



Association of *EGFR* Variant Allele Frequency with Survival Outcomes in Metastatic Lung Adenocarcinoma

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ABSTRACT

Objective: Variant allele frequency (VAF) from tissue-based next-generation sequencing (NGS) can approximate mutational burden, yet its interpretation is sensitive to tumor cellularity.

Material and Methods: We examined whether a tumor-cellularity-adjusted epidermal growth factor receptor (EGFR) VAF (aVAF) at baseline was associated with outcomes in metastatic *EGFR*-mutant lung adenocarcinoma treated with EGFR-tyrosine kinase inhibitors.

Results: Forty-five patients were included; exon 19 deletions accounted for 58% of *EGFR* alterations, and exon 21 L858R substitutions accounted for 33%. When stratified by aVAF, patients with higher values (>64.4%) experienced more favorable outcomes than those with lower values. Median progression-free survival was 31.0 months in the high-aVAF group versus 8.6 months in the low-aVAF group. Median overall survival showed a similar separation (47.6 vs. 14.2 months). The objective response rate (78.3%) and the disease control rate (100%) were also markedly higher in patients with elevated aVAF. In multivariable Cox regression analyses, aVAF remained an independent predictor of both PFS and OS. In addition to aVAF, higher Charlson Comorbidity Index scores, poor performance status (Eastern Cooperative Oncology Group ≥ 2), non-exon 19 *EGFR* mutation subtypes, and elevated baseline carcinoembryonic antigen levels were associated with inferior survival outcomes.

Conclusion: Biologically, higher aVAF values may reflect early truncal *EGFR* mutations, indicating greater oncogenic dependence on *EGFR* signaling. Overall, these findings suggest that baseline *EGFR* aVAF is a clinically meaningful and accessible prognostic biomarker that may improve risk stratification and outcome prediction in metastatic *EGFR*-mutant lung adenocarcinoma.

Keywords: *EGFR*-mutant non-small cell lung cancer; variant allele frequency; adjusted variant allele frequency; tyrosine kinase inhibitors; prognostic biomarkers; next-generation sequencing; tumor heterogeneity

INTRODUCTION

Lung cancer continues to be a major global health burden, with the highest incidence and mortality of all malignancies. Non-small cell lung cancer (NSCLC) constitutes roughly 85% of cases, and most patients present with advanced disease at diagnosis.¹ Within lung adenocarcinoma, activating alterations in the epidermal growth factor receptor (EGFR) are among the most common actionable drivers, occurring in about one-fifth to one-quarter of tumors, although reported frequencies vary substantially across regions and populations.^{2,3} *EGFR*-tyrosine

kinase inhibitors (TKIs) have reshaped first-line treatment for advanced *EGFR*-mutant NSCLC. In routine practice, they offer higher response rates and longer survival than conventional chemotherapy, while generally being better tolerated.^{2,4,5} However, clinical responses to *EGFR*-targeted therapies remain highly variable. A substantial subset of patients derives little or no benefit from *EGFR*-TKIs from the outset, while many others experience an initial response followed by disease progression, most commonly within 8 to 18 months of treatment initiation.⁶⁻⁹

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Performance status, metastatic burden (oligometastatic versus more disseminated cancer), baseline carcinoembryonic antigen (CEA), and PET/CT-derived metabolic activity (primary tumor SUV_{max}) are among the variables that have been connected to outcomes in *EGFR*-mutant NSCLC.^{6,10} Concurrent changes in tumor suppressor genes, such as *TP53*, *RB1* or *PTEN*, have been associated with earlier resistance and poorer survival, proving that molecular context is also important.¹¹⁻¹³ Nevertheless, the range of progression-free survival (PFS) and overall survival (OS) observed with *EGFR*-TKIs is not entirely explained by these characteristics. The fact that tissue-based biomarkers rely on both the material and tumor biology presents a practical hurdle.¹⁴

One of the additional promising prognostic factor might be variant allele frequency (VAF).¹⁵⁻¹⁹ VAF represents the proportion of sequencing reads carrying a given mutation and can serve as a proxy for mutational burden and clonal representation. Interpreting the results of next-generation sequencing (NGS) is impacted by the considerable variation in tumor cellularity among specimens.¹⁵ Comparisons across patients are more difficult since the tissue VAF is sensitive to tumor cellularity, copy-number variations, and the actual fraction of mutation-bearing cells. By adjusting the reported VAF to the neoplastic cell fraction, a adjusted *EGFR* VAF (aVAF) has been proposed as a way to lessen this variability.¹⁵ From a conceptual standpoint, a larger aVAF could indicate a more truncal early event and possibly a stronger reliance on *EGFR* signaling. *EGFR*-mutant risk classification could be improved with this quantitative method, which could go beyond the straightforward “mutation present/absent” paradigm.¹⁵

On this basis, we hypothesized that baseline-aVAF is associated with clinical outcomes in metastatic *EGFR*-mutant lung adenocarcinoma treated with *EGFR*-TKIs. The primary objective of this study was to evaluate the association of aVAF with PFS and OS. Secondary objectives included assessing the relationship between aVAF and objective response rate (ORR), disease control rate (DCR), co-mutation profiles, and established clinical prognostic factors. Additionally, we explored whether baseline aVAF and other molecular or clinical characteristics were associated with the development of acquired resistance following *EGFR*-TKI therapy, including the emergence of the *EGFR* T790M mutation.

