratio for death, overall survival, and progression-free survival).

Conclusion

The results of this study indicated PFS was shorter in male gender, ECOG status ≥2, former smoking, brain metastasis, second-line with EGFR-TKI other than osimertinib or chemotherapy, and NLR ≥5.0. Osimertinib as the second-line treatment was an independent predictor of PFS. These results suggest that the benefits of osimertinib necessitates that aggressive re-biopsy after progression on first- or second-generation EGFR-TKI treatment is merited for appropriate second-line treatments to provide better outcomes for these patients.

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 human participants were reviewed and approved by E-Da Hospital EMRP-110-080, National Taiwan University Hospital NTUH-201611059RINB, Far Eastern Memorial Hospital FEMH-111075-E, and Yang-Ming Chiao Tung University Hospital YMUH-2021A022. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conception and design: Che-YC, Chu-YC, S-CC, Chi-YC, and Y-FW; Acquisition of data: Che-YC, Chu-YC, Chi-YC, Y-CL, and C-FC; Analysis and interpretation of data: Che-YC, Chu-YC, S-CC, Chi-YC, Y-CL, C-FC, and Y-FW; Drafting the article: Che-YC, Chu-YC, S-CC, and Y-FW; Revising the article critically for important intellectual content: Che-YC, Chu-YC, S-CC, Chi-YC, Y-CL, C-FC, and Y-FW.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1104098/full#supplementary-material

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Predicting cancer relapse following lung stereotactic radiotherapy: an external validation study using real-world evidence

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Purpose: For patients receiving lung stereotactic ablative radiotherapy (SABR), evidence suggests that high peritumor density predicts an increased risk of microscopic disease (MDE) and local-regional failure, but only if there is low or heterogenous *incidental* dose surrounding the tumor (GTV). A data-mining method (*Cox-per-radius*) has been developed to investigate this dose-density interaction. We apply the method to predict local relapse (LR) and regional failure (RF) in patients with non-small cell lung cancer.

Methods: 199 patients treated in a routine setting were collated from a single institution for training, and 76 patients from an external institution for validation. Three density metrics (mean, 90th percentile, standard deviation (SD)) were studied in 1mm annuli between 0.5cm inside and 2cm outside the GTV boundary. Dose SD and fraction of volume receiving less than 30Gy were studied in annuli 0.5-2cm outside the GTV to describe *incidental* MDE dosage. Heat-maps were created that correlate with changes in LR and RF rates due to the interaction between dose heterogeneity and density at each distance combination. Regions of significant improvement were studied in Cox proportional hazards models, and explored with and without re-fitting in external data. Correlations between the dose component of the interaction and common dose metrics were reported.

Results: Local relapse occurred at a rate of 6.5% in the training cohort, and 18% in the validation cohort, which included larger and more centrally located tumors. High peritumor density in combination with high dose variability (0.5 - 1.6cm) predicts LR. No interactions predicted RF. The LR interaction improved the predictive ability compared to using clinical variables alone (optimism-adjusted C-index; 0.82 vs 0.76). Re-fitting model coefficients in external data confirmed the importance of this interaction (C-index; 0.86 vs 0.76). Dose variability in the 0.5-1.6 cm annular region strongly correlates with heterogeneity inside the target volume (SD; ρ = 0.53 training, ρ = 0.65 validation).

Conclusion: In these real-world cohorts, the combination of relatively high peritumor density and high dose variability predicts increase in LR, but not RF, following lung SABR. This external validation justifies potential use of the model to increase low-dose CTV margins for high-risk patients.

KEYWORDS

image-based data mining, real world data, biomarker-by-treatment interactions, local relapse, NSCLC, stereotactic ablative body radiotherapy (SABR), personalized medicine, external validation

1 Introduction

Patients with early-stage lung cancer who are medically inoperable or refuse surgery will receive stereotactic body radiotherapy (SABR) as standard of care (1). It is a well-tolerated and successful treatment, with five-year local relapse (LR), regional failure (RF), and distant metastasis (DM) rates at ranges between 8-11%, 10-13%, and 11-22% respectively (2–5). SABR is characterized by high dose radiation delivered by multiple conformal beams to precisely target the tumor and avoid surrounding tissue. To maintain a conformal dose distribution, dose heterogeneity inside the planning target volume (PTV) is permitted (6). It is still unknown what part of the tumoral dose distribution is affecting tumor control the most (7), and further considerable institutional differences exist in treatment planning approaches (8). Better understanding of the level of tumor dose homogeneity/heterogeneity could lead to changes in radiotherapy planning to improve patient outcomes. In some situations, data from real-world settings allows us to test hypotheses on the impact of changes that can be made to the treatment planning process as an alternative to costly clinical trials. Real word data also has the advantage of being more inclusive of a general population. This is particularly relevant in the context of patients with lung cancer treated with SABR as most of them are elderly, frail and with multiple comorbidities. These patients are typically excluded or underrepresented in clinical trials (9).

The association between dose and LR has been well investigated through the link between the prescription dose and tumor control probability. Such studies report dose associations with the isodose surface encompassing the PTV (10, 11), the isocenter (12) and the average of the two (13). A study on 1500 patients emphasized the importance of ensuring high doses within the gross tumor volume (GTV) to promote local control (13). Reports have also detailed the importance of high dose *outside* the GTV as means of treating microscopic disease (MDE) and nodal micro-metastases that could be responsible for treatment failure (14, 15). Further studying this effect may identify associations that can be utilized to personalize treatments for patients at *high-risk* of failure.

Assessing pre-treatment imaging biomarkers is a non-invasive approach to identify high-risk patients who could be candidates for treatment adaptation. Salguero et al. demonstrated that CT-based GTV circularity and surface density predict high-risk of MDE (14). High-risk of MDE also translated into an increased risk of local-regional failure if patients had low dose within 1.5cm from the GTV (16). Previous efforts have, however, focused on using tumor dose parameterizations, histological, or clinical characteristics alone to study and stratify risk of MDE (10–13, 17).

