The impact of adjuvant EGFR-TKIs and 14-gene molecular assay on stage I non–small cell lung cancer with sensitive EGFR mutations

Yu Jiang,[a,b,g] Yuechun Lin,[a,b,g] Wenhai Fu,[a,b,g] Qihua He,[a,b,g] Hengrui Liang,[a,b] Ran Zhong,[a,b] Ran Cheng,[a,b] Bingliang Li,[c] Yaokai Wen,[d] Huiting Wang,[a,b] Jianfu Li,[a,b] Caichen Li,[a,b] Shan Xiong,[a,b] Songan Chen,[e] Jianxing Xiang,[e] Michael J. Mann,[f,h,***] Jianxing He,[a,b,h,**] and Wenhua Liang[a,b,h,*]

[a]National Clinical Research Center for Respiratory Disease, Guangzhou, China
[b]Departments of Thoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China
[c]Department of Cardiac Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China
[d]Department of Medical Oncology, Shanghai Pulmonary Hospital, Tongji University Medical School Cancer Institute, Tongji University School of Medicine, Shanghai, China
[e]Burning Rock Biotech, Guangzhou, China
[f]Department of Surgery, Division of Cardiothoracic Surgery, University of California San Francisco, San Francisco, CA, USA

eClinicalMedicine 2023;64: 102205
Published Online xxx https://doi.org/10.1016/j.eclinm.2023.102205

Summary

Background Currently, the role of EGFR-TKIs as adjuvant therapy for stage I, especially IA NSCLC, after surgical resection remains unclear. We aimed to compare the effect of adjuvant EGFR-TKIs with observation in such patients by incorporating an established 14-gene molecular assay for risk stratification.

Methods This retrospective cohort study was conducted at the First Affiliated Hospital of Guangzhou Medical University (Study ID: ChNCRCRD-2022-GZ01). From March 2013 to February 2019, completely resected stage I NSCLC (8th TNM staging) patients with sensitive EGFR mutation were included. Patients with eligible samples for molecular risk stratification were subjected to the 14-gene prognostic assay. Inverse probability of treatment weighting (IPTW) was employed to minimize imbalances in baseline characteristics.

Findings A total of 227 stage I NSCLC patients were enrolled, with 55 in EGFR-TKI group and 172 in the observation group. The median duration of follow-up was 78.4 months. After IPTW, the 5-year DFS (HR = 0.30, 95% CI, 0.14–0.67; P = 0.003) and OS (HR = 0.26, 95% CI, 0.07–0.96; P = 0.044) of the EGFR-TKI group were significantly better than the observation group. For subgroup analyses, adjuvant EGFR-TKIs were associated with favorable 5-year DFS rates in both IA (100.0% vs. 84.5%; P = 0.007), and IB group (98.8% vs. 75.3%; P = 0.008). The 14-gene assay was performed in 180 patients. Among intermediate-high-risk patients, EGFR-TKIs were associated with a significant improvement in 5-year DFS rates compared to observation (96.0% vs. 70.5%; P = 0.012), while no difference was found in low-risk patients (100.0% vs. 94.9%; P = 0.360).

Interpretation Our study suggested that adjuvant EGFR-TKI might improve DFS and OS of stage IA and IB EGFR-mutated NSCLC, and the 14-gene molecular assay could help patients that would benefit the most from treatment.

Funding This work was supported by China National Science Foundation (82022048, 82373121).

Keywords: Adjuvant therapy; EGFR-TKI; Non–small-cell lung cancer; Stage I; Risk stratification

*Corresponding author. National Clinical Research Center for Respiratory Disease, The Department of Thoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510120, China.
**Corresponding author. National Clinical Research Center for Respiratory Disease, The Department of Thoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510120, China.
***Corresponding author. Department of Surgery, Division of Cardiothoracic Surgery, University of California San Francisco, San Francisco, CA, USA.
E-mail addresses: liangwh1987@163.com (W. Liang), drjianxing.he@gmail.com (J. He), michael.mann@ucsf.edu (M.J. Mann).
[g]Drs Jiang, Lin, He and Fu contributed equally to this article.
[h]Drs Liang, He and Mann are joint corresponding authors.

Research in context

Evidence before this study

Although increasing evidence suggests that adjuvant epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) may be an effective treatment option for a specific population following complete resection of early-stage NSCLC, most studies have predominantly excluded patients with stage I NSCLC. As of December 31st, 2022, our exhaustive search of PubMed using relevant keywords such as "stage I," "NSCLC," "EGFR-TKI," and "adjuvant" has yielded limited studies reporting on the efficacy of adjuvant EGFR-TKI therapy specifically in stage I, and in particular, stage IA, NSCLC.

Added value of this study

Our research findings demonstrate the substantial anti-tumor effects of adjuvant EGFR-TKIs in patients with stage IA and IB NSCLC. The introduction of the 14-gene prognostic assay further augments the clinical utility of our research by identifying high-risk patients who can be targeted for personalized adjuvant treatment, thus paving the way for more effective and tailored therapeutic strategies in the management of early-stage NSCLC.

Implications of all the available evidence

The study demonstrates that adjuvant EGFR-TKIs is associated with improved prognosis, especially in early-stage NSCLC patients with high molecular risk profiles, with no tumor recurrence or metastasis in stage IA patients within a 5-year follow-up. The evidence presented in our study underscores the potential of adjuvant EGFR-TKIs as an effective therapeutic strategy for patients with completely resected stage IA and IB NSCLC.

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality globally.[1] Currently, surgical resection represents the preferred therapeutic modality for early-stage lung cancer.[2] Nevertheless, stage I NSCLC exhibits considerable heterogeneity in terms of clinical outcomes. According to the current eighth edition of the tumor-node-metastasis (TNM) staging system, the 5-year overall survival (OS) rate for stage I NSCLC has been reported to range from 73% to 90%,[3] implying that the long-term survival outcomes after complete surgical resection of stage I NSCLC remained unsatisfactory. Notably, approximately 20%–40% of stage I NSCLC patients experience local recurrence or distant metastasis within 5 years after surgery,[3] which may be attributed to locally or systemically undetectable residual viable tumor cells that were not eliminated by surgery,[4] making adjuvant treatment for stage I NSCLC a major clinical challenge.

Adjuvant chemotherapy has emerged as the standard postoperative treatment regimen for patients with stage II to IIIA and select stage IB NSCLC, resulting in a 16% reduction in the risk of recurrence or death and a 5% improvement in OS.[5,6] However, it is neither beneficial nor recommended for patients with stage I lung cancer.[7] Therefore, better treatment strategies to further improve the long-term survival of patients with stage I NSCLC are urgently expected. Epidermal growth factor receptor (EGFR) mutations, particularly exon 19 deletions (19 Del) and exon 21 codon p.Leu858Arg (L858R) point mutations, have been established as predictive factors for response to EGFR tyrosine kinase inhibitors (TKIs) in NSCLC.[8,9] Several studies have reported that resected NSCLC patients with sensitive EGFR mutations who receive adjuvant EGFR-TKIs experience longer disease-free survival (DFS) and fewer adverse events compared to those receiving adjuvant chemotherapy or placebo.[10,11] Although previous studies suggested a potential role for first-generation EGFR-TKIs in postoperative therapy,[11–13] identification of target populations that may benefit from adjuvant EGFR-TKIs in the context of stage I NSCLC remains to be explored.

A 14-gene molecular assay has been established to stratify the risk of recurrence in patients with NSCLC after surgical resection.[14] Previous studies have demonstrated that among patients classified as molecular high-risk, those who received adjuvant chemotherapy exhibited significantly longer disease-free survival DFS compared to those who did not.[15] For one thing, it would be helpful to accurately identify high-risk patients who may benefit from adjuvant therapy. For another, it may identify patients with a low risk of recurrence who are more likely cured with surgical resection alone, thereby obviating the need for additional interventions.