MATERIAL AND METHODS

Study Design and Patient Population

This retrospective observational study included patients who were diagnosed with metastatic lung adenocarcinoma harboring activating *EGFR* mutations and who were treated and followed at a tertiary academic oncology center.

Patients were eligible if they met the following criteria: (i) histologically or cytologically confirmed metastatic non-small cell lung cancer of the adenocarcinoma subtype; (ii) documented sensitizing *EGFR* mutations (including exon 19 deletions, L858R, and other less common activating variants); (iii) receipt of at least one line of *EGFR* TKI therapy; and (iv) availability of molecular pathology data allowing assessment of VAF. Patients with missing key clinical data, insufficient tumor material for molecular analysis, or unclear primary tumor origin were excluded. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ankara University Human Researchs Ethics Committee (approval no: I03-359-26, date: 07.04.2026).

Demographic and clinical variables were retrieved from electronic medical records. Data collected included age at diagnosis, sex, Eastern Cooperative Oncology Group (ECOG) performance status, Charlson Comorbidity Index, smoking status, number of metastatic organ sites, presence of brain metastases, baseline serum CEA, *EGFR* mutation subtype, and *EGFR*-TKI generation (first/second vs. third). Treatment course, radiologic response assessments, and survival outcomes were reviewed in a standardized manner.

Pathological Assessment and Molecular Analysis

Tumor material was obtained from diagnostic procedures performed in routine clinical practice. Specimens included cytological smears and cell blocks derived from endobronchial ultrasound-guided transbronchial needle aspiration or transthoracic fine-needle aspiration biopsy, as well as formalin-fixed paraffin-embedded tissue from bronchoscopic or surgical biopsies and from resection specimens. DNA extraction was carried out using commercially available kits in accordance with the manufacturer's instructions. DNA concentration was measured using fluorometric quantification (Qubit). NGS was performed with targeted multigene panels (Qiagen-based and related platforms) designed to capture common driver alterations and resistance-associated genes. The tested gene set included, among others, *AKT1*, *ALK*, *BRAF*, *TP53*, *DDR2*, *EGFR*, *ERBB2/HER2*, *ESR1*, *FGFR1*, *KIT*, *KRAS*, *MAP2K1*, *MET*, *MYD88*, *NRAS*, *NTRK1*, *PDGFRA*, *PIK3CA*, *PTEN*, *RICTOR*, and *ROS1*. Because testing occurred over different time periods and depended on tissue adequacy, the exact panel composition could vary between cases, reflecting real-world conditions.

To reduce the impact of variable tumor cellularity on VAF interpretation, an aVAF was calculated by normalizing the reported baseline *EGFR* VAF to the neoplastic cell fraction estimated in the corresponding pathology report.

aVAF formula has shown below:

$\text{aVAF}(\%) = \text{reported baseline EGFR VAF}(\%) / (\text{tumor cellularity}(\%) / 100)$

Baseline co-occurring alterations were recorded. For patients who experienced disease progression during *EGFR*-TKI treatment and underwent repeat tissue sampling, secondary resistance alterations—particularly *EGFR* T790M—were documented when available.

Outcome Definitions

ORR was defined as the proportion of patients achieving complete response or partial response (PR). DCR included CR, PR, or stable disease. PFS was calculated from *EGFR*-TKI initiation to radiologic progression or death from any cause, whichever occurred first. OS was defined as the time from treatment initiation to death from any cause.

Statistical Analysis

Baseline characteristics were summarized using descriptive statistics. Survival distributions were estimated with the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards models were used to explore associations with PFS and OS. Variables considered clinically relevant or that showed significance in univariable testing were entered into multivariable models. Effect estimates were reported as hazard ratios (HRs) with 95% confidence intervals (CIs). Analyses were conducted using IBM SPSS Statistics, version 23.0 (IBM Corp., NY, USA). A two-sided *p*-value <0.05 was considered statistically significant.