Ignoring risk stratification can lead to incorrectly claiming a lack of association (18). In our previous work, we developed a *Cox-per-radius* method to investigate the interaction between imaging density biomarkers and dose in independent annuli surrounding the GTV (19). In that study we demonstrated that the interaction between CT-based imaging biomarkers and dose far outside the tumor is linked to DM. In this study, we will use our previously developed method to assess whether a similar associations can be identified for LR and RF using two real-world patients cohorts. We will utilize real-world data from geographically separate institutions to explore the generalizability of any identified patterns.

2 Methods

2.1 Clinical data and patient follow-up

2.1.1 Training data

Data was available for 195 patients with T1-2 N0M0 non-small cell lung cancer (confirmed histologically or suspected based on radiology) who were treated with SABR for primary lung cancer during 2011-2017 at The Christie NHS Foundation Trust, with 60 Gy in 5 fractions on consecutive weekdays. Institutional approval was granted to collect and analyse this data (REC reference: 17/NW/0060). All patients were staged with both a CT and 18F-FDG positron emission tomography (PET) scan, but did not always receive a histological diagnosis. Four-dimensional computed tomography scans (4D-CT) and three-dimensional dose distributions were available, as described previously and in Supplementary Material (SM), Section 1 (19). Clinical data was retrospectively collected from structured e-forms completed in routine practice. Clinical data was available on tumour lobe location, age, sex, Eastern Cooperative Oncology Group (ECOG) performance status (functional ability), ACE27 comorbidity score (describing the presence and severity of existing medical conditions), and histological sub-type. Where

available, histological sub-type diagnosis was classified as 'adenocarcinoma not otherwise specified (ADC NOS)', 'squamous cell carcinoma (SCC)', 'carcinoma NOS' or other. Information on tumor centrality was not available.

In routine practice, patients were followed-up every three months for the first year, and six monthly thereafter. At the discretion of the clinician, a free-breathing CT is performed, PET or biopsy recommended when treatment failure is suspected. Recorded data on treatment failure was retrospectively collected from electronic records. Local relapse (LR) was defined as progression in or adjacent to the original treatment volume, based on clinical interpretation of 'adjacent' following reported definitions as guidance (20). Regional failure (RF) was defined as recurrence in regional lymph nodes (hilar or mediastinal). Time to failure was recorded from the start of radiotherapy to the date of the first scan that showed progression. Patients were censored at the most recent follow-up in the absence of failure.

2.1.2 Validation data

For validation, data were available for 139 patients with T1-2 NOM0 early-stage NSCLC treated with a range of fractionation regimes (see SM, Table 1) treated at Memorial Sloan Kettering Cancer Center (MSKCC) between 2014 and 2017. There was no agreement in treatment schedules between the training and validation data. For validation, we limited selection to patients treated with 50Gy in 5 fractions treated on consecutive weekdays, as the most common treatment schedule, and included all such patients treated with that regime during this time frame. All patients included had been staged with a CT and PET scan. A data-sharing agreement was in place and a study analysis plan made available online at the start of the collaboration (21). Clinical data was collected retrospectively from structured e-forms completed in routine practice. Clinical data was available on tumor lobe location, age, sex, Karnofsky performance status (a rating of 0-100 measuring a patient's ability to perform daily tasks), and histological sub-type (SCC, ADC, or other). Information on

TABLE 1 A table to demonstrate patient demographic differences in training and validation data.

Characteristic	Training, N = 195[1]	Validation, N = 76[1]	p-value[2]
Tumor volume (cc)	4 (0 - 31)	14 (0 - 158)	<0.001
Tumor motion (cm)	0.56 (0.00 - 3.43)	0.36 (0.00 - 2.73)	0.016
Tumor lobe location			0.7
Lower	69 (35%)	29 (38%)	
Upper	126 (65%)	47 (62%)	
Age (years)	75 (45 - 92)	78 (52 - 92)	0.036
Biological sex			0.3
Female	99 (51%)	44 (58%)	
Male	96 (49%)	32 (42%)	
Histological subtype			<0.001
Adenocarcinoma, NOS	35 (37%)	45 (59%)	
Squamous cell carcinoma	37 (39%)	24 (32%)	
Carcinoma, NOS	14 (15%)	0 (0%)	
Other	8 (8.5%)	7 (9.2%)	
Unknown	101	0	
Performance status (ECOG)			<0.001
0	3 (1.8%)	29 (38%)	
1	64 (37%)	43 (57%)	
2	83 (49%)	4 (5.3%)	
3	21 (12%)	0 (0%)	
Unknown	24	0	
Local relapse	13 (6.7%)	12 (16%)	0.020
Regional failure	15 (7.7%)	5 (6.6%)	0.8

[1]Statistics presented: n (%); Median (range).
[2]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.
Tumours were larger and less mobile in the validation data compared to training. Worse performance status was reported in the training set. Differences were also identified in histological sub-type with a larger proportion of adenocarcinoma in the validation data, but this could be influenced by the missing data reported for training. In the validation set there were significantly more local relapses but a similar percentage of regional failures.

comorbidity score or tumor centrality was not available. The Karnofsky performance status was scaled to the ECOG gradings to match the training cohort using a published guide (22).

Data was also collected on recurrences for this cohort. Local relapse was defined as new tumor growth at the site of prior CT; and recurrences were typically confirmed by PET (to demonstrate local FDG avidity) and/or biopsy. Regional failure was defined as recurrence in regional lymph nodes.