In this study, we conducted a preliminary analysis in the real-word practice scenario to investigate the potential benefits of adjuvant EGFR-TKIs to stage I NSCLC patients following complete surgical resection, and to identify the dominant population with the optimal clinical outcome.

Methods

Study design and population

This retrospective cohort analysis was conducted at the First Affiliated Hospital of Guangzhou Medical University, spanning from March 2013 to February 2019. The study design is presented in Fig. 1. Eligible patients were (I) aged ≥18 years; (II) histopathologically diagnosed stage I non-squamous NSCLC (8th TNM staging); (III) presence of a sensitizing EGFR mutation (19 Del or L858R); (IV) underwent complete surgical resections via lobectomy or pneumonectomy with systemic

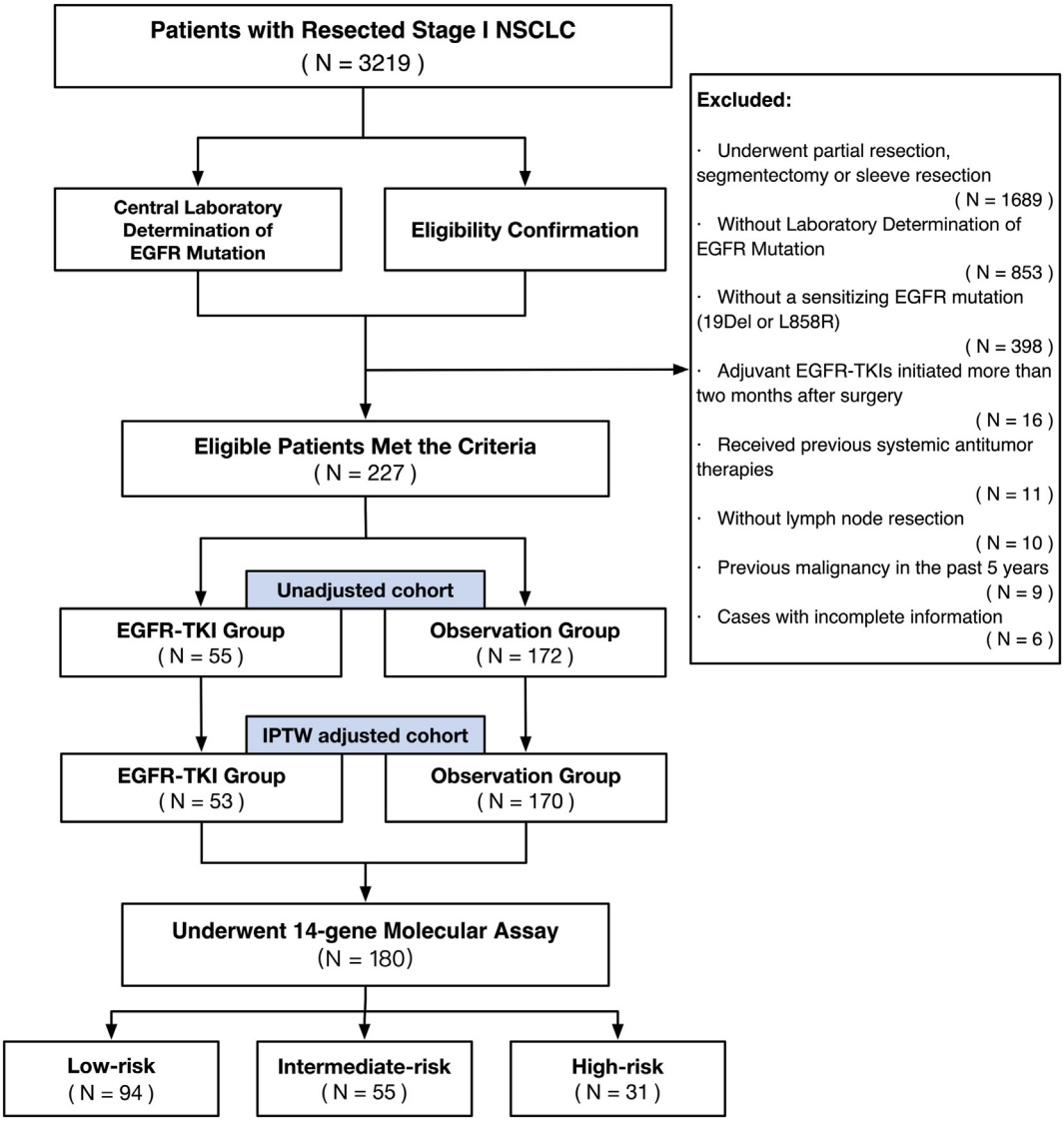

Fig. 1: Study profile. Data cutoff on February 28, 2019. Ab*breviations:* EGFR, epidermal growth factor receptor; IPTW, inverse probability of treatment weighting; N, number; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor.

intrathoracic lymph node dissection; (V) availability of clinicopathological data, (VI) complete postoperative follow-up of at least 5 years for patients without disease progression, while for patients who experienced disease progression, their follow-up duration was not necessarily 5 years but rather the time until progression within the 5-year period. The following patients were excluded: (I) underwent incomplete surgical resection, as per the Chinese guidelines on the diagnosis and treatment of primary lung cancer (2019); (II) adjuvant EGFR-TKIs initiated more than two months after surgery; (III) previous malignancy in the past 5 years; (IV) received previous systemic antitumor therapies (chemotherapy, radiotherapy, or target therapy).

Decisions about whether patients would receive adjuvant EGFR-TKIs were collaboratively made by the patients and their attending physicians. This process encompassed a comprehensive evaluation of various factors, including the patient's economic circumstances and treatment preferences. Moreover, guidance was sought from the National Comprehensive Cancer Network (NCCN) guidelines, which outline specific high-risk factors. By incorporating these considerations, a personalized recommendation was formulated regarding the use of adjuvant EGFR-TKIs. The daily dosages of icotinib, erlotinib, and gefitinib were 150 mg three times, 150 mg once, and 250 mg once, respectively.

Data collection

The baseline clinical data and the postoperative follow-up information were extracted from the electronic medical records system, including patient demographics (age, sex, smoking status), cancer information (tumor location, tumor size, TNM stage, pathology, histology, differentiation grade, EGFR status), and postoperative information (survival status, disease progression, and any subsequent treatments received). The TNM stage was reclassified according to the 8th edition of the TNM classification by the International Association for the Study of Lung Cancer.[3] EGFR mutations were detected using either the amplification-refractory mutation polymerase chain reaction (PCR) system or next-generation sequencing (NGS).

For patients without recorded survival status in the postoperative medical records, we conducted telephone interviews with the patients. While telephone interviews may not provide as comprehensive information as the electronic medical records system, they serve as a valuable means to collect patient-reported outcomes and determine their overall well-being and disease status.

Disease recurrence was assessed based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, including chest computed tomography (CT) scan every 3 months during treatment, and every 6 months or as needed during long-term follow-up period based on symptoms. Patients were followed up from diagnosis until death or their last follow-up date. Data collection ended on July 31, 2022.

14-Gene molecular assay

A Clinical Laboratory Improvement Amendments (CLIA)-certified 14-gene quantitative PCR expression assay (DetermaRx™, Burning Rock Biotech) has been developed and validated globally to evaluate the recurrence risk of non-squamous NSCLC after surgical resection. The complete methodology for RNA extraction, quality control, quantitative reverse transcription PCR, and analytical validation have been thoroughly described in previous studies.[14,16]

To explore the prognostic value of 14-gene assay in adjuvant molecular targeted therapies, we retrospectively collected formalin-fixed, paraffin-embedded tumor samples submitted for molecular testing to obtain risk stratification. The molecular prognostic assay stratified patients into low, intermediate, or high-risk groups based on the risk score, where intermediate- and high-risk patients were grouped together and considered as molecular high-risk for recurrence.

