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Original Article

The role of immunotherapy and chemotherapy combinations in TKI-resistant EGFR-mutant non-small cell lung cancer: insights from real-world evidence

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Abstract: Purpose: Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are the first-line treatment for advanced *EGFR*-mutant non-small cell lung cancer (NSCLC). Despite this, most patients experience tumor progression. The optimal immunotherapy (IO)-based strategy and its timing after EGFR-TKI failure remains under debate. Materials and methods: A retrospective analysis was performed to assess the outcomes for patients with *EGFR*-mutant NSCLC who were treated with either IO alone or in combination with chemotherapy (C/T) following disease progression. Data from January 2014 to December 2022 at Taipei Veterans General Hospital were reviewed. The Kaplan-Meier method was used to evaluate overall survival (OS) and time to treatment failure (TTF), while a Cox proportional hazards model evaluated the impact of clinical factors on survival. Results: This study enrolled 107 patients with advanced *EGFR*-mutant NSCLC, all of whom had previously been treated with first- to second-generation EGFR-TKIs. The IO alone group included 33 patients, while 74 patients were in the IO combined with chemotherapy (IO+C/T) group. The median number of prior treatment lines before immunotherapy was 2. The IO+C/T group demonstrated a trend toward longer OS compared to the IO alone group (OS: 20 vs. 16 months, P=0.70). Patients with more than four lines of treatment before IO-based therapy had significantly worse OS (6 vs. 29 months, P<0.001) and TTF (2 vs. 5, P=0.018) than those less than 4 lines of treatment. Multivariate analysis revealed that patients who had undergone more than 4 lines of treatment before IO-based therapy had poorer OS (HR 2.21, 95% CI 1.16-4.21, P=0.01) and TTF (HR 1.89, 95% CI 1.11-3.19, P=0.019) compared to those with fewer than 4 lines of treatment. The HRs for OS were 4.32 (95% CI 1.95-9.61, P<0.001) for patients with more than 4 lines of treatment and 2.05 (95% CI 1.04-4.05, P=0.038) for those with 2-4 lines of treatment, in comparison to patients who had 0-1 lines of treatment. Conclusion: This study highlights the potential benefits of early initiation of IO-based regimens in advanced *EGFR*-mutant NSCLC following EGFR-TKI failure. Combination therapy with chemotherapy showed a trend toward improved survival compared to IO monotherapy, although not statistically significant. Moreover, poorer outcomes associated with multiple prior treatments underscore the importance of timely implementation of IO-based strategies to optimize clinical benefit in this patient population.

Keywords: Epidermal growth factor receptor, non-small cell lung cancer, immunotherapy, tyrosine kinase inhibitors

Introduction

Lung cancer remains the leading cause of cancer incidence and mortality globally, with approximately 2.2 million new cases and 1.8 million deaths reported annually. Among these, non-small cell lung cancer (NSCLC) harboring mutations in the epidermal growth factor receptor (EGFR) gene accounts for 10-15% of cases in Caucasian populations and 30-50% in Asian populations [1]. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been established as the standard first-line treatment for EGFR-mutant advanced NSCLC, offering superior response rates and survival benefits compared to conventional chemotherapy [2]. However, despite the efficacy of osimertinib as the first-line treatment, resistance invariably develops, leading to disease progression after approximately 19 months of therapy

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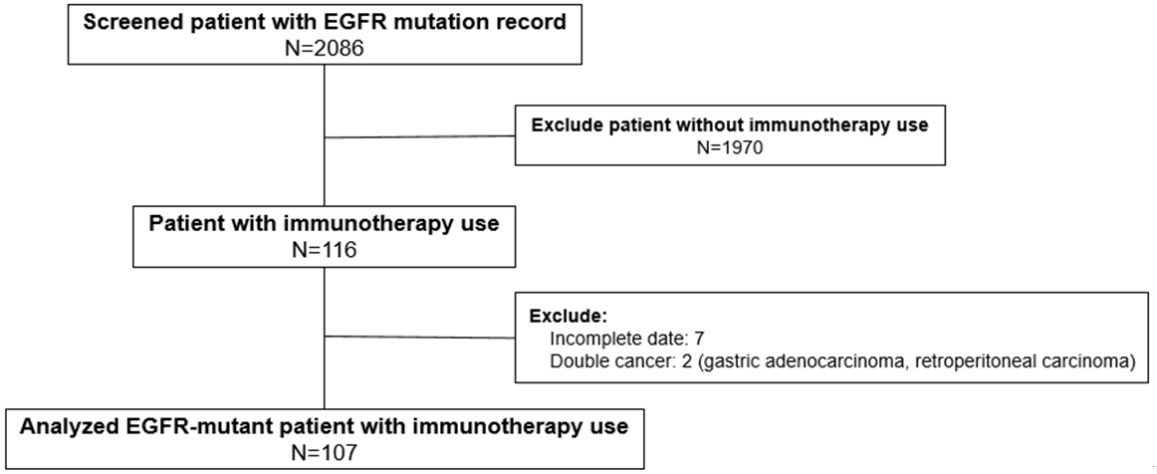

Figure 1. Flow chart of the study population. Flowchart illustrating the study population, including patient inclusion and exclusion criteria. Acronyms: EGFR, epidermal growth factor receptor.

[3]. Treatment options following progression remain limited in efficacy and include local therapies such as stereotactic ablative radiotherapy (SABR) or surgery, continuation of osimertinib beyond progression, the combination of amivantamab and chemotherapy, platinum-based doublet chemotherapy, and regimens incorporating atezolizumab, bevacizumab, and chemotherapy [2, 4].

Emerging evidence suggests that EGFR-TKI therapy can modulate the tumor immune microenvironment, including increased CD8 tumor-infiltrating lymphocyte density, elevated tumor mutation burden, and upregulation of programmed death-ligand 1 (PD-L1) expression on tumor cells [5, 6]. This finding suggests the possibility of immune checkpoint inhibitors (ICI)-based therapy for this population. While immunotherapy approaches such as single-agent immune checkpoint inhibitors (e.g., pembrolizumab, nivolumab, atezolizumab), dual regimens (e.g., ipilimumab plus nivolumab), and immunotherapy-chemotherapy combinations have shown promise in metastatic NSCLC, patients with *EGFR* mutations are often excluded from clinical trials and meta-analyses [7-11]. Results from IMpower150 and ORIENT31 trials support the use of ICI-based combination therapies in patients with *EGFR*-mutant metastatic NSCLC previously treated with EGFR-TKIs [8, 12]. However, recent phase 3 trials, including CheckMate-722 and KEYNOT-789, which evaluated immunotherapy in combination with chemotherapy versus chemotherapy alone, failed to demonstrate statistically significant benefits [13, 14].