2.2 Imaging and dosimetric data

Both training and validation cohorts had treatment plans that included a 'motion-adapted' GTV (iGTV) which incorporates the GTV combined across all respiratory phases. In both institutions, this is outlined on the maximum intensity projection (MIP) and edited on individual respiratory phases. The PTV was also recorded, which represents the iGTV plus a 5mm expansion (with a 2-3mm additional CTV in the validation cohort). To extract the tumor volume (GTV) for every phase, an in-house process was applied to estimate and remove the motion adaptation using rigid tumor registration across 4D phases (23). As a result, a GTV contour was available for every phase and two additional clinical variables recorded: tumor volume and tumor motion amplitude. Results of the registration were assessed visually on a movie-loop of registered phases, and all GTV contours were visually approved by a single observer.

Three-dimensional dose distributions were available for all patients and were converted to biologically equivalent doses in 2Gy fractions (EQD2) using α/β=10. Independently, the same set of distributions were blurred according to respiratory motion (derived from the registration above) and then converted to EQD2 – which represents the *blurred* dose represented on a reference phase (for which we used the middle, i.e., 50% phase). The mean, maximum, minimum, and standard deviation (SD) of the dose inside the PTV was calculated in both cohorts based on the planned dose. The *blurred* dose provides an indication of the *planned* tumor dose over the respiratory cycle. From this distribution, the mean, maximum, minimum, and SD dose on the generated GTV was calculated.

2.2.1 Density metrics

The radial histogram framework described in our previous work was implemented to measure density and dose at radial distances from the generated GTV for all patients (19). A 2D cross-histogram of density vs distance in bins of 1mm annuli from -0.5 to 2cm from the GTV was formed for each 4D phase considering lung tissue only. Only lung tissue was considered for density metrics so that higher density is not just a surrogate for nearby organs-at-risk. For each patient, a single phase was selected as the most stable compared to neighboring phases and used for density analysis in the remaining analysis (as detailed in previous work) (19, 24). Summary curves were then extracted from the 2D histogram for each patient to describe the mean, 90th percentile, and SD density at the defined distances from the GTV. The summary curves were smoothed with Gaussian smoothing (σ = 1.5mm) (as annuli thickness is less than slice thickness) and stored for each patient.

2.2.2 Dose metrics

To extract dosimetric information, scans were cropped based on the body contour, and dose-volume histograms were extracted in 1mm annuli from the *blurred* dose distribution in the region 0.5-4cm radially from the GTV on the reference phase (19). Smoothed curves were summarized by dose SD, and the fraction of volume in each 1mm rim that receives EQD2 of less than 30Gy, which is a dose threshold reported for controlling MDE (25). An additional curve of mean dose was extracted for visualization only.

2.2.3 Exploratory comparison

The average curve across patients for each metric as function of radius was calculated in training and validation. The curves did not inform selection of which variables to use in the remaining analysis but assist in interpretating the results. We visually assessed the images for all patients to note any qualitative characteristics that may lead to differences between the two groups.

2.3 Model development

2.3.1 Clinical model and 'standard' dose metrics

For training, a reference Cox model was derived for both LR and RF including clinical variables with complete data for all patients – which were tumor lobe location, age, sex, tumor motion amplitude, and tumor volume. The concordance index (C-index), Akaike Information Criterion (AIC), and any variables that statistically significantly predicted LR and/or RF were recorded. Each GTV and PTV dose-related parameter extracted in Section 2.2 was then included in the Cox model individually to determine whether it was associated with RF and LR, and a likelihood-ratio test was performed for models with and without the dose parameter to determine if there was a significant improvement in model performance.

2.3.2 Interaction maps

The *Cox-per-radius* method was applied for each outcome separately (LR and RF); full details reported in previous work (19). Briefly, for each combination of dose and density in independent annuli, the density feature, dose metric, and interaction term (*density*dose*) were added to the clinical model. A likelihood-ratio test was performed for models with and without the interaction to produce a heat-map of p-values describing the benefit of the interaction for prediction in each dose-density annulus combination. Regions of statistical significance (defined by $p<0.05$) were highlighted on the heat-map.

Region size post-processing was then used as multiple-testing correction on the heat-maps to ensure regions were truly not less than 3mm thickness in either the dose or density scale (likely representing spurious associations) (26, 27). The average height and width of each region defined boxes on a heat-map that represent the significant distances for dose and density independently. From the

defined independent annuli, the dose and density values in the identified regions were extracted and included in overall Cox models. Internal validation was performed over 500 bootstrap resamples to estimate the interaction coefficient stability and model performance. A secondary post-processing step was then performed, which removed unstable interaction coefficients on internal validation or interactions that did not improve the performance of the clinical model (C-index). Unstable coefficients are those that show both a negative and positive effect within the 95% confidence interval across resamples.

2.3.3 Final models, internal validation, and interpretation

Coefficients and p-values were reported for models with and without the interaction term. To interpret the direction and size of the association between density and outcome, *contrast plots* were created with log(hazard ratio) on the y-axis and different density values on the x-axis for the 10^{th} percentile, median, and 90^{th} percentile value of the dose parameter in the relevant region (28).

Based on the results from the internal validation, the concordance-index was adjusted for optimism. For each bootstrapped model we calculated an estimate of the C-index and calculated the difference between the C-index of the model in the bootstrapped data and in the original data. To calculate the optimism-adjusted C-index, the median difference across all resamples was subtracted from the original C-index. This internal validation was performed for the clinical model, and the new models with and without an interaction term for comparison. For all models, the median and 95% confidence interval of the C-index across the bootstrap resamples were recorded.

For interpretation of the identified dose location, correlations were investigated between the relevant dose parameter and the metrics extracted in Section 2.2.

2.4 External validation

No formal advice is currently reported for external *'validation'* in an image-based data mining framework. Following the stricter advice of Royston et al. on validation in prediction modelling (29), the model coefficients developed on the training data were applied in the new data-set to build a validation Cox model. The C-index of the validation model was recorded and compared to that identified from the internal validation. This process was performed for the clinical model and repeated for the new models with and without the interaction terms.