Outcomes

The primary endpoint of this study was 5-year disease-free survival (DFS), which was calculated as the interval from the initiation of treatment to the first recurrence or death. The secondary end point was overall survival (OS), which was defined as the time from treatment to death from any cause. Patients who survived without recurrence or were lost to follow-up were censored at the time of their last available assessment, while patients who died from other causes without prior recurrence were censored at the date of death.

Statistics

The study population was divided into two groups based on postoperative treatment: adjuvant EGFR-TKI and observation. Given the nonrandomized nature of the study design, inverse probability of treatment weighting (IPTW) was applied to balance baseline covariates and minimize potential confounding factors between these two groups. The propensity score was derived by incorporating baseline variables that were deemed clinically relevant, along with logistic regression results aimed at exploring the association with adjuvant EGFR-TKIs. These variables encompassed the following factors: age, sex, smoking status, tumor location, examined lymph node (ELN), TNM stage, histology, differentiation grade, EGFR status, visceral pleural invasion (VPI), ground glass opacity (GGO), and multiple primary lung cancer (MPLC). Each patient was assigned a weight based on the inverse of their probability of being assigned to a specific treatment group. A stabilized inverse probability was then calculated using the propensity score. Standardized differences were computed to assess the balance of covariates before and after stabilized IPTW. Following the application of IPTW, all covariates demonstrated standardized differences of less than 0.10, indicating a satisfactory balance between the two groups.

To summarize patient characteristics, continuous variables were summarized as means with standard deviations (for normally distributed data) or medians with interquartile ranges (for non-normally distributed data), and differences between groups were evaluated using either Student's t-test or Mann–Whitney U-test. Categorical variables were presented as frequencies and percentages and compared between groups using Pearson's chi-square test or Fisher's exact test.

The DFS and OS were estimated using the Kaplan–Meier method and compared using the log-rank test. Independent prognostic factors were identified using univariable and multivariable Cox proportional hazard regression analysis, and hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. Subgroup results are presented as a forest plot, illustrating the clinical efficacy of adjuvant EGFR-TKIs across distinct clinicopathological variables. To estimate the number needed to treat (NNT) values, which represent the number of individuals that need to be treated to prevent the progression of one case, the inverse of the 5-year incidence rate differences was calculated. Statistical significance was set at a P-value of less than 0.05, and all analyses were conducted using IBM SPSS Statistics (version 26.0), R software (version 4.2.2), and GraphPad Prism (version 9.5.1).

Ethics

The study was registered at the National Clinical Research Center for Respiratory Disease on December 29, 2021 (Study ID: ChNCRCRD-2022-GZ01). The study was approved by the Institutional Ethics Committee for Clinical Investigation of First Affiliated Hospital of Guangzhou Medical University (No. KLS-2020), enabling the use of extracted data for research purposes. As a retrospective study, written informed consents were waived by the ethical committee. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

Role of the funding source

The funders had no role in the study design, data collection, data analyses, interpretation, or writing of report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication.

Results

Patient characteristics

A total of 227 eligible patients were included in the study (Fig. 1), with 55 (24.2%) receiving adjuvant EGFR-TKIs therapy and 172 (75.8%) in the observation group. The median follow-up time was 78.4 (IQR, 67.0–89.5) months. The median age of the study population was 61 years. The demographic and clinical characteristics of all patients are presented in Table 1. All patients underwent R0 surgical resection by lobectomy with negative margins. Before weighting, pronounced differences in stage, VPI, and MPLC were noted between the EGFR-TKIs and observation groups, indicating the imbalance baseline characteristics between the two groups. After weighting, 223 stage I NSCLC patients were included in the survival analysis, with 53 patients receiving adjuvant EGFR-TKIs and 170 patients in the observation group. Of these, 111 (49.8%) patients were in stage IB, while 112 (50.2%) were in stage IA. Leu858 and 19 Del were identified in 143 (64.1%) and 80 (35.9%) patients, respectively. Cancer cell differentiation was classified as poor (85, 38.1%), moderate (96, 43.0%) and well (42, 18.9%). The mean standard differences were inferior to 10% for all baseline characteristics as presented in Tables 1 and in the love plot (Fig. 2).

Impact of EGFR-TKIs on survival outcomes in stage I NSCLC patients

In total, 18 of 110 patients (16.4%) with stage IA received adjuvant EGFR-TKIs, which increased to 37 of 117 patients (31.6%) with stage IB. Of 55 patients with adjuvant EGFR-TKIs, 27 (49.1%) received icotinib, 13 (23.6%) received erlotinib, and 15 (27.3%) received gefitinib. The median duration of treatment for icotinib, erlotinib and gefitinib were 12.2 months [interquartile range (IQR), 9.2–16.8 months], 14.8 months (IQR, 11.6–17.2 months) and 13.1 months (IQR, 8.6–17.8 months), respectively. Logistic regression was performed to determine the variables associated with adjuvant EGFR-TKIs therapy in stage I NSCLC (Table S1). For one thing, compared to patients with well- and moderate-differentiated NSCLC, those with poorly differentiated were more likely to receive adjuvant EGFR-TKIs (HR = 1.94, 95% CI, 1.02–3.70; P = 0.044). For another, patients with IB NSCLC (HR = 2.22, 95% CI, 1.12–4.40; P = 0.023) and MPLC (HR = 4.04, 95% CI, 2.06–7.91; P < 0.001) were also more likely to undergo adjuvant EGFR-TKIs therapies, reflecting a higher proportion of high-risk population in the EGFR-TKI group.

In the Kaplan–Meier analyses and log-rank test, the EGFR-TKI group was associated with significantly higher 5-year DFS rates [99.4% (95% CI, 98.1%–100.0%) vs. 79.6% (95% CI, 73.3%–86.5%); P = 0.002] and OS rates [100.0% (95% CI, 100.0%–100.0%) vs. 93.7% (95% CI, 89.9%–97.7%); P = 0.032] than the observation group (Fig. 3A–B). The adjusted risk difference in 5-year progression rate between these two groups was 19.8% (95% CI, 13.5%–24.8%), which translated into a number needed to treat of 5 in patients with stage I NSCLC.

In the observation group, a total of 31 patients (18.0%) experienced recurrences, whereas only 1 patient (1.8%) in the EGFR-TKI group experienced locoregional recurrence (P = 0.003). Among the 31 patients who experienced recurrence in the observation group, 11 cases were identified as locoregional recurrences, while the remaining 20 cases were categorized as distant recurrences. The most frequently observed sites of locoregional recurrence were the ipsilateral lung, lymph nodes, and pleura. Regarding distant recurrence, 11 patients (55.0%) exhibited brain metastasis, 6 patients (30.0%) showed bone metastasis, and 3 patients (15.0%) had contralateral lung metastasis.

Univariable Cox proportional hazards regression analysis found that adjuvant EGFR-TKIs were significantly associated with improved DFS in matched patients (HR = 0.39, 95% CI, 0.15–0.96; P = 0.048). Multivariable Cox proportional hazards regression analysis showed that adjuvant EGFR-TKIs remained independently associated with improved DFS (HR = 0.30, 95% CI, 0.14–0.67; P = 0.003) (Table S2). Further analysis of OS was limited due to a low number of deaths.