Given the suboptimal outcomes of immunotherapy in *EGFR*-mutant metastatic NSCLC observed in prior studies, this study was designed to determine the optimal ICI-based treatment regimen and timing following EGFR-TKIs failure. To address this gap, we conducted a retrospective study to compare the efficacy and clinical outcomes of immunotherapy alone versus its combination with chemotherapy in patients with advanced *EGFR*-mutant NSCLC after disease progression.

Materials and methods

Patient cohort

The medical records of patients with advanced NSCLC who had received EGFR-TKIs at Taipei Veterans General Hospital in Taiwan between January 2014 and December 2022 were retrospectively reviewed to assess eligibility for enrollment (**Figure 1**). Relevant clinical information, including demographic characteristics and survival outcomes, was extracted for analysis. Lung cancer staging was determined according to the eighth edition of the tumor-node-metastasis (TNM) classification system for NSCLC [15]. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (IRB-TPEVGH No.: 2022-08-010BC; 2018-07-021C). Patients were eligible for inclusion if they met the following criteria: (1) histo-

logically confirmed stage IV NSCLC harboring *EGFR* mutation; and (2) receipt of EGFR-TKIs therapy followed by immunotherapy-based regimens due to disease progression. *EGFR* mutations included alterations occurring between exon 18 and exon 21. Mutation testing was performed as part of routine clinical practice using either polymerase chain reaction (PCR)-based assays or next-generation sequencing (NGS) platforms [16]. Exclusion criteria included patients with *EGFR* wild-type NSCLC, those who had never received EGFR-TKIs, or individuals with incomplete medical records or double cancers. The decision to prescribe immunotherapy-containing regimens was based on clinical judgment.

Study design

The clinical characteristics, including gender, smoking history, age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), *EGFR* mutation status, cancer staging, and lines of treatment were retrospectively reviewed. The enrolled patients were categorized into two groups: the IO (immunotherapy) group (IO alone group) and the IO + chemotherapy (C/T) group (immunotherapy combined with chemotherapy group). Immunotherapy included PD-1 and PD-L1 inhibitors, such as nivolumab, pembrolizumab, atezolizumab, and durvalumab. Overall survival (OS) was defined as the time from initiation of treatment to death or last follow-up. Time to treatment failure (TTF) was defined as the interval from treatment initiation to the date of treatment discontinuation due to any reason, including disease progression, drug toxicity, or death. Treatment responses were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Peripheral blood mononuclear cells (PBMCs) were isolated from patients to check lymphocyte subpopulation profiles. Immunophenotyping of lymphocyte subpopulations, such as regulatory T cells ($CD4^+CD25^+FoxP3^+$), cytotoxic T cells ($CD3^+CD8^+$), helper T cells ($CD3^+CD4^+$), exhausted T cells ($CD3^+CD8^+PD\text{-}1^+$, $TIM\text{-}3^+$, $1B11^+$, or $LAG\text{-}3^+$), anergic T cells ($CD3^+BTLA^+$), and monocytes ($HLA\text{-}DR^+$ $CD14^+$), was performed using flow cytometry [17-19].

Statistical analysis

Comparisons of baseline characteristics were conducted using Pearson's chi-square test or Fisher's exact test, as appropriate. Kaplan-Meier survival curves were used to analyze OS and TTF, while Cox proportional hazards regression and logistic regression were employed to assess clinical features and outcomes. A *p*-value of <0.05 was considered statistically significant, and all statistical analyses were performed using SPSS software (version 25.0, IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

This study included 107 patients with advanced *EGFR*-mutant NSCLC, all of whom had previously been treated with first- or second-generation EGFR-TKIs. The IO alone group included 33 patients, while 74 patients were in IO+C/T group. The median age was 58.1 years (range: 55.8-60.3 years). Most patients (80.4%) were never-smokers, and 41.1% were male. The majority (93.5%) had favorable performance status (ECOG 0-1). All patients harbored confirmed *EGFR* mutations, with exon 19 deletions identified in 47.7%, L858R mutations in 41.1%, and uncommon *EGFR* mutations in 11.2% of patients. PD-L1 testing was performed in 42.1% of patients. The median number of prior lines of therapy before initiation of immunotherapy was two. Notably, 61% of patients had received osimertinib prior to IO-based treatment. Baseline characteristics were generally balanced between the two groups, except for anti-vascular endothelial growth factor (anti-VEGF) antibody and T790M examination assessments, which were more frequently observed in the IO+C/T group (**Table 1**).

Treatment outcomes of immunotherapy and combination chemotherapy

The objective response rate (ORR) was 20% in both the IO and IO+C/T groups. However, the disease control rate (DCR) was higher in the IO+C/T group compared to the IO group (69% vs. 53%; Figure S1). Immunotherapy-related adverse events were observed in four patients and were limited to grade 1-2 toxicities, including pneumonitis and cutaneous rash. Patients receiving platinum-based chemotherapy exhibited a higher ORR compared to those treated with non-platinum-based regimens (27.9% vs. 7.1%, P=0.03). Similarly, pemetrexed-containing regimens were associated with a signifi-

Table 1. Baseline characteristics of patients with EGFR mutations (N=107)