As a second comparison (as opposed to strict prediction-model validation) we re-fit the model coefficients in the new data set to determine if the significance of the interaction would still be identified. Contrast plots were created for this interaction, and the same correlations between other dose metrics performed for interpretation. As a further validation step, we also studied the stratification of patients with Kaplan-Meier plots in both training and validation using an arbitrary cut off for the median peritumour density, and the lowest and highest third of the identified dose metrics.

3 Results

3.1 Clinical, imaging and dosimetric data

For training, 195 patients with complete clinical, image and dose data were available (19). Thirteen patients had LR (6.5%) and 15 patients (8%) had RF. Complete clinical data was available on tumor lobe location, age, sex, tumor motion amplitude, and tumor volume. In the training data, we report 52% missing data on histological sub-type and 12% on performance status – hence these were excluded from further analysis.

In the validation set, we first selected patients who were treated with the same number of fractions as the training data-set, this left 95 patients treated with 50Gy in 5 fractions. Out of the remaining patients ten more were excluded due to: missing radiotherapy data (n = 1), missing 4D-CT phases (n = 5), alternative planning approach (breath-hold; n = 2), or no iGTV (n = 2). On visual assessment of the GTV generation, we observed seven failures related to registration and three related to segmentation. Overall, 76 patients were available for analysis with complete data, and the difference between the training and the validation cohort are shown in Table 1. Twelve patients had LR (16%) and five RF (6.6%). In the validation cohort, the tumours were larger with a mean tumour volume of 14cc compared to 4cc, and a maximum volume of 158cc compared to 31cc. The tumours in the validation cohort also were more centrally located on visual assessment, which contributed to a significantly lower tumour motion amplitude. In the validation data, a larger distribution of adenocarcinoma compared to squamous cell carcinoma was recorded, and patients had better performance status.

3.2 Clinical models for training data

Firstly, using the training data, multivariable Cox models were built to predict LR and RF containing all clinical variables in Table 1, and the full tables are reported in SM Table 2. The summary of these models in Table 2 demonstrates that only

TABLE 2 Clinical model for local and regional failure in training data with significant variables, C-index, and Akaike Information Criterion (AIC) reported. Performance metrics were evaluated on the full data-set.

Outcome	Multivariable prognostic factors (p<=0.05)	C-index	AIC
Regional	None	0.74	142.3
Local	ln(Tumour volume) *(HR = 3.05, p = 0.006)*	0.79	100.0

tumor volume is a prognostic factor for LR, and there are no prognostic factors for RF. Including 'standard' dose metrics in each model did not significantly improve model performance (SM, Table 3).

3.3 Radial dose and density metrics

Three density metrics (mean, 90th percentile, SD) and three dose metrics (mean, SD, fraction volume receiving less than 30Gy) were extracted from both training and validation cohorts. These metrics were averaged across all patients and the differences visualised between cohorts as shown in Figure 1. An increased density heterogeneity inside and outside the tumour in the validation data was observed. The mean dose is lower in the validation cohort as the total prescription was 50Gy in 5 fractions compared to 60Gy in 5 fractions delivered in the training cohort. Differences in the other curves (Figure 1) include variation in both dose and density heterogeneity which likely represent institutional planning differences.

3.4 Dose-density interaction maps

Using the dose and density curves extracted for all patients, six *Cox-per-radius* maps were produced from combinations of the three density metrics and two dose metrics (SD, fraction volume receiving less than 30Gy) extracted for this stage of the analysis.

In the analysis of LR, post-processing 1-40% of significant pixels were removed (SM, Figure 1 and 2). After this, up to nine candidate regions were identified on each map. Of these nine regions, three were below the size threshold, one had unstable coefficients on internal validation, and four did not improve the clinical model performance (SM, Table 3), therefore only one consistently identified region remained (Figure 2).

This region indicates that 90th percentile density at the peritumor border (-0.1 to 0.3cm) interacts with the dose SD 0.5-1.6cm and jointly predict LR. The interaction term was significant in 74% of bootstrap resamples and the interaction model performance had a median of 0.81 (95% confidence interval: 0.70 - 0.85) on internal validation. The larger region (light pink border; Figure 2) was not considered important as it did not improve the clinical model performance with a median C-index of 0.79.

For RF, the maps before and after post-processing are shown in SM, Figures 3 and 4 where between 25-100% of spurious significant pixels were removed across maps. Two regions remained (SM, Table 4), but neither met the 3mm threshold for annuli size and hence no regions were considered for further analysis of regional failure.

3.5 Final training and validation models

The first column of Table 3 reports the median and 95% confidence interval of the training C-index for the clinical LR model, the model with additional dosimetric and density information, and the final model with an interaction term. In the second column, we record the optimism-adjusted estimates of these values. In both cases, the interaction model out-performs the other models. The strict prediction-model validation results (without refitting) demonstrate that the interaction model does not directly translate to the external data, but the dose and density terms alone do improve on the clinical model in the validation data-set. However, when model coefficients were re-fitted, the importance of the interaction term was re-established.

3.6 Model interpretation

The model coefficients for the interaction model in the training data and for the re-fitted model in the validation data are shown in Table 4. The HRs for the interaction term are reported at the 90th percentile of SD dose (11.7Gy) and peritumor density (-107.4HU) as this is a multiplicative model, the hazard can only be interpreted as a single value at specified values of the interacting variables.

For interpretation on the direction of effect we plot the log (hazard ratio) for peritumor 90th percentile density as a continuous variable at three reference values of dose SD in Figure 3. The plot demonstrates that high peritumor density and high dose SD in the identified region is linked to increased risk of LR. At other values of dose SD there is no significant association between density and LR.

An example stratification with these models is demonstrated in Figure 4, where it can be seen that only at high dose SD, peritumour density is linked to worse outcome in training and validation cohorts.

TABLE 3 Concordance index of models on training and validation data.