Subgroup analysis

In the subgroup analyses, we evaluated the association of adjuvant EGFR-TKIs with survival outcomes in patients with IA and IB NSCLC. Patients with IA NSCLC undergoing EGFR-TKIs experienced higher 5-year DFS rates than the observation group [100.0% (95% CI, 100.0%–100.0%) vs. 84.5% (95% CI, 77.0%–92.7%); P = 0.007]. Similar conclusions were obtained in the IB

Characteristic–n (%)	Before weighting				After weighting			
	EGFR-TKIs (n = 55)	Observation (n = 172)	P-value	SMD	EGFR-TKIs (n = 53)	Observation (n = 170)	P-value	SMD
Age								
<60	19 (34.5)	70 (40.7)	0.513	0.154	24 (45.3)	69 (40.6)	0.605	0.088
≥60	36 (65.5)	102 (59.3)			29 (54.7)	101 (59.4)		
Sex								
Female	30 (54.5)	100 (58.1)	0.755	0.073	28 (52.8)	98 (57.6)	0.612	0.091
Male	25 (45.5)	72 (41.9)			25 (47.2)	72 (42.4)		
Smoking								
Never	43 (78.2)	139 (80.8)	0.817	0.065	41 (77.4)	136 (80.0)	0.639	0.080
Ever	12 (21.8)	33 (19.2)			12 (22.6)	34 (20.0)		
Location								
Upper	37 (67.3)	95 (55.2)	0.259	0.262	30 (56.6)	97 (57.1)	0.915	0.077
Middle	5 (9.1)	17 (9.9)			7 (13.2)	17 (10.0)		
Lower	13 (23.6)	60 (34.9)			16 (30.2)	56 (32.9)		
ELN								
<16	23 (41.8)	88 (51.2)	0.293	0.188	27 (50.9)	84 (49.4)	0.911	0.020
≥16	32 (58.2)	84 (48.8)			26 (49.1)	86 (50.6)		
Pathology stage								
IA	18 (32.7)	92 (53.5)	**0.012**	0.429	28 (52.8)	84 (49.4)	0.731	0.060
IB	37 (67.3)	80 (46.5)			25 (47.2)	86 (50.6)		
Differentiation grade								
Well	8 (14.5)	36 (20.9)	0.159	0.297	9 (17.0)	33 (19.4)	0.928	0.071
Moderate	20 (36.4)	76 (44.2)			23 (43.4)	73 (42.9)		
Poor	27 (49.1)	60 (34.9)			21 (39.6)	64 (37.6)		
Histology								
Adenocarcinoma	54 (98.2)	172 (100.0)	0.547	0.192	53 (100.0)	170 (100.0)	0.081	0.095
Adenosquamous carcinoma	1 (1.8)	0 (0.0)			0 (0.0)	0 (0.0)		
EGFR mutation								
Exon 19 deletions	20 (36.4)	63 (36.6)	1.000	0.005	18 (34.0)	62 (36.5)	0.758	0.055
Exon 21 Leu858Arg	35 (63.6)	109 (63.4)			35 (66.0)	108 (63.5)		
VPI								
Yes	36 (65.5)	76 (44.2)	**0.010**	0.437	28 (52.8)	83 (48.8)	0.722	0.067
No	19 (34.5)	96 (55.8)			25 (47.2)	87 (51.2)		
GGO								
Yes	18 (32.7)	43 (25.0)	0.342	0.171	15 (28.3)	47 (27.6)	0.815	0.041
No	37 (67.3)	129 (75.0)			38 (71.7)	123 (72.4)		
MPLC								
Yes	23 (53.5)	32 (23.4)	**<0.001**	0.659	19 (34.0)	55 (32.4)	0.778	0.047
No	20 (46.5)	105 (76.6)			35 (66.0)	115 (67.6)		
LVI								
Yes	5 (9.1)	9 (5.2)	0.476	0.150	3 (5.7)	10 (5.9)	0.940	0.012
No	50 (90.9)	163 (94.8)			50 (94.3)	160 (94.1)		

Abbreviations: EGFR, epidermal growth factor receptor; ELN, examined lymph node; GGO, ground glass opacity; IPTW, inverse probability of treatment weighting; LVI, lympho-vascular invasion; MPLC, multiple primary lung cancer; SMD, standardized mean difference; TKI, tyrosine kinase inhibitor; VPI, visceral pleural invasion.

Table 1: **Baseline demographic and clinical characteristics of patients before and after IPTW.**

group, showing a better 5-year DFS rates in patients with EGFR-TKIs [98.8% (95% CI, 96.5%–100.0%) vs. 75.3% (95% CI, 65.6%–86.5%); P = 0.008]. In terms of OS outcomes, the IB group exhibited a notable clinical advantage between EGFR-TKI and observation group [100.0% (95% CI, 100.0%–100.0%) vs. 91.2% (95% CI, 85.0%–97.8%); P = 0.030)], whereas the IA group did not [100.0% (95% CI, 100.0%–100.0%) vs. 96.8% (95% CI, 93.3%–100.0%); P = 0.560) (Fig. 3C–F). Additionally, the clinical efficacy of adjuvant EGFR-TKIs was consistently favorable in most of the prespecified subgroups (Figure S1). The adjusted risk difference in 5-year

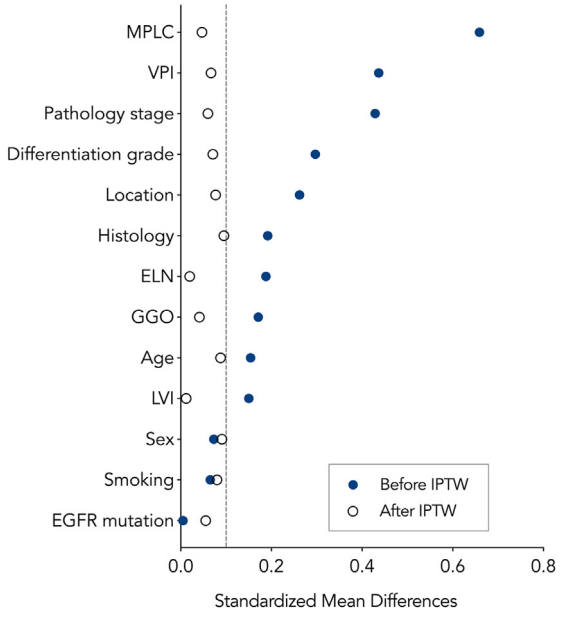

Fig. 2: Love plots for standardized mean differences comparing covariate values before and after IPTW. Abb*reviations:* EGFR, epidermal growth factor receptor; ELN, examined lymph node; GGO, ground glass opacity; IPTW, inverse probability of treatment weighting; LVI, lympho-vascular invasion; MPLC, multiple primary lung cancer; VPI, visceral pleural invasion.

progression rate between the two groups was 15.5% (95% CI, 7.3%–23.0%) in IA NSCLC and 23.5% (95% CI, 13.5%–30.9%) in IB NSCLC, which translated into a number needed to treat of 6 and 4 in IA and IB NSCLC.

Association of 14-gene molecular risk stratification with prognosis

The 14-gene assay was performed in 180 patients, stratifying 31 (17.2%), 55 (30.6%), and 94 (52.2%) patients as high, intermediate, and low risk of recurrence, respectively (Figure S2). In the observation group, patients in the high-risk group (HR = 11.72; P < 0.001) and the intermediate-risk group (HR = 4.19; P = 0.019) both showed a notably reduced DFS compared to those in the low-risk group. Consequently, these two risk groups were combined and designated as the intermediate-high-risk group, representing individuals with a molecular high-risk of recurrence (Fig. 4A–B). The 5-year DFS rates for the low-risk and intermediate-high-risk group were 94.9% and 70.5%, respectively (P < 0.001) (Fig. 4C). Moreover, the 5-year OS for the low-risk and intermediate-high-risk group were 97.4% and 91.3%, respectively (P = 0.002) (Fig. 4D). These findings indicated the independent association of the 14-gene molecular risk stratification with prognosis, and demonstrated that the 14-gene assay remained a significant prognostic indicator after stratification by clinicopathological variables.