	All patients (n=107)	IO (n=36)	IO+C/T (n=71)	*P* value
Gender				
Male	44 (41.1%)	11 (30.6%)	33 (46.5%)	0.11
Female	63 (58.9%)	25 (69.4%)	38 (53.5%)	
Median age	58.1 (55.8-60.3)	59.6 (55.6-63.6)	57.4 (54.6-60.2)	0.09
Smoking status				
Never smoker	86 (80.4%)	32 (88.9%)	54 (76.1%)	0.13
Ever smoker	21 (19.6%)	4 (11.1%)	17 (23.9%)	
Stage IV status				
Stage IVA	39 (36.4%)	12 (33.3%)	27 (38.0%)	0.63
Stage IVB	68 (63.6%)	24 (66.7%)	44 (62.0%)	
ECOG PS				
0-1	100 (93.5%)	34 (94.4%)	66 (93.0%)	1.00
≥2	7 (6.5%)	2 (5.6%)	5 (7.0%)	
Median of previous treatment lines	2 (1-4)	3 (1-4)	2 (1-4)	0.29
EGFR mutation				
Exon 19 deletion	51 (47.7%)	15 (41.7%)	36 (50.7%)	0.63
L858R	44 (41.1%)	16 (44.4%)	28 (39.4%)	
Uncommon	12 (11.2%)	5 (13.9%)	7 (9.9%)	
Brain metastasis				
No	71 (66.4%)	24 (66.7%)	47 (66.2%)	0.96
Yes	36 (33.6%)	12 (33.3%)	24 (33.8%)	
Liver metastasis				
No	89 (83.2%)	31 (86.1%)	58 (81.7%)	0.56
Yes	18 (16.8%)	5 (13.9%)	13 (18.3%)	
Bone metastasis				
No	57 (53.3%)	21 (58.3%)	36 (50.7%)	0.45
Yes	50 (46.7%)	15 (41.7%)	35 (49.3%)	
Histology				
Adenocarcinoma	96 (89.7%)	32 (88.9%)	64 (90.1%)	0.95
Squamous cell carcinoma	5 (4.7%)	2 (5.6%)	3 (4.2%)	
Others	6 (5.6%)	2 (5.6%)	4 (5.6%)	
PD-L1 level (%)				
<1%	23 (21.5%)	6 (16.7%)	17 (23.9%)	0.77
1-49%	17 (15.9%)	7 (19.4%)	10 (14.1%)	
≥50%	5 (4.7%)	2 (5.6%)	3 (42%)	
No examination	62 (57.9%)	21 (58.3%)	41 (57.7%)	
Osimertinib use before IO				
No	46 (43.0%)	19 (52.8%)	27 (38.0%)	0.14
Yes	61 (57.0%)	17 (47.2%)	44 (62.0%)	
With anti-VEGF antibody				
No	80 (74.8%)	33 (91.7%)	47 (66.2%)	0.004
Yes	27 (25.2%)	3 (8.3%)	24 (33.8%)	
T790M mutation[a]				
Without T790M	32 (47.8%)	15 (68.2%)	17 (37.8%)	0.019
With T790M	35 (52.2%)	7 (31.8%)	28 (62.2%)	

[a]Patient received further gene mutation examination (n=67). Acronyms: C/T, chemotherapy; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; IO, immunotherapy; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

Table 2. Treatment response for different chemotherapy regiment

	Objective response rate (ORR)	Disease control rate (DCR)
Platinum-based (n=43)	27.9% (12/43)	74.4% (32/43)
Without Platinum-based (n=28)	7.1% (2/43)	60.7% (17/28)
p value	0.03	0.22
Chemotherapy with pemetrexed (n=39)	33.3% (13/39)	79.5% (31/39)
Chemotherapy without pemetrexed (n=32)	3.1% (1/32)	56.3% (18/32)
p value	0.002	0.04
Platinum-Pemetrexed (n=31)	35.5% (11/31)	80.6% (25/31)
Without Platinum-Pemetrexed (n=40)	7.5% (3/40)	60.0% (24/40)
p value	0.006	0.06

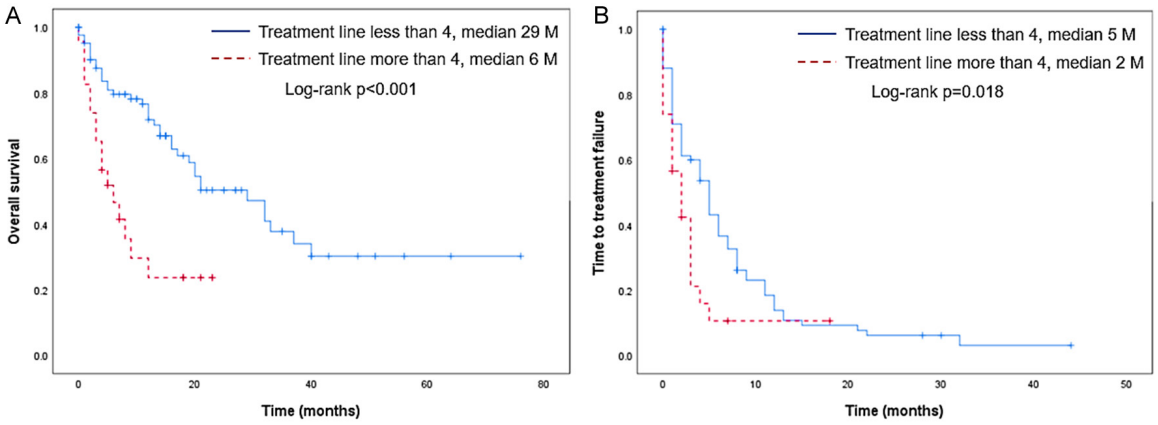

Figure 2. Kaplan-Meier curve of overall survival and time to treatment failure for patients with different previous treatment line. The image illustrates the impact of different previous treatment line on overall survival (OS) and time to treatment failure (TTF) in patients receiving ICI-based therapy (N=107). (A) Patients with more than 4 lines of treatment before ICI-based therapy had significantly poor overall all (OS) (6 vs. 29 months, P<0.001) and (B) time to treatment failure (TTF) (2 vs. 5, P=0.018) than those less than 4 lines of treatment.

cantly higher ORR compared to non-pemetrexed regimens (33.3% vs. 3.1%, P=0.002). Notable, patients receiving platinum-pemetrexed combinations demonstrated superior response rates compared to those treated with non-pemetrexed regimens (35.5% vs. 7.5%, P=0.006) (**Table 2**).

In terms of clinical outcomes, the IO+C/T group exhibited a trend toward longer OS (20 vs. 16 months, P=0.70) and TTF (4 vs. 2 months, P=0.46) compared to the IO group (Figure S2). Patients with more than 4 lines of treatment before ICI-based therapy had significantly poor OS (6 vs. 29 months, P<0.001) (**Figure 2A**) and TTF (2 vs. 5 months, P=0.018) than those less than 4 lines of treatment (**Figure 2B**). In detail, hazard ratios (HRs) for OS were 4.32 (95% confidence interval [CI] 1.95-9.61, P<0.001) for patients with more than 4 prior lines of therapy and 2.05 (95% CI 1.04-4.05, P=0.038) for those with 2-4 lines, compared to patients with 0-1 line of therapy. Similarly, HRs for TTF were 1.97 (95% CI 1.06-3.65, P=0.03) for patients with more than 4 lines of therapy and 1.11 (95% CI 0.69-1.79, P=0.67) for those with 2-4 lines, relative to those with 0-1 prior treatment line (Figure S3).

Association between clinical significance and treatment outcomes

Multivariate analysis revealed that patients undergoing more than 4 lines of treatment prior to IO-based therapy had shorter OS (HR 2.21, 95% CI 1.16-4.21, P=0.01) and TTF (HR 1.89, 95% CI 1.11-3.19, P=0.019) compared to those with fewer than 4 lines of treatment (**Table 3**). Additionally, patients with age over 65 were associated with poorer TTF (HR 1.59, 95% CI 1.00-2.54, P=0.049) (**Table 4**).