Model C-index	Training	Optimism-adjusted	Validation (without refitting)	Validation (with refitting)
Clinical	0.81 *(0.66-0.94)*	0.76 *(0.61-0.80)*	0.58 *(0.37–0.76)*	0.76 *(0.58–0.91)*
Clinical + SD dose + 90th percentile density	0.84 *(0.71-0.95)*	0.77 *(0.61-0.82)*	0.64 *(0.37-0.80)*	0.81 *(0.66-0.99)*
*Clinical + SD dose + 90th percentile density + SD dose*90th percentile density*	0.88 *(0.79-0.99)*	0.82 *(0.69-0.93)*	0.56 *(0.41-0.70)*	0.86 *(0.71-1.00)*

Optimism-adjusted results represent the internally validated models on the training data. Validation without re-fitting represents a strict prediction model validation procedure, whereas with re-fitting is building a new model with all the identified variables in the training data for comparison.

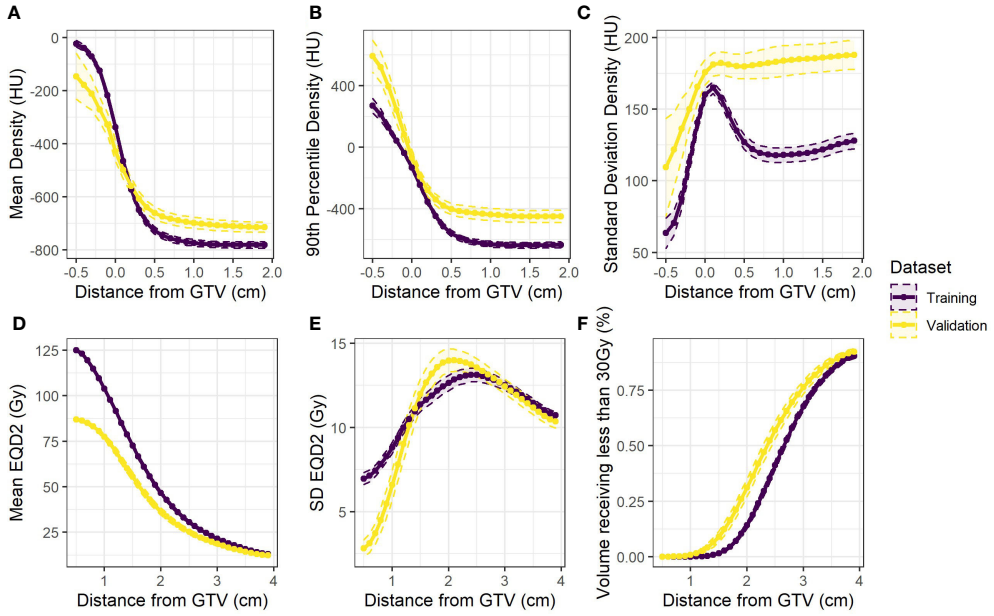

FIGURE 1
Population averages (dashed 95% confidence intervals) for the extracted variables against distance from the GTV for the training data (purple) and the validation data (yellow) **(A)** mean **(B)** 90th percentile and **(C)** standard deviation of density. Also **(D)** mean and **(E)** standard deviation of equivalent dose, and **(F)** the fraction of volume in each rim receiving less than 30Gy.

3.7 Dosimetric correlations

The correlations between dose in the PTV and GTV and the SD dose in the identified region was studied to investigate the relationship between *incidental* dose and more 'standard' dose metrics. The correlations among PTV dose metrics are shown in Figure 5, and among GTV metrics in SM, Figure 5. Dose heterogeneity (SD) in the identified region (0.5 – 1.6cm from GTV) was positively correlated with mean PTV and mean GTV dose in training data, but the correlation was weaker in the validation data. Similar observations are seen for SD, max and min dose to PTV and GTV.

4 Discussion

In this study based on real world datasets, as illustrated by the median age of patients and high performance status, the `*Cox-per-radius*` method developed in previous work was applied to investigate spatial interactions of density and dose for the purpose of better understanding local relapse (LR) and regional failure (RF) among early-stage lung cancer patients treated with SABR (19). We identified that higher peritumor density is significantly associated with increased chance of LR for patients who have high dose variability 0.5-1.6cm outside of the GTV. Higher dose variability

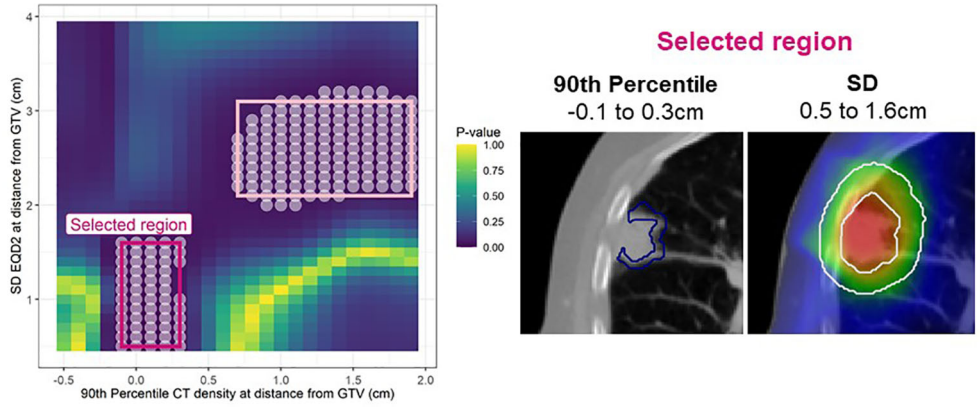

FIGURE 2
Left: Cox per radius significance map of interaction between 90th percentile density vs SD EQD2 at distance from the GTV. The p-value reflects a likelihood-ratio test of improvement in model performance due to inclusion of the interaction between dose and density at each location. All significant points are shown with a white circle and the regions extracted for assessment in bootstrap are highlighted in pink. After post-processing only one region remained (bright pink). Right: The annuli volumes defined by the selected regions overlayed on an example patient.