Association of 14-gene molecular risk stratification with adjuvant EGFR-TKIs benefits

The interaction test between the 14-gene molecular risk stratification and adjuvant EGFR-TKIs demonstrated that molecular high-risk patients had a more favorable response to adjuvant EGFR-TKIs compared with molecular low-risk patients. Specifically, for high-risk patients, adjuvant EGFR-TKIs were associated with a statistically significant improvement in both 5-year DFS [96.0% (95% CI, 88.6%–100.0%) vs. 70.5% (95% CI, 59.9%–82.9%); P = 0.012] and OS rates [100.0% (100.0%–100.0%) vs. 91.3% (95% CI, 84.3%–98.9%); P = 0.048] compared to observation alone. Conversely, no statistically significant differences were observed in either DFS [100.0% (95% CI, 100.0%–100.0%) vs. 94.9% (95% CI, 90.1%–99.9%); P = 0.360] or OS [100.0% (100.0%–100.0%) vs. 97.4% (95% CI, 93.9%–100.0%); P = 0.520] among the low-risk population (Fig. 5).

Subgroup analyses according to TNM stage revealed that molecular high-risk patients had superior survival benefits in stage IA and IB NSCLC subgroups. However, low-risk patients showed clinically smaller benefits in the stage IB NSCLC subgroup, and no differences were identified in the stage IA NSCLC subgroup (Figures S3–S5).

Discussion

Adjuvant EGFR-TKIs have been established as effective therapeutic options for improving survival outcomes in patients diagnosed with stage II to IIIA NSCLC harboring EGFR mutations,[17] as these patients are at high risk for harboring occult micrometastases.[18–20] Notably, approximately 30% of stage IA patients ultimately succumb to occult metastasis, a potential target for eradication through systemic adjuvant treatment.[15] Despite these promising prospects, owing to the lackluster data from previous clinical trials, these advances have not been successfully applied to stage I, especially stage IA NSCLC patients.

In the present study, although the median DFS was not reached in either group at the data cutoff, adjuvant EGFR-TKIs demonstrated a sustained and clinically significant 5-year DFS benefit in stage I NSCLC patients, particularly in those identified as high-risk based on both clinicopathological and molecular stratification criteria. This finding holds considerable significance as it figured out the dominant population that may benefit from adjuvant EGFR-TKIs that has previously been overlooked. To the best of our knowledge, this is the first study evaluating the efficacy of adjuvant EGFR-TKIs in both stage IA and IB NSCLC patients.

Our findings corroborate the observations made in previous studies that have compared adjuvant EGFR-TKIs with observation in stage IB NSCLC. The updated analysis of the final and mature ADAURA trial revealed that the third-generation EGFR-TKI

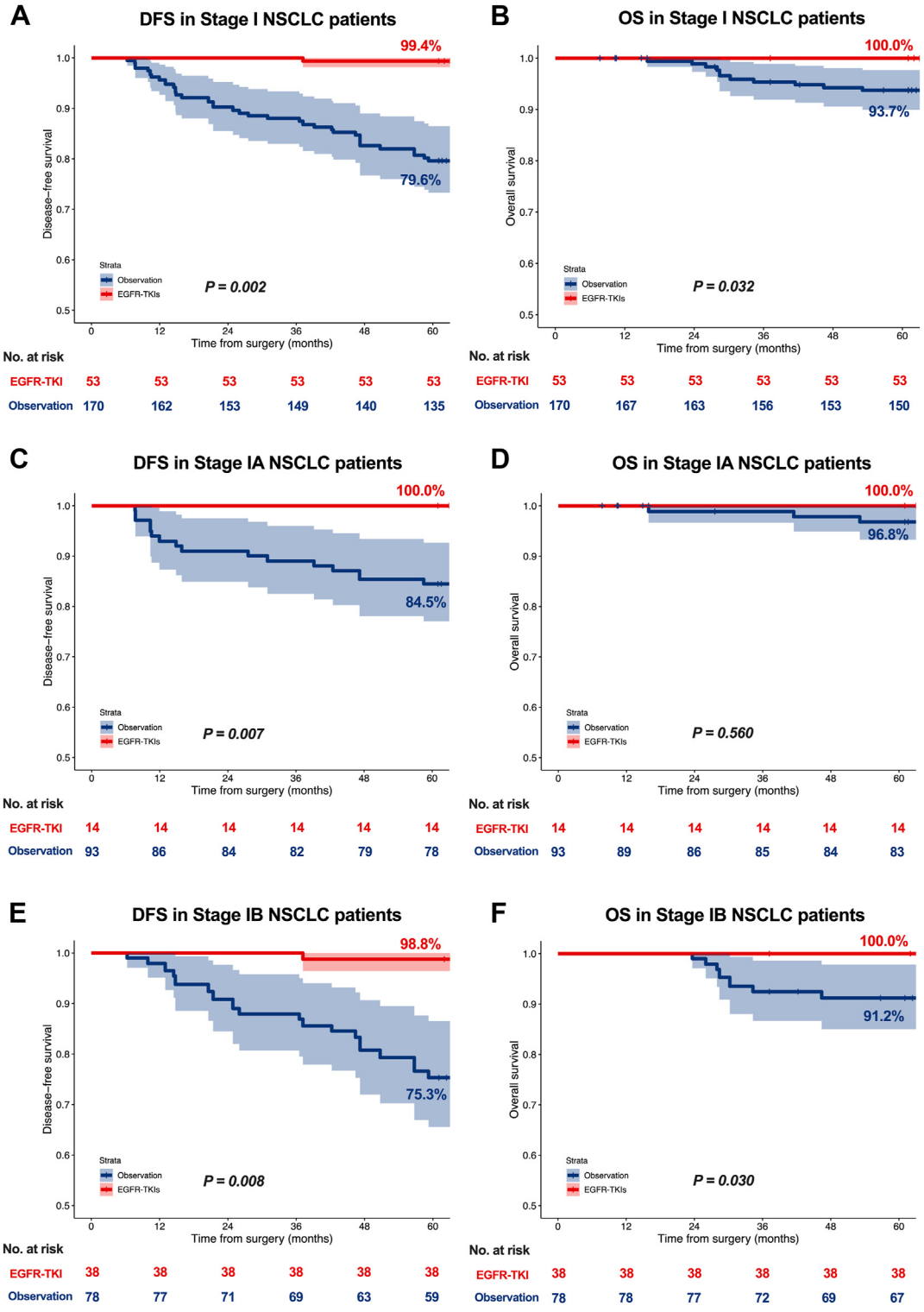

Fig. 3: Kaplan–Meier survival analysis for stage I NSCLC patients according to treatment. (A) DFS in stage I NSCLC patients. (B) OS in stage I NSCLC patients. (C) DFS in stage IA NSCLC patients. (D) OS in stage IA NSCLC patients. (E) DFS in stage IB NSCLC patients. (F) OS in stage IB NSCLC patients. The shaded area represents the 95% CI. *Abbreviations:* CI, confidence interval; DFS, disease-free survival; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; OS, overall survival; TKI, tyrosine kinase inhibitor.