Table 3. Cox regression of factors related to overall survival (N=107)

Predictive variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Female	0.79 (0.47-1.36)	0.41		
Age over 65	0.92 (0.49-1.76)	0.81		
Smoking history	1.01 (0.52-1.97)	0.97		
ECOG PS ≥2	1.25 (0.45-3.50)	0.67		
PD-L1 level ≥1%	1.28 (0.55-2.96)	0.57		
Brain metastasis	1.13 (0.63-2.00)	0.68		
Liver metastasis	1.09 (0.53-2.23)	0.81		
Bone metastasis	0.62 (0.36-1.07)	0.08	0.74 (0.42-1.30)	0.29
Previous treatment line 0 and 1	0.39 (0.21-0.75)	0.005	0.53 (0.26-1.05)	0.07
Previous treatment line 2-4	1.13 (0.66-1.94)	0.65		
Previous treatment line >4	2.92 (1.58-5.39)	0.001	2.21 (1.16-4.21)	0.01
With chemotherapy	0.89 (0.51-1.58)	0.71		
Third generation TKI	1.10 (0.64-1.89)	0.72		
With anti-VEGF antibody	0.65 (0.32-1.28)	0.21		
EGFR exon 19 deletion	1.23 (0.72-2.12)	0.45		
EGFR L858R mutation	0.87 (0.49-1.53)	0.64		
T790M mutation	0.86 (0.43-1.72)	0.67		
iRAE	0.68 (0.09-4.96)	0.71		

Acronyms: ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; iRAE, immunotherapy-related adverse events; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

Table 4. Cox regression of factors related to time to treatment failure (N=107)

Predictive variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Female	0.79 (0.52-1.21)	0.28		
Age over 65	1.46 (0.92-2.31)	0.1	1.59 (1.00-2.54)	0.049
Smoking history	1.12 (0.69-1.96)	0.57		
ECOG PS ≥2	1.20 (0.51-2.83)	0.67		
PD-L1 level ≥1%	1.05 (0.56-1.97)	0.88		
Brain meta	1.09 (0.70-1.69)	0.7		
Liver meta	0.89 (0.49-1.66)	0.73		
Bone meta	0.78 (0.52-1.18)	0.24		
Previous treatment line 0 and 1	0.79 (0.51-1.24)	0.31		
Previous treatment line 2-4	0.90 (0.59-1.36)	0.63		
Previous treatment line >4	1.77 (1.05-2.99)	0.03	1.89 (1.11-3.19)	0.019
With chemotherapy	0.86 (0.56-1.33)	0.5		
Third generation TKI	1.01 (0.67-1.54)	0.95		
Anti-VEGF	0.66 (0.41-1.07)	0.09	0.63 (0.38-1.03)	0.065
EGFR exon 19 del	1.12 (0.74-1.69)	0.59		
EGFR L858R	0.83 (0.54-1.26)	0.37		
T790M mutation	0.80 (0.47-1.36)	0.41		
iRAE	0.53 (0.13-2.17)	0.38		

Acronyms: ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; iRAE, immunotherapy-related adverse events; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

Among patients harboring *EGFR* L858R mutations, those with more than four lines of prior therapy had significantly shorter TTF in both univariate (HR 2.91, 95% CI 1.19-7.09, P=0.02)

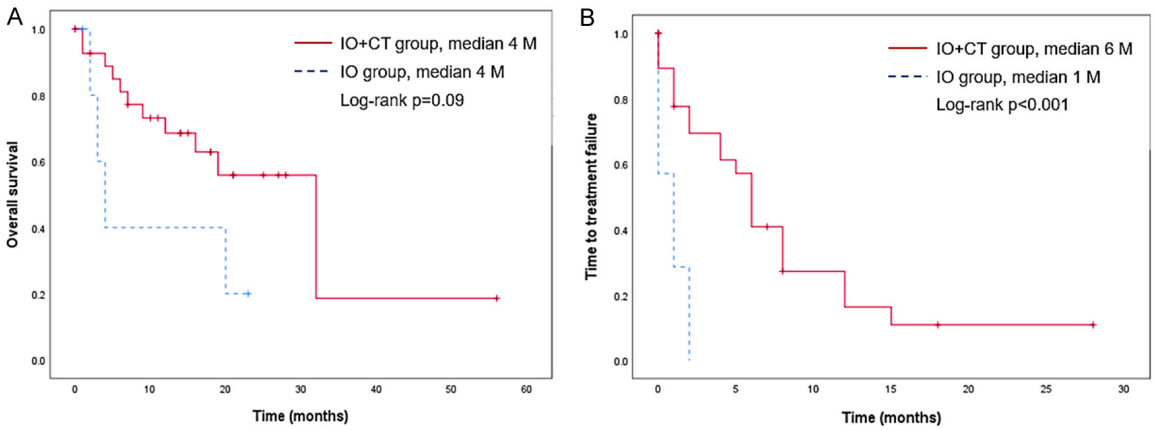

Figure 3. Kaplan-Meier curve of overall survival and time to treatment failure for patients with T790M mutation. The image illustrates the impact of immunotherapy (IO) alone versus immunotherapy (IO) + chemotherapy (C/T) on overall survival (OS) and time to treatment failure (TTF) in patients with T790M mutation (N=35). A. In patients with the T790M mutation, a trend toward improved (OS was observed in the IO+C/T group compared with IO alone group (32 vs. 4 months, P=0.09), but the difference did not reach statistical significance. B. The IO+C/T group demonstrated significantly longer TTF compared to IO alone group (6 vs. 1 months, P<0.001).

and multivariate analysis (adjusted HR 2.58, 95% CI 1.04-6.43, P=0.042), suggesting that extensive prior treatment is an independent negative prognostic factor (Table S1). Similarly, in patients with EGFR exon 19 deletion, multivariate analysis identified both extensive prior therapy (HR 2.99, 95% CI 1.25-7.13, P=0.014) and a history of smoking (HR 2.84, 95% CI 1.22-6.63, P=0.016) as independent predictors of shorter OS (Table S2).

Furthermore, the use of anti-VEGF antibodies was associated with a trend toward reduced risk of treatment failure, although this did not reach statistical significance in the multivariate analysis (HR 0.63, 95% CI 0.38-1.03, P=0.065) (**Table 4**). Other clinical variables, including sex, performance status, and distant metastasis site, were not associated with either OS or TTF in the multivariate analysis.