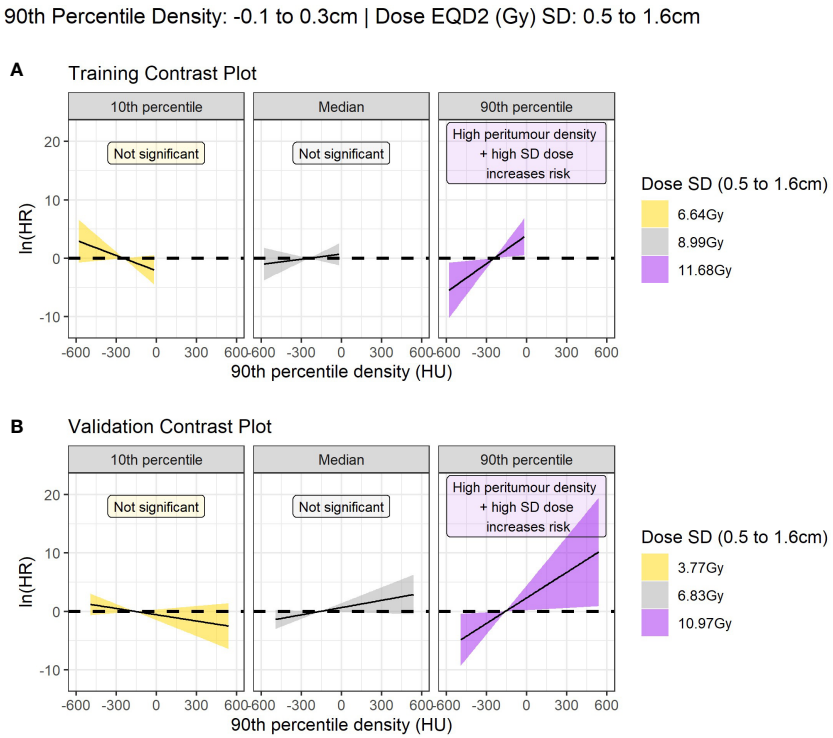

FIGURE 3
Contrast plot displaying the log(hazard ratio) versus 90th percentile peritumour density at different values of dose SD (standard deviation). Significant association between density and dose is only detected at high dose variability. In this case, higher peritumour density is associated with increased risk. **(A)** Shows the results in the training data, and **(B)** shows the validation results.

in this region is likely driven by increased heterogeneity in the PTV leading to steeper dose fall-off, as shown by correlations between the PTV dose and dose in this region further out. Similarly, Salguero et al. (14) found that patients with high risk of MDE (based on higher GTV surface density and more complex shape) had increased risk of local-regional failure if receiving a low minimum dose up to 1.5cm from the GTV. Both results suggest that microscopic disease coverage is important to prevent LR and could potentially be customized according to peritumor density. Using a second real-world validation data-set, we confirmed the importance of the interaction between peritumor density and dose variability in the same location despite large demographic differences observed in the two cohorts.

Interestingly, the <1.6cm region identified is considerably narrower compared to the corresponding region discovered by our group for DM, in which dose variability and underdosage ~3cm was predominant (19). The direct mechanisms to DM, LR, and RF are not fully understood, but the idea of these being assigned to different locations of importance is supported.

No dose-density interactions were found to provide predictive ability for RF. In other work, reduced risk of RF has been linked to higher *incidental* dose to the ipsilateral hilum, which suggests RF may be a result of microscopic disease presence at the site of failure or undiagnosed nodal metastases (15). This finding could not be confirmed in this work as the radial approach removes information of proximity to specific anatomy. However, regions were identified prior to post-processing, so sensitivity testing is required to ensure we are not removing insightful information in this process. As the work of Salguero et al. reports only on local-regional failure it is impossible to determine whether it is the RF or LR that dominate their result or whether it is a combination thereof (14). The results of our study suggest considering LR and RF independently. To better understand RF, anatomical information could be included by combining density interactions with a voxel-based dose analysis (30), but this was beyond the scope of this work.

It is promising that the results of our study generalize to an external validation cohort despite differences in CT acquisition parameters. We did not apply any correction for scanner differences, since inter-scanner variability is typically small compared to inter-patient variability for density metrics (otherwise known as first-order radiomic features) (31). Differences between cohorts may require controlling for more complex texture features, such as, the Grey Level Run Length Matrix features. In this study, the focus on density features was motivated by interpretability and preliminary radiomic analysis that shown relation between 90th percentile density and metastasis prediction (24). In addition to other radiomic features, different imaging modalities could be considered to improve prediction of recurrence, e.g. 18-FDG PET (32). Imaging that tracks changes during treatment (e.g., cone-beam CT) could also be utilized to consider tumor reduction (33), and the location of the surrounding microscopic disease (34).