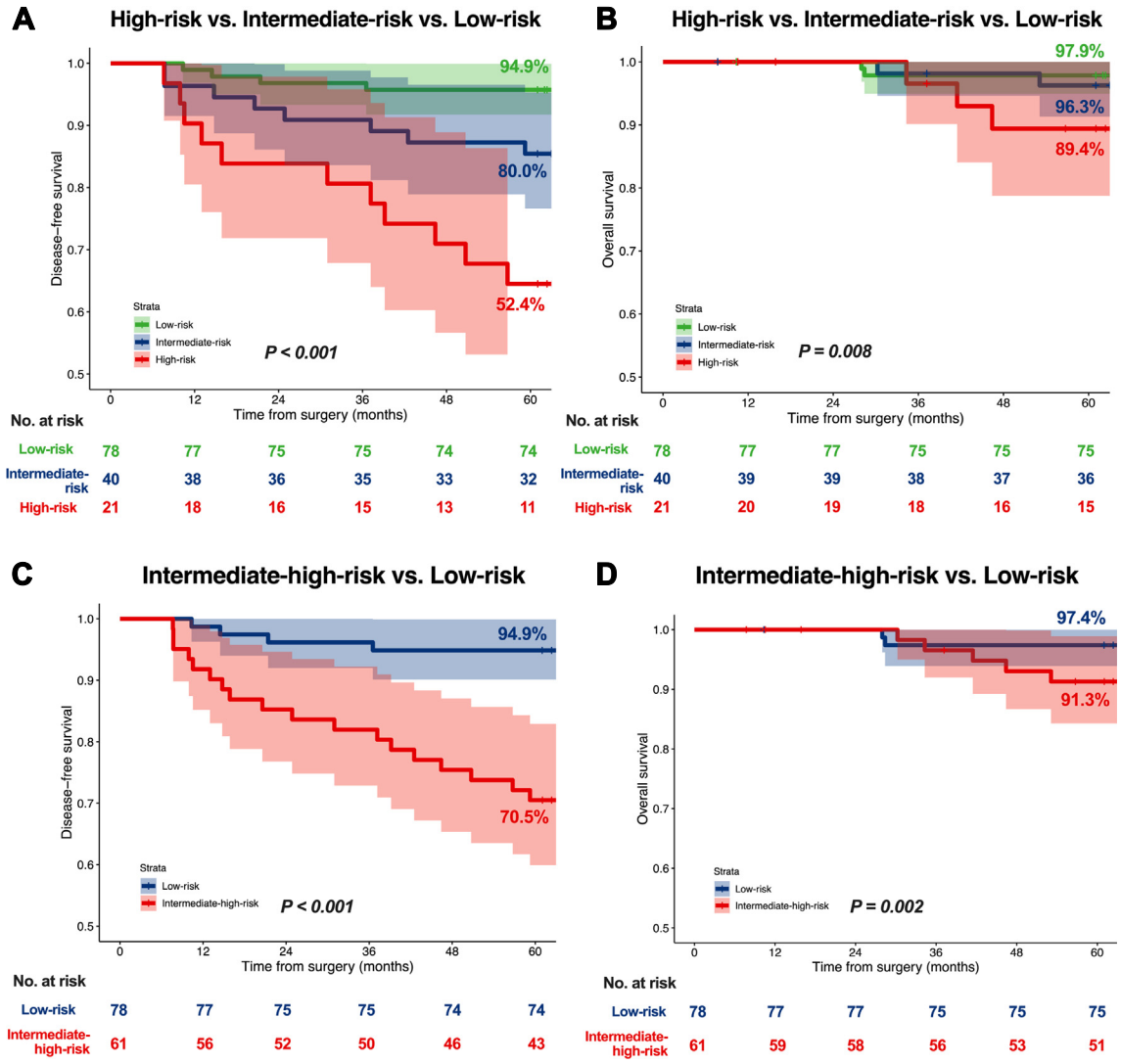

Fig. 4: Kaplan–Meier survival analysis of the observational cohorts, stratified by the 14-gene assay. (A) DFS in high-, intermediate-, and low-risk stage I NSCLC patients. (B) OS in high-, intermediate-, and low-risk stage I NSCLC patients. (C) DFS in intermediate-high- and low-risk stage I NSCLC patients. (D) OS in intermediate-high- and low-risk stage I NSCLC patients. The shaded area represents the 95% CI. *Abbreviations:* CI, confidence interval; DFS, disease-free survival; OS, overall survival.

osimertinib yielded improvements in DFS (HR = 0.44, 95% CI, 0.25–0.76) and OS (HR = 0.44, 95% CI, 0.17–1.02) when compared to placebo in surgically resected stage IB EGFR-mutant NSCLC.[21,22] Similarly, the CORIN trial demonstrated that adjuvant icotinib was associated with a remarkable 75% reduction in the 3-year risk of disease relapse or mortality (HR = 0.25, 95% CI, 0.07–0.87) according to the 8th edition of the TNM staging system.[23]

The efficacy of adjuvant EGFR-TKI in this study is consistently maintained until the last observed date, as evidenced by the sustained separation of the Kaplan–Meier curves during the 5-year follow-up period. This finding suggests that some patients may continue to derive benefits from adjuvant EGFR-TKIs even after the discontinuation of these agents. The survival advantage associated with adjuvant EGFR-TKIs in early-stage NSCLC can be attributed to several potential mechanisms. Firstly, adjuvant EGFR-TKIs prevent the establishment of dormant micrometastases originating from circulating tumor cells (CTCs) following surgical intervention. Secondly, highly potent and selective EGFR-TKIs have demonstrated their capacity to induce apoptosis with long-lasting effects in xenograft and transgenic tumor models harboring EGFR mutations.[24,25] In addition, our previous study revealed a lower frequency of cell cycle gene co-mutation frequency in stage IB NSCLC compared to those in stage