T790M mutation and treatment response

Among the 107 patients included in the study, *EGFR* mutation status was re-assessed in 67 individuals prior to the initiation of immunotherapy. Of these, 35 patients were found to harbor the T790M mutation, while the remaining 32 patients tested negative for T790M. In the subgroup of patients with the T790M mutation, those who received immunotherapy and chemotherapy demonstrated a trend toward prolonged OS compared to those treated with immunotherapy alone (32 vs. 4 months, P=0.09); however, the difference did not reach statistical significance (**Figure 3A**). Notably, the IO+C/T group exhibited a significantly longer TTF compared to the IO alone group (6 vs. 1 months, P<0.001) (**Figure 3B**). These findings suggest that the presence of the T790M mutation may influence treatment responses and underscore the potential clinical benefit of combination therapy strategies in this molecularly defined patient subgroup.

Case-based analysis of peripheral lymphocyte profiles in EGFR-mutant NSCLC

Peripheral lymphocyte subpopulation profiles of three *EGFR*-mutant NSCLC patients treated with ICI-based therapy were analyzed using flow cytometry (**Figure 4**). Case I received nivolumab and experienced progressive disease (PD). This patient exhibited moderate levels of circulating $CD4^+$ and $CD8^+$ T cells but showed the highest levels of $1B11^+CD8^+$ T cells (exhausted T cells), and $BTLA^+CD8^+$ T cells (anergic T cells), indicating a profoundly immunosuppressive or exhausted phenotype consistent with disease progression (PD). Case II, treated with pembrolizumab combined with vinorelbine, achieved a partial response (PR). Among these three cases, Case II had the highest levels of $CD4^+$ T cells (helper T cells), $CD4^+CD25^+Foxp3^+$ T cells (regulatory T cells), and $CD8^+$ T cells (cytotoxic T cells), while

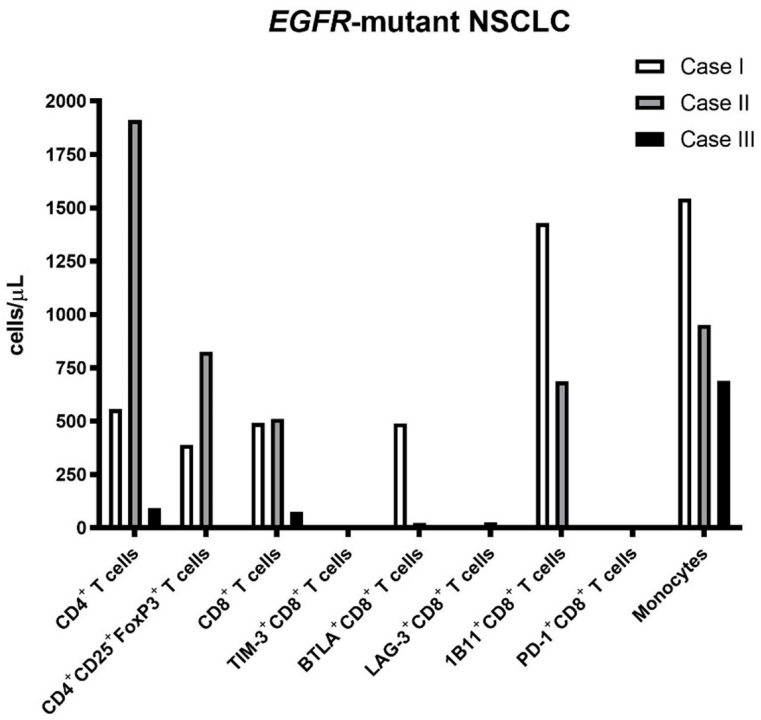

Figure 4. Peripheral immune cell subsets in EGFR-mutant NSCLC patients. The image illustrates peripheral immune cell subsets were quantified in three patients with EGFR-mutant non-small cell lung cancer (NSCLC). Cell populations were measured by flow cytometry and include $CD4^+$ T cells (helper T cells), $CD3^+CD25^+FoxP3^+$ T cells (regulatory T cells), $CD8^+$ T cells (cytotoxic T cells), and various $CD8^+$ T cell subsets expressing immune checkpoint markers. The cell counts per microliter (cells/μL) of the lymphocyte subpopulations for each case are presented.

$PD\text{-}1^+CD8^+$ T cell was undetectable. Case III, who received nivolumab plus paclitaxel and achieved stable disease (SD), exhibited relatively low $CD4^+$ and $CD8^+$ T cell levels. However, inhibitory checkpoint-expressing $CD8^+$ T cells, including $TIM\text{-}3^+$, $BTLA^+$, $LAG\text{-}3^+$, $1B11^+$, and $PD\text{-}1^+$ subsets, were virtually undetectable, possibly reflecting a modest yet balanced immune state sufficient to maintain disease control. Notably, PD-1 expression on T cells was nearly absent across all three cases. This may reflect the limited activation or exhaustion of circulating T cells in *EGFR*-mutant NSCLC, which is known to exhibit a non-inflamed immune phenotype [20]. In summary, PD was potentially associated with elevated levels of exhausted and anergic T cells, PR with robust and diversified T cell activity, and SD with a relatively low but possibly regulated immune profile. These findings suggest that the qualitative composition and distribution of immune cell subsets, rather than PD-1 expression alone, may serve as a potentially useful predictor of therapeutic response in *EGFR*-mutant NSCLC.

Discussion

This study presents real-world clinical outcomes of patients with *EGFR*-mutant NSCLC treated with immunotherapy-based regimens following disease progression on EGFR-TKIs. Although a trend toward improved OS and PFS was observed in patients receiving immunotherapy combined with chemotherapy compared to those treated with immune checkpoint inhibitor monotherapy, these differences did not reach statistical significance. These findings are consistent with recent large-scale phase 3 clinical trials, including CheckMate-722 and KEYNOTE-789, which similarly failed to demonstrate a survival benefit for immunotherapy plus chemotherapy in TKI-refractory *EGFR*-mutant NSCLC [13, 14]. However, within our cohort, patients receiving platinum-based chemotherapy exhibited significantly higher ORR compared with those treated with non-platinum-based regimens. Furthermore, platinum-based chemotherapy combination with pemetrexed was associated with superior ORR compared to non-pemetrexed regimens. Notably, patients who had received more than four prior lines of treatment before initiating IO-based therapy had significantly worse OS and TTF than those with fewer prior treatments, suggesting that earlier initiation of IO-based strategies may confer a survival benefit.