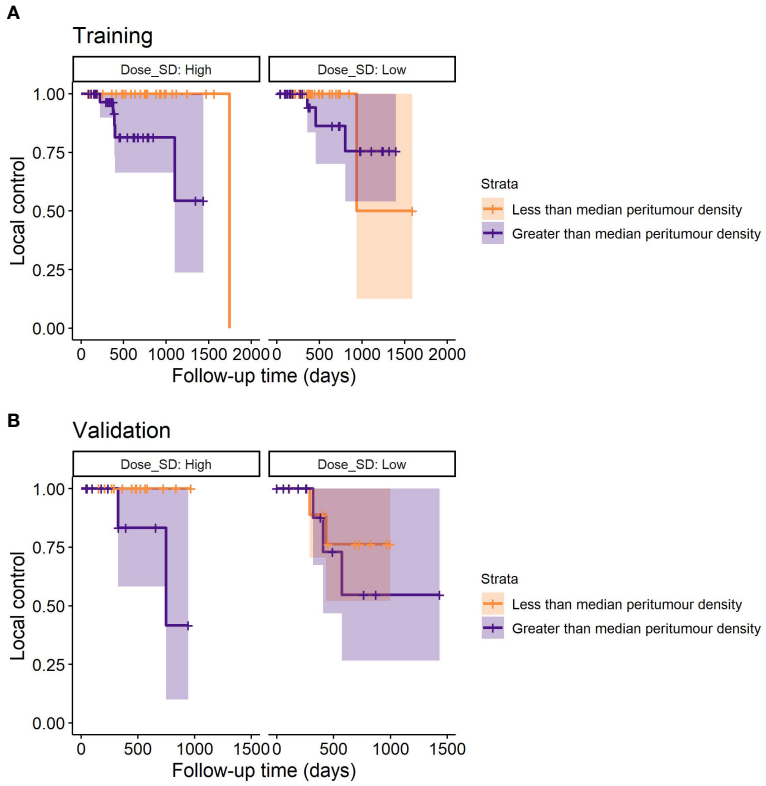

FIGURE 4
Example stratification of LR based on the median peritumour density for the lowest and highest third of dose SD *(discarding uninformative middle values)*. For low dose SD (assumed adequate MDE coverage), peritumour density no longer stratifies patients for LR – suggesting that peritumour density is a potential predictor for MDE. **(A)** Shows the survival plots on the training data, and **(B)** shows the validation data.

A limitation of the methodology presented in this work is the potential risk of spurious correlations between density at the border of an automatically generated GTV and the spatially offset dose annuli. In particular, density at the peritumor border is closely linked to the ability to accurately define the GTV. Higher peritumor density could be representative of high density spiculations that may or may not be included in the GTV or demonstrate an under-sampled iGTV for tumors with large motion amplitude (35, 36). Such contour variation could link to under-treatment of the GTV as opposed to microscopic disease. In addition, an organ-at-risk abutting the GTV could be associated with an increase in peritumor density. Although care was taken to visually check all

TABLE 4 The hazard ratios (HR) and p-values for the final models built in the training and validation data for predicting LR.

	Training		Validation	
	HR	P-value	HR	P-value
ln(Tumour volume)	4.29	**0.008**	0.53	0.095
Tumour motion [cm]	1.68	0.334	1.22	0.784
Tumour location (lower lobe reference)	10.3	**0.047**	0.52	0.454
Age [years]	1.01	0.885	0.97	0.552
Sex (female reference)	1.4	0.619	8.36	0.059
90th percentile density -0.1 to 0.3cm from GTV [unit: 100HU]	0.02	**0.013**	0.27	**0.057**
Dose variability 0.5 to 1.6cm from GTV [Gy]	2.90	**0.02**	1.20	0.184
90th percentile density * Dose variability [100HU * Gy]	1.64	**0.009**	1.29	**0.035**

The bold values are those that are significant predictors in the model ($p<0.05$).

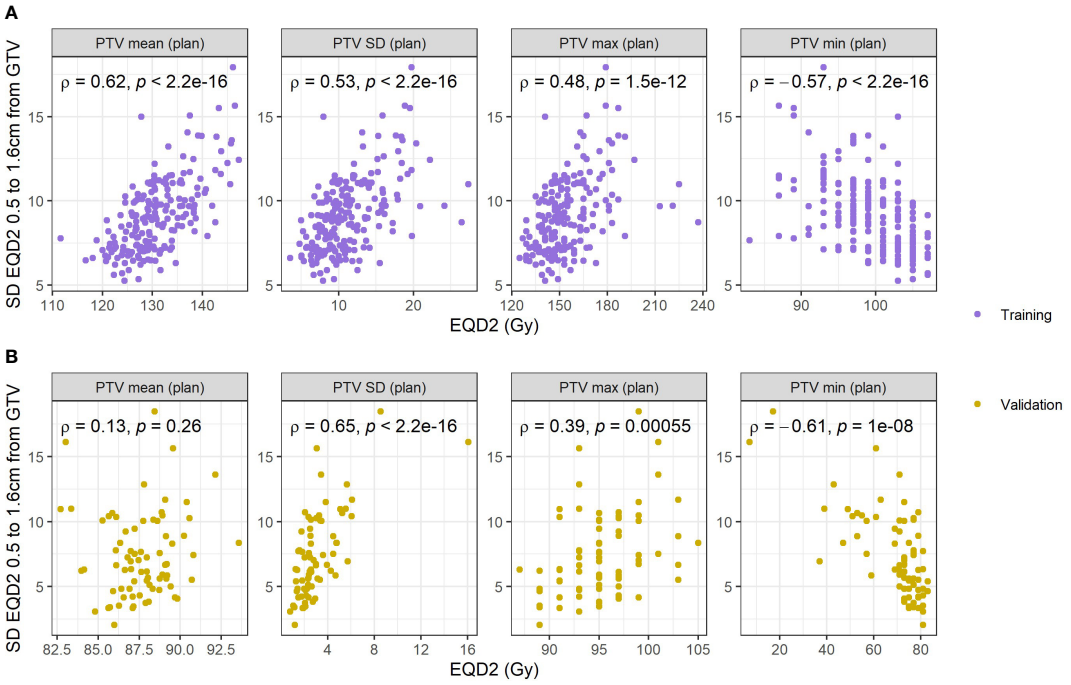

FIGURE 5
Correlations with standard deviation of the respiratory blurred dose extracted from the region identified and different dose metrics extracted from the PTV on the planned dose distribution. **(A)** Reports correlations in the training data, and **(B)** reports correlations in the validation data.

contours this cannot be fully excluded as a potential confounding factor. The influence of these factors on the association between high peritumor density and LR could be further studied by exploring the impact of contour variation on LR (37). Further, adopting a physics-approach of annuli at set distances regardless of surrounding anatomy means the dose annuli assessed in this study are not restricted to specific anatomical locations. The annuli can, therefore, include regions for some patients where microscopic disease is unlikely to be a biologically plausible route to LR (i.e., chest-wall) (14). To investigate the sensitivity of the method, a further improvement could include using dose information sampled from the lungs only (similar to density biomarkers), but one would have to be cautious as this could also lead to bias due to loss of different amounts of data when performing lung cropping for peripheral tumors or those close to surrounding organs. The radial data-mining technique demonstrated may provide information on routes to LR, but it would be important to assess histological characteristics of the tumor when making biological conclusions. This was limited by access to such information in routine practice.