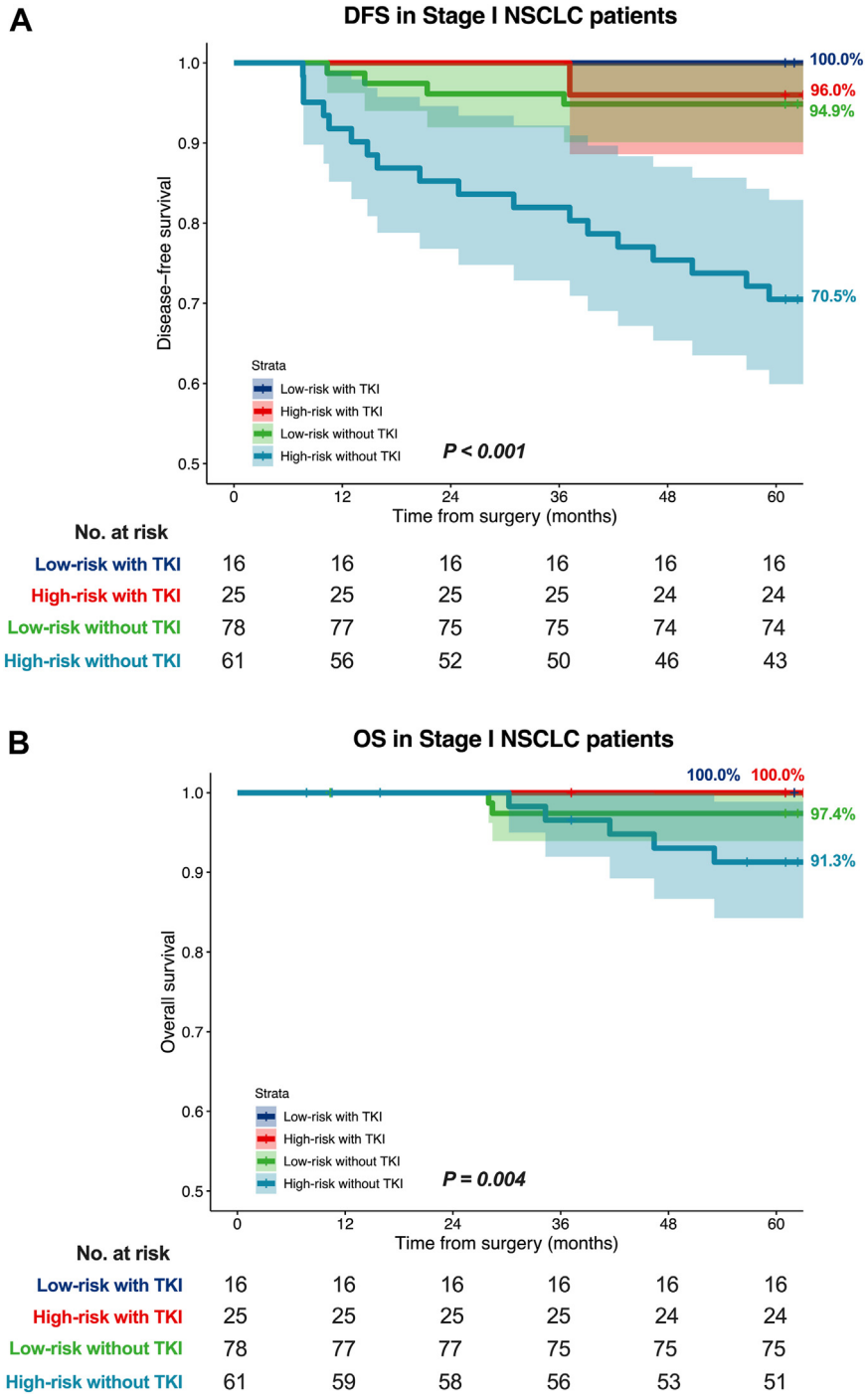

Fig. 5: Association of adjuvant EGFR-TKIs with DFS and OS in stage I NSCLC patients, stratified by the 14-gene assay. (A) DFS in stage I NSCLC patients. (B) OS in stage I NSCLC patients. The shaded area represents the 95% CI. *Abbreviations:* CI, confidence interval; DFS, disease-free survival; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; OS, overall survival; TKI, tyrosine kinase inhibitor.

IIIB-IV (13.3% vs. 25.5%). This discrepancy suggests a potentially favorable treatment response in early-stage NSCLC when compared to patients with advanced-stage disease.[26]

The overall incidence of recurrences, encompassing both locoregional and distant recurrences, was significantly lower in the EGFR-TKI group compared to the observation group. Notably, in the stage IA cohort,

patients who received adjuvant EGFR-TKIs exhibited no signs of tumor recurrence or metastatic disease within the 5-year post-surgery period. This finding is distinct from previous clinical trials where a decline in recurrence-free survival after 2 or 3 years was frequently observed, possibly due to the discontinuation of EGFR-TKIs.[13,27–32] Remarkably, such waning treatment effect was not observed in stage IA patients in the present study. These data provide initial insights into the patterns of recurrence and raise questions regarding the impact of adjuvant EGFR-TKIs on the natural progression of the disease, particularly whether they improve cure rates or merely delay relapse. Although the underlying mechanism remains unclear, the clinical significance of these findings should not be overlooked.

As reported in previous literature, the 14-gene assay was found to be a superior predictor of recurrence rates compared to conventional clinicopathologic and NCCN risk criteria, which was consistent with our cohort of 227 patients.[14,15,33,34] Specifically, in our study, improved survival outcomes were observed exclusively in the high-risk group, while the low-risk group exhibited favorable long-term survival irrespective of adjuvant EGFR-TKI treatment. Furthermore, the differentiation between the survival curves of the EGFR-TKI and observation groups became more distinct after molecular risk stratification using the 14-gene assay. This observation suggests that the pronounced survival benefit observed in the EGFR-TKI group may be attributed to the inclusion of a large number of high-risk patients who are currently receiving inadequate adjuvant EGFR-TKI therapy. These results provide preliminary evidence for the clinical utility of the 14-gene assay and support its accuracy in categorizing patients into low- and high-risk populations.

Several studies have demonstrated that molecular profiling and monitoring of minimal residual disease (MRD) may provide valuable insights into the risk of recurrence after curative surgery for NSCLC.[35–39] In our study, we have employed the 14-gene assay as a valuable tool to guide risk stratification for postoperative recurrence and predict the potential benefits of adjuvant EGFR-TKI therapy in patients with stage I NSCLC, thereby complementing existing clinical-pathological stratification and NCCN guidelines. However, the sensitivity of MRD detection methods still requires improvement. In cases where MRD detection presents false negativity, a comprehensive assessment of post-operative recurrence risk in NSCLC patients can also be achieved through the integration of other molecular markers.

Our study has several limitations that should be acknowledged. Firstly, given the nature of our study, which focuses on stage I NSCLC patients receiving adjuvant EGFR-TKI therapy as an off-label treatment, the eligible patient population itself is relatively small, which resulted in limited statistical power for survival analysis and a restricted number of recurrence events. Also, due to the limitations of the available data and the primary objectives of our study, we were unable to provide comprehensive results on tolerability and toxicity of adjuvant EGFR-TKI. While our study focused on the 14-gene panel, which has shown potential in distinguishing between EGFR wild-type and mutant variants in previous study,[40] there may be other biomarkers that could provide further insights into treatment outcomes and guide personalized therapeutic strategies. Exploring and identifying predictive markers and models specifically tailored for EGFR-mutated NSCLC should be a focus of future investigations in the field. Also, as a single-center study, the generalizability of the observed survival benefit to a broader population of stage I NSCLC patients remains to be confirmed. Moreover, the retrospective nature of the study introduced potential selection bias and hindered the determination of the optimal treatment regimen and duration for adjuvant EGFR-TKIs in high-risk stage I NSCLC patients. These findings should, in this respect, be interpreted with caution. Furthermore, it was not feasible at the time to conduct routine large-panel NGS testing, resulting in insufficient data on co-mutations and their potential influence on treatment outcomes. However, despite these limitations, our data provide valuable evidence supporting the efficacy of adjuvant EGFR-TKIs for patients with surgically resected stage I NSCLC harboring EGFR mutations. To obtain more robust and definitive conclusions, further prospective interventional trials with larger sample sizes and rigorous study designs are currently ongoing. These trials aim to address the existing limitations and provide more comprehensive insights into the role of adjuvant EGFR-TKIs in the management of stage I NSCLC.

In summary, adjuvant EGFR-TKIs is associated with improved survival outcomes in patients with stage I EGFR-mutated NSCLC after complete tumor resection, particularly in those identified as high risk for recurrence. These findings highlight the potential clinical importance of incorporating the 14-gene molecular assay into the decision-making process for adjuvant EGFR-TKIs. Additional large-scale randomized clinical trials are highly warranted to validate the observed survival benefits with adjuvant EGFR-TKIs in patients with early-stage NSCLC.

Contributors

WHL, JXH and MJM conceived the idea for the study. YJ, YCL, WHF, QHH, HRL, RZ, RC and BLL performed the research and collected data. YJ, YCL, RZ, YKW, HTW, JFL, CCL and SX have verified the underlying data. YJ, YCL and WHF drafted the initial draft of the manuscript. All authors contributed to the design, data analysis, and results interpretations. The corresponding authors had full access to the data in

the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final version of the manuscript.

Data sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author on a reasonable request. The raw image and follow-up data are not publicly available because they contain sensitive information that could compromise patient privacy.

Declaration of interests

There are no conflicts of interest to declare.

Acknowledgements

The authors would like to thank all the oncologists who participated in this study. This study received funding from China National Science Foundation (82022048, 82373121).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102205.