In contrast to our results, previous studies have reported more favorable outcomes for immunotherapy-chemotherapy combinations in this population [8, 12, 21]. Several factors may explain these discrepancies, including differences in study populations, geographic and demographic variability, *EGFR* mutation subtypes, and prior exposure to third-generation

EGFR-TKIs such as osimertinib. Additionally, *EGFR*-mutant NSCLC is typically characterized by an "immune-cold" tumor microenvironment, marked by low tumor immunogenicity, reduced tumor mutation burden (TMB), and increased infiltration by immunosuppressive cells, such as regulatory T cells, myeloid-derived suppressor cells, and tumor associated macrophages [5, 22, 23]. These factors contribute to a diminished antitumor immune response and may undermine the efficacy of ICI monotherapy in this context. Moreover, EGFR-TKIs may modulate the tumor microenvironment and affect PD-L1 expression, potentially influencing subsequent response to immunotherapy. Although immune checkpoint inhibitors have demonstrated efficacy in NSCLC overall, their performance in *EGFR*-mutant populations remains limited [20]. For instance, the CheckMate-012 trial reported lower response rates and shorter PFS for nivolumab in *EGFR*-mutant patients compared to *EGFR* wild-type counterparts (ORR: 14% vs. 30%, median PFS: 1.8 vs. 6.6 months) [24]. Similarly, in the KEYNOTE-001 study, pembrolizumab was less effective in patients with *EGFR* mutations, with shorter median OS compared to *EGFR* wild-type NSCLC (median OS: 6.0 vs. 11.9 months) [25]. In the OAK trial, atezolizumab improved survival in the overall population, but this benefit did not extend to patients with *EGFR* mutations (10.5 vs. 16.2 months) [26]. Meta-analyses of several pivotal trials (CheckMate-057, POPLAR, KEYNOTE-010 and OAK) also conclude that PD-l/PD-L1 inhibitors failed to improve OS relative to chemotherapy in the second-line setting for *EGFR*-mutant NSCLC [27, 28]. Collectively, these findings emphasize the urgent need for optimized immunotherapy-based treatment strategies in advanced *EGFR*-mutant NSCLC. Our multivariate analysis reinforces this, demonstrating that receiving more than four lines of treatment prior to IO-based therapy independently predicted poorer OS and TTF. There was no previous study focus on optimal timing for immunotherapy-based treatment in TKI-refractory *EGFR*-mutant NSCLC [5, 29, 30]. Our results suggest that earlier administration of immunotherapy-based regimens may significantly reduce the risk of death. Additionally, the superior ORR observed in patients treated with platinum-pemetrexed combinations supports the potential benefit of early initiation of such regimens in improving patient outcomes.

Mutations in the *EGFR* gene predominantly occur in exons 18-21, with exon 19 deletions (19del) and the L858R point mutation in exon 21 accounting for approximately 85% of all *EGFR* alterations. However, the relationship between these common mutations and PD-L1 expression remains unclear. Preclinical evidence suggests that 19del and L858R mutations may upregulate PD-L1 expression through activation of the p-ERK1/2 and c-Jun signaling pathways; paradoxically, clinical studies have reported lower PD-L1 positivity in tumors harboring these mutations compared to other less common *EGFR* variants [31]. Patients with uncommon mutations, including G719X, p.L861Q, S768I, and exon 20 insertions, have demonstrated higher PD-L1 expression than those with 19del or L858R mutations [32]. In our study, multivariate analysis revealed that patients with *EGFR* L858R mutations who had received more than four lines of prior therapy experienced significantly shorter TTF. Similarly, among patients with exon 19 deletions, those with more than four lines of treatment before initiating immunotherapy-based therapy had shorter OS. These findings suggest that extensive prior treatment is an independent predictor of worse outcomes in these molecular subgroups.

Tyrosine kinase inhibitor (TKI) therapy has been shown to modulate the immune system in *EGFR*-mutant NSCLC, with reports indicating altered immune cell infiltration following the development of TKI resistance. This may reflect an enhanced immune response characterized by increased infiltration of effector immune cells [33].

Nonetheless, different resistance mechanisms may variably impact the immune microenvironment. For instance, PD-L1 expression has been reported to be higher in T790M-negative tumors compared to T790M-positive cases after progression on EGFR-TKIs, potentially due to the activation of alternative oncogenic signaling pathways, which may enhance sensitivity to immune checkpoint inhibitors [34]. Conversely, the *EGFR* T790M mutation has been shown to activate the STAT3 pathway through elevated interleukin-6 (IL-6) levels, thereby promoting the IL-6/JAK/STAT3 signaling cascade and contributing to immune resistance [35, 36]. Consistent with these

mechanistic insights, our findings indicate that T790M-positive patients treated with IO+C/T experienced significantly longer TTF and a trend toward improved OS compared to those receiving immunotherapy alone. These results support the notion that ICI monotherapy is less effective in *EGFR*-mutant NSCLC harboring the T790M mutation and underscore the potential therapeutic benefit of combination strategies in this patient subgroup.

The interplay between EGFR and VEGF signaling pathways plays a critical role in the pathogenesis and progression of NSCLC. EGFR activation promotes hypoxia-inducible factor 1-alpha (HIF1α) signaling independently of hypoxic conditions, leading to sustained VEGF-A expression [37]. Resistance to EGFR-targeted therapies has been associated with increased VEGF-A levels and enhanced activation of the VEGF receptor (VEGFR) signaling axis. In addition to its pro-angiogenic effects, VEGF-A contributes to immune suppression by impairing lymphocyte trafficking, inhibiting dendritic cell maturation, and recruiting immunosuppressive cell populations, including regulatory T cells and myeloid-derived suppressor cells [38, 39]. Several studies have demonstrated that dual inhibition of EGFR and VEGF-VEGFR pathways can enhance ORR and PFS [40, 41]. The IMpower150 trial reported that patients with *EGFR*-mutant NSCLC who progressed on prior EGFR-TKI therapy derived PFS benefit from a regimen combining chemotherapy, atezolizumab, and bevacizumab, underscoring the potential of VEGF blockade to enhance immune checkpoint inhibitor efficacy [8, 42]. In our analysis, the use of anti-VEGF antibodies in combination with IO-based therapy was associated with a trend toward reduced risk of treatment failure in the multivariate model. Recent developments in bispecific antibody therapies offer additional promise. Ivonescimab, a bispecific antibody targeting PD-1 and VEGFA, has shown encouraging efficacy in metastatic *EGFR*-mutant NSCLC following TKI failure. Its dual-binding mechanism enhances PD-1 affinity in the presence of VEGF, resulting in synergistic immunomodulatory effects [43]. The HARMONi-A trial demonstrated a significant improvement in PFS with ivonescimab, reporting a hazard ratio of 0.46 (95% CI 0.34-0.62, P<0.001), with 37.9% of patients remaining progression-free at nine months [44]. Thus, HARMONi-A reported comparable PFS benefits (HR 0.46, 6-month PFS 55.4%), suggesting ivonescimab, a PD-1/VEGFA antibody, as a promising treatment option. In general, *EGFR*-mutant NSCLC has a poor response to ICIs. Future research should prioritize developing combination therapies that integrate anti-angiogenesis agents, immunomodulatory cytokines, chemotherapy, and radiotherapy [20].