There was also limited access to information on baseline pathologies that are common in lung cancer patients (e.g., chronic obstructive pulmonary disease (COPD) and emphysema) that have CT density presentations (e.g., fibrosis and bullae) that could make SABR planning more difficult. Such pathologies could influence the dose-density analysis, so care was taken to review images, dose, and density curves to detect possible outliers. In future study, this information such be incorporated in the analysis to understand causal links behind the associations identified in this work. Another potential avenue of further investigation could involve the underlying reason of the importance of dose alone (13, 38), while in this study and others the dose and density interaction is required to identify a similar association with outcomes (14, 19). This may in part be due to cohort differences, including tumor size or planning techniques combined with typically small cohort sizes and low number of events all contributing to limited power to determine true effects. Despite the low event rates, we were able to both produce a predictive model for LR with an optimism-adjusted performance of 0.82 in two independent data sets. In particular, the two cohorts included difference in demographics (i.e., performance status), tumor volume, and tumor location, which is representative of the challenges faced using real-world data as opposed to carefully selected patients enrolled on a clinical trial. Despite these challenges, we identified significant support towards the peritumor density as a predictive image biomarker, and increased dose variability up to 1.6cm for high-risk patients leads to worse clinical outcomes. The larger SD in density outside of the GTV in the validation cohort suggests that GTV delineations are tighter, which is consistent with the higher observed LR rate.

Furthermore, the higher LR rate observed in the validation data could also be explained by the lower overall dose (50Gy in 5 compared to 60Gy in 5). Here it is relevant to note that the model of Jeong et al., available online at https://tcp4rt.info, which predicts local failure rates quite close to those observed (39). Namely: predicted failure rates: 6.3% (12Gy x 5), 13% (10Gy x 5); observed rates: 6.7% (12Gy x 5), 16% (10Gy x 5). The Jeong fit to early-stage lung cancer SBRT saturates at a failure rate of 5%, even

for very high biological effective dose. It seems reasonable to hypothesize that the mechanism identified in this paper, namely, underdosing of peripheral local disease, is partly responsible for these commonly observed high-dose failures.

The interaction between peritumoral density and dose variability to the identified region (0.5 – 1.6cm from GTV) maintained significance in external validation despite differences in planning approaches implemented at both institutions. The difference in planning approaches was demonstrated by difference in dosimetric correlations between the two cohorts. In training data, a positive correlation was found between standard deviation of dose to identified region, and mean PTV and mean GTV dose. This correlation was weaker in the validation data. In future, it would be worth comparing planning approaches to determine how to best homogenize dose to the identified region.

The inclusion of interaction terms in analysis of real-world data-sets has so far been under investigated. Whilst the current gold-standard evidence for a predictive biomarker is a significant *'interaction test'* in a randomized controlled trial (40), we have demonstrated inclusion of dose-density interactions in retrospective analyses could allow us to explore and hypothesize on the impact of personalized radiotherapy using real-world data-sets. As randomized controlled trials have long timescales and are limited to specific populations, this complementary method is beneficial to assess the impact of smaller changes to clinical practice in a real-world cohort (41). The discovery and validation of a significant interaction suggests that patients could potentially be stratified based on risk of local relapse pre-treatment which could lead to changes in radiotherapy delivery (e.g., increased margins or dose intensification) or selection for immunotherapy to improve patient outcome.

5 Conclusion

In summary, we have applied a previously described data-mining technique to predict LR and RF following lung SABR. High peritumor density was found to interact with dose variability up to 1.6cm outside the GTV and they jointly predicted LR in two independent data-sets from different institutions. No direct association with clinical outcome was found in GTV and PTV dose metrics, but correlations demonstrated that within PTV heterogeneity may be chiefly responsible for this interaction. Overall, external confirmation of the model supports the use of density biomarkers to predict risk of microscopic disease extension, and that adequate dose coverage outside the intended treatment region of the tumor could reduce the risk of local failure. The proposed density x dose biomarker could be investigated in the future to personalize SABR dose distributions and possibly to reduce the rate of residual failures observed even at high doses.

Data availability statement

The datasets presented in this article are not readily available because ethical permission was not granted for general publication of the dataset. Requests to access the datasets should be directed to Dr Alan McWilliam, alan.mcwilliam@manchester.ac.uk and Dr Maria Thor, thorm@mskcc.org.

Ethics statement

Our retrospective analysis of anonymized routine data was approved by institutional information governance and research ethics committee (The Christie NHS Foundation Trust and Caldicott Committee). The research was carried out according to a protocol approved by the Caldicott committee. A data-sharing agreement was in-place for remote analysis of the data from Memorial Sloan Kettering Cancer Center.

Author contributions

AD developed the methodology, collated the data, performed primary analysis, and wrote the manuscript. MT collated the validation data and was involved in detailed discussions about the planned validation work. AM and MH provided expert insight at all stages of the experiment. CF-F provided expert clinical insight to the project. AR and JD provided insight into the presentation of results and supported the validation work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

AR: Research funding from AstraZeneca, Merck, Pfizer, Boehringer Ingelheim, and Varian Medical Systems. Consulting fees/advisory boards for AstraZeneca, Merck and MoreHealth.

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1156389/full#supplementary-material

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