References

1 Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17–48.
2 Chansky K, Detterbeck FC, Nicholson AG, et al. The IASLC lung cancer staging project: external validation of the revision of the TNM stage groupings in the eighth edition of the TNM classification of lung cancer. *J Thorac Oncol*. 2017;12(7):1109–1121.
3 Goldstraw P, Chansky K, Crowley J, et al. The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (Eighth) edition of the TNM classification for lung cancer. *J Thorac Oncol*. 2016;11(1):39–51.
4 Park JH, Lee C-T, Lee HW, Baek HJ, Zo JI, Shim YM. Postoperative adjuvant chemotherapy for stage I non-small cell lung cancer. *Eur J Cardiothorac Surg*. 2005;27(6):1086–1091.
5 Pignon J-P, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol*. 2008;26(21):3552–3559.
6 Kris MG, Gaspar LE, Chaft JE, et al. Adjuvant systemic therapy and adjuvant radiation therapy for stage I to IIIA completely resected non-small-cell lung cancers: American society of clinical oncology/cancer care ontario clinical practice guideline update. *J Clin Oncol*. 2017;35(25):2960–2974.
7 Arriagada R, Auperin A, Burdett S, et al. Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. *Lancet*. 2010;375(9722):1267–1277.
8 Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer*. 2007;7(3):169–181.
9 Sholl LM, Aisner DL, Varella-Garcia M, et al. Multi-institutional oncogenic driver mutation analysis in lung adenocarcinoma: the lung cancer mutation consortium experience. *J Thorac Oncol*. 2015;10(5):768–777.
10 Cheng H, Li X-J, Wang X-J, et al. A meta-analysis of adjuvant EGFR-TKIs for patients with resected non-small cell lung cancer. *Lung Cancer*. 2019;137:7–13.
11 Huang Q, Li J, Sun Y, Wang R, Cheng X, Chen H. Efficacy of EGFR tyrosine kinase inhibitors in the adjuvant treatment for operable non-small cell lung cancer by a meta-analysis. *Chest*. 2016;149(6):1384–1392.
12 Zhong W-Z, Wang Q, Mao W-M, et al. Gefitinib versus vinorelbine plus cisplatin as adjuvant treatment for stage II-IIIA (N1-N2) EGFR-mutant NSCLC: final overall survival analysis of CTONG1104 phase III trial. *J Clin Oncol*. 2021;39(7):713–722.
13 Kelly K, Altorki NK, Eberhardt WEE, et al. Adjuvant erlotinib versus placebo in patients with stage IB-IIIA non-small-cell lung cancer (RADIANT): a randomized, double-blind, phase III trial. *J Clin Oncol*. 2015;33(34):4007–4014.
14 Kratz JR, He J, Van Den Eeden SK, et al. A practical molecular assay to predict survival in resected non-squamous, non-small-cell lung cancer: development and international validation studies. *Lancet*. 2012;379(9818):823–832.
15 Woodard GA, Wang SX, Kratz JR, et al. Adjuvant chemotherapy guided by molecular profiling and improved outcomes in early stage, non-small-cell lung cancer. *Clin Lung Cancer*. 2018;19(1):58–64.
16 Kratz JR, Tham PT, Mulvihill MS, et al. Analytical validation of a practical molecular assay prognostic of survival in non-squamous non-small cell lung cancer. *Diagn Mol Pathol*. 2013;22(2):65–69.
17 Wu Y-L, Tsuboi M, He J, et al. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. *N Engl J Med*. 2020;383(18):1711–1723.
18 Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med*. 2005;352(25):2589–2597.
19 Douillard J-Y, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. *Lancet Oncol*. 2006;7(9):719–727.
20 Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon J-P, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med*. 2004;350(4):351–360.
21 Herbst RS, Wu Y-L, John T, et al. Adjuvant osimertinib for resected EGFR-mutated stage IB-IIIA non-small-cell lung cancer: updated results from the phase III randomized ADAURA trial. *J Clin Oncol*. 2023;41(10):1830–1840.
22 Tsuboi M, Herbst RS, John T, et al. Overall survival with osimertinib in resected EGFR-mutated NSCLC. *N Engl J Med*. 2023;389(2):137–147.
23 Ou W, Li N, Wang B-X, et al. Adjuvant icotinib versus observation in patients with completely resected EGFR-mutated stage IB NSCLC (GASTO1003, CORIN): a randomised, open-label, phase 2 trial. *eClinicalMedicine*. 2023;57:101839.
24 Shi P, Zhang S, Zhu L, et al. The third-generation EGFR inhibitor, osimertinib, promotes c-FLIP degradation, enhancing apoptosis including TRAIL-induced apoptosis in NSCLC cells with activating EGFR mutations. *Transl Oncol*. 2019;12(5):705–713.
25 Cross DAE, Ashton SE, Ghiorghiu S, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov*. 2014;4(9):1046–1061.
26 Li F, Zhao Y, Zhong R, et al. No benefit of chemotherapy in Osimertinib-treated postoperative non-small cell lung cancer patients. *Transl Lung Cancer Res*. 2021;10(8):3689–3693.
27 Yue D, Xu S, Wang Q, et al. Erlotinib versus vinorelbine plus cisplatin as adjuvant therapy in Chinese patients with stage IIIA EGFR mutation-positive non-small-cell lung cancer (EVAN): a randomised, open-label, phase 2 trial. *Lancet Respir Med*. 2018;6(11):863–873.
28 Pennell NA, Neal JW, Chaft JE, et al. SELECT: a phase II trial of adjuvant erlotinib in patients with resected epidermal growth factor receptor-mutant non-small-cell lung cancer. *J Clin Oncol*. 2019;37(2):97–104.
29 Zhong W-Z, Wang Q, Mao W-M, et al. Gefitinib versus vinorelbine plus cisplatin as adjuvant treatment for stage II-IIIA (N1-N2) EGFR-mutant NSCLC (ADJUVANT/CTONG1104): a randomised, open-label, phase 3 study. *Lancet Oncol*. 2018;19(1):139–148.
30 Tada H, Mitsudomi T, Misumi T, et al. Randomized phase III study of gefitinib versus cisplatin plus vinorelbine for patients with resected stage II-IIIA non-small-cell lung cancer with EGFR mutation (IMPACT). *J Clin Oncol*. 2022;40(3):231–241.
31 He J, Su C, Liang W, et al. Icotinib versus chemotherapy as adjuvant treatment for stage II-IIIA EGFR-mutant non-small-cell lung cancer (EVIDENCE): a randomised, open-label, phase 3 trial. *Lancet Respir Med*. 2021;9(9):1021–1029.
32 Tsuboi M, Wu Y-L, Grohe C, et al. LBA47 Osimertinib as adjuvant therapy in patients (pts) with resected EGFR-mutated (EGFRm) stage IB-IIIA non-small cell lung cancer (NSCLC): updated results from ADAURA. *Ann Oncol*. 2022;33:S1413–S1414.

33 Woodard GA, Gubens MA, Jahan TM, et al. Prognostic molecular assay might improve identification of patients at risk for recurrence in early-stage non-small-cell lung cancer. *Clin Lung Cancer*. 2014; 15(6):426–432.

34 Kratz JR, Van den Eeden SK, He J, Jablons DM, Mann MJ. A prognostic assay to identify patients at high risk of mortality despite small, node-negative lung tumors. *JAMA*. 2012;308(16): 1629–1631.

35 Al Bakir M, Huebner A, Martínez-Ruiz C, et al. The evolution of non-small cell lung cancer metastases in TRACERx. *Nature*. 2023;616(7957):534–542.

36 Chaudhuri AA, Chabon JJ, Lovejoy AF, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. *Cancer Discov*. 2017;7(12):1394–1403.

37 Zviran A, Schulman RC, Shah M, et al. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. *Nat Med*. 2020;26(7):1114–1124.

38 Chen K, Zhao H, Shi Y, et al. Perioperative dynamic changes in circulating tumor DNA in patients with lung cancer (DYNAMIC). *Clin Cancer Res*. 2019;25(23):7058–7067.

39 Abbosh C, Birkbak NJ, Wilson GA, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature*. 2017;545(7655):446–451.

40 Woodard GA, Kratz JR, Haro G, et al. Molecular risk stratification is independent of EGFR mutation status in identifying early-stage non-squamous non-small cell lung cancer patients at risk for recurrence and likely to benefit from adjuvant chemotherapy. *Clin Lung Cancer*. 2021;22(6):587–595.