In our lymphocyte subpopulation analysis of EGFR-mutant NSCLC patients following immunotherapy revealed that the patient who achieved a partial response exhibited elevated levels of helper T cells, and cytotoxic T cells, while $PD\text{-}1^{+}CD8^{+}$ T cells were undetectable. This immune profile aligns with findings from previous studies. Circulating helper T cells have been identified as predictors of treatment response and prognosis in advanced NSCLC patients undergoing combination immunotherapy and chemotherapy [45]. Higher baseline levels of circulating cytotoxic T cells have also been associated with prolonged survival in advanced NSCLC patients treated with PD-1/PD-L1 inhibitors [46]. Furthermore, reductions in $PD\text{-}1^{+}CD8^{+}$ T cells have been correlated with greater clinical benefit and improved survival in patients receiving PD-1 inhibitors, highlighting a potentially valuable biomarker for identifying those most likely to benefit from PD-1 blockade [47, 48]. Another case in our study, referred to as Case I, exhibited moderate levels of circulating $CD4^{+}$ and $CD8^{+}$ T lymphocytes, but showed disproportionately high frequencies of $1B11^{+}CD8^{+}$ and $BTLA^{+}CD8^{+}$ T cells, which phenotypically correspond to exhausted and anergic cytotoxic T cells, respectively. This immune profile suggests a dysfunctional effector T cell compartment and an immunosuppressive systemic environment, which may underlie the patient's lack of clinical response and subsequent disease progression [49, 50].

Despite its strengths, our study has several limitations. First, its retrospective and single-center design may limit the generalizability of the findings, and potential selection bias cannot be excluded. Second, key molecular biomarkers, such as PD-L1 expression and T790M mutation status, were assessed in only a subset of patients. While subgroup analyses from the ATLANTIC trial have suggested that PD-L1 expression may predict ICI efficacy in *EGFR*-mutant NSCLC [51], other studies, such as a paired analysis of 1,586 patients with lung

adenocarcinoma, have indicated that *EGFR* mutations may impair the predictive value of PD-L1 [52]. Thus, the role of PD-L1 expression as a biomarker in this patient population remains uncertain. Third, the chemotherapy regimens employed in our cohort were heterogeneous, including single agents (e.g., pemetrexed, navelbine, or docetaxel) and doublet combinations (e.g., platinum plus pemetrexed or gemcitabine plus navelbine). This variability reflects the absence of a standardized treatment protocol for later-line therapies. Future prospective studies are warranted to establish optimal treatment strategies and to further investigate the utility of molecular biomarkers in guiding therapy selection for *EGFR*-mutant NSCLC.

Conclusion

The early incorporation of immunotherapy-based regimens, particularly in combination with chemotherapy, may enhance clinical outcomes in patients with *EGFR*-mutant NSCLC following progression on EGFR-TKI therapy. Delayed initiation or administration of multiple prior lines of treatment has been associated with inferior survival outcomes, highlighting the critical importance of timely therapeutic intervention.

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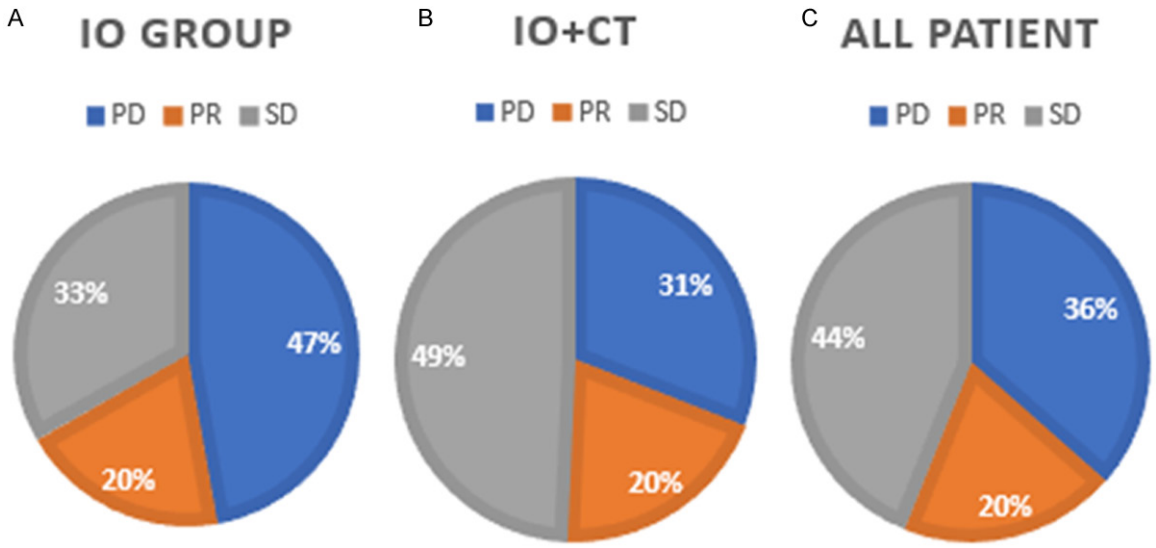

Figure S1. Treatment response to immunotherapy alone (A), immunotherapy combined with chemotherapy (B) and all patient (C). (A) Immunotherapy alone group (N=36): overall response rate (ORR): 20%, disease control rate (DCR): 53%. (B) Immunotherapy combined with chemotherapy group (N=71): ORR 20%, DCR 69%. (C) All patient (N=107): ORR: 20%, DCR: 64%. Acronyms: PD, progressive disease; PR: partial regression; SD, stable disease.

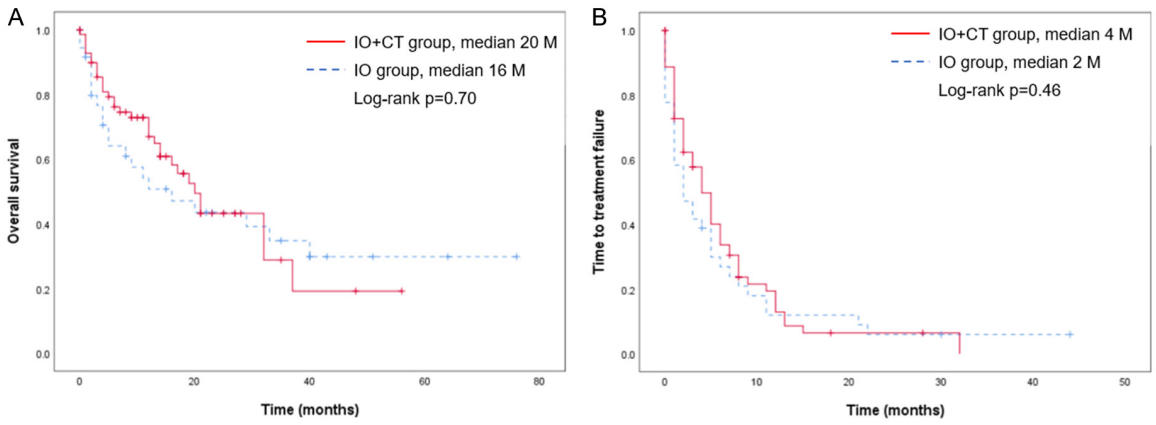

Figure S2. Kaplan-Meier curve of overall survival and time to treatment failure for all patients. The image illustrates the impact of immunotherapy (IO) alone versus immunotherapy (IO) + chemotherapy (C/T) on overall survival (OS) and time to treatment failure (TTF) in all patients (N=107). (A) The IO+C/T group exhibited a trend toward longer OS (20 vs. 16 months, P=0.70) and (B) TTF (4 vs. 2 months, P=0.46) compared to the IO group.

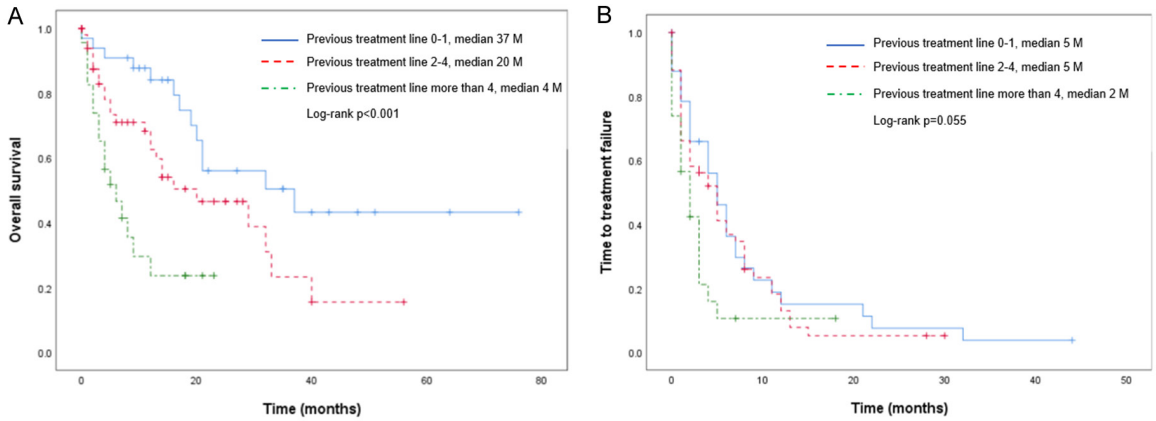

Figure S3. Kaplan-Meier curve of overall survival and time to treatment failure for different previous treatment lines. The image illustrates overall all survival (OS) and time to treatment failure (TTF) between different previous treatment lines in all patients (N=107). A. Hazard ratios for OS were 4.32 (95% confidence interval [CI] 1.95-9.61, P<0.001) for patients with more than 4 lines of treatment and 2.05 (95% CI 1.04-4.05, p=0.038) for those with 2-4 lines, compared to patients with 0-1 line of treatment. B. Similarly, HRs for TTF were 1.97 (95% CI 1.06-3.65, P=0.03) for patients with more than 4 lines of treatment and 1.11 (95% CI 0.69-1.79, P=0.67) for those with 2-4 lines, compared to patients with 0-1 line of treatment.

Table S1. Cox regression of factors related to TTF in EGFR L858R group (N=44)

Predictive variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Female	1.09 (0.56-2.12)	0.81		
Age over 65	1.09 (0.53-2.25)	0.82		
Smoking history	1.12 (0.48-2.59)	0.79		
ECOG ≥2	0.37 (0.05-2.71)	0.32		
PD-L1 ≥1%	1.36 (0.50-3.69)	0.54		
Brain meta	1.07 (0.54-2.13)	0.85		
Liver meta	1.31 (0.46-3.76)	0.62		
Bone meta	0.61 (0.31-1.19)	0.10	0.69 (0.35-1.38)	0.29
T790M mutation	0.70 (0.30-1.63)	0.41		
Previous treatment line 0 and 1	0.70 (0.35-1.40)	0.31		
Previous treatment line 2-4	0.89 (0.47-1.72)	0.75		
Previous treatment line >4	2.91 (1.19-7.09)	0.02	2.58 (1.04-6.43)	0.042
With chemotherapy	0.77 (0.39-1.49)	0.43		
Third generation TKI	0.91 (0.48-1.74)	0.77		
Anti-VEGF	0.64 (0.30-1.32)	0.22		

Acronyms: ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

Table S2. Cox regression of factors related to OS in EGFR exon 19 del group (N=51)

Predictive variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Female	0.57 (0.28-1.16)	0.12		
Age over 65	0.74 (0.30-1.81)	0.51		
Smoking history	2.16 (0.99-4.71)	0.053	2.84 (1.22-6.63)	0.016
ECOG ≥2	1.02 (0.24-4.34)	0.98		
PD-L1 ≥1%	0.63 (0.17-2.34)	0.49		
Brain meta	0.72 (0.32-1.62)	0.43		
Liver meta	1.06 (0.40-2.78)	0.91		
Bone meta	0.87 (0.42-1.79)	0.71		
Previous treatment line 0 and 1	0.49 (0.22-1.11)	0.08	0.51 (0.19-1.33)	0.17
Previous treatment line 2-4	0.78 (0.38-1.61)	0.50		
Previous treatment line >4	3.71 (1.67-8.26)	0.001	2.99 (1.25-7.13)	0.014
With chemotherapy	1.30 (0.59-2.86)	0.51		
Third generation TKI	1.49 (0.68-3.28)	0.31		
Anti-VEGF	0.61 (0.23-1.60)	0.32		

Acronyms: ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.