RESULTS

A total of 45 patients with metastatic non-small-cell lung adenocarcinoma harboring *EGFR* mutations who received *EGFR*-TKI were included in the study. At baseline, the median age of the study cohort was 70 years (range, 45-84), and two-thirds of the patients were female. Most individuals had preserved functional status, with 82% having ECOG performance status 0-1, and 18% presenting with ECOG ≥ 2 . Intracranial involvement was documented in one-third of cases, while disease had spread to more than two organs in 18%. Liver metastases were detected in 16% of the patients. By definition, all participants had metastatic disease, which corresponds to a minimum Charlson Comorbidity Index of 6. Regarding systemic therapy, the vast majority of patients (96%) received first- or second-generation *EGFR*-TKIs, and only two patients (4%) received a third-generation agent. Approximately one-third had previously undergone chemotherapy or radiotherapy. The distribution of *EGFR* alterations showed that exon 19 deletions were the most common (58%), followed by L858R substitutions (33%); 9%

carried other sensitizing variants. Median baseline CEA was 11.7 ng/mL (range, 0.65 to >1000 ng/mL), and two-thirds of patients had values >5 ng/mL. The median aVAF was 64.4% [interquartile range (IQR): 37.5-110], and co-mutations affecting *TP53*, *MET*, or the PI3K pathway were observed in 44% of the cohort (Table 1).

At baseline, the distribution of *EGFR* aVAF showed substantial variability across the cohort. The median aVAF was 64.4%, with an IQR of 37.5-110.0% and a range of 6.5-720% (Figure 1).

When patients were stratified according to aVAF (median cut-off), no significant differences were observed in baseline demographic characteristics. Baseline CEA levels were comparable (median 16.8 vs. 11.7 ng/mL; *p*=0.84), although a higher proportion of patients with elevated CEA ≥ 5 ng/mL was observed in the high aVAF group (78% vs. 55%; *p*=0.12). By

TABLE 1: Baseline demographic, clinical, and molecular characteristics of patients with metastatic *EGFR*-mutant lung adenocarcinoma.

Characteristic	n (%) or median (range)
Demographics	
Age, years	Median 70 (range 45-84)
Sex (male/female)	15 (33.3%) male/30 (66.7%) female
Clinical features	
ECOG performance status 0-1/ ≥ 2	37 (82.2%)/8 (17.8%)
Brain metastases (yes/no)	15 (33.3%)/30 (66.7%)
Number of metastatic organs <2/ ≥ 2	37 (82.2%)/8 (17.8%)
Liver metastases (yes/no)	7 (15.6%)/38 (84.4%)
Charlson Comorbidity Index	Median 6 (all ≥ 6 , metastatic disease)
Treatment	
<i>EGFR</i> -TKI generation (1 st /2 nd vs. 3 rd)	43 (95.6%)/2 (4.4%)
Prior chemotherapy or radiotherapy	15 (33.3%)
Molecular features	
<i>EGFR</i> mutation type: exon 19 del/L858R/other	26 (57.8%)/15 (33.3%)/4 (8.9%)
CEA at baseline, ng/mL	Median 11.7 (range 0.65->1000)
CEA ≥ 5 ng/mL	30 (66.7%)
Adjusted <i>EGFR</i> VAF, %	Median 64.4 (IQR: 37.5-110)
High adjusted <i>EGFR</i> VAF (>median, 64.4%)	23 (51.1%)
Comutation (any <i>TP53</i> , <i>MET</i> , or PI3K pathway)	20 (44.4%)

ECOG: Eastern Cooperative Oncology Group; *EGFR*: Epidermal growth factor receptor; TKI: Tyrosine kinase inhibitor; CEA: Carcinoembryonic antigen; VAF: Variant allele frequency; IQR: Interquartile range; *TP53*: Tumor protein p53; *MET*: MET proto-oncogene, receptor tyrosine kinase; PI3K: Phosphatidylinositol 3-kinase.

definition, adjusted VAF values differed significantly between categories (medians: 37.5% vs. 110%; $p<0.01$). Importantly, co-mutations involving *TP53*, *MET*, or PI3K pathway genes were substantially more common in patients with high aVAF (65% vs. 23%; $p<0.01$) (Table 2).

Exon 19 deletions were the most frequent (58%), followed by L858R substitutions in exon 21(33%), and other sensitizing mutations (9%) (Supplementary Figure 1). Among patients with different EGFR mutation subtypes, those harboring exon 19 deletions demonstrated a numerically longer median PFS (15.7 months) and median OS (21.8 months) compared

TABLE 2: Baseline clinical characteristics according to adjusted EGFR VAF category.

Characteristic	Low aVAF ($\leq$ median, n=22)	High aVAF ($>$ median, n=23)	p-value
Demographics			
Age, years	68.5 (45-84)	70 (48-80)	0.83
Sex (male/female)	8 (36.4%)/14 (63.6%)	7 (30.4%)/16 (69.6%)	0.76
Clinical features			
ECOG PS 0-1/ ≥ 2	17 (77.3%)/5 (22.7%)	20 (87.0%)/3 (13.0%)	0.46
Brain metastases (yes/no)	7 (31.8%)/15 (68.2%)	8 (34.8%)/15 (65.2%)	1.00
Number of metastatic organs ≤ 2 / > 2	18 (81.8%)/4 (18.2%)	21 (91.3%)/2 (8.7%)	0.41
Liver metastases (yes/no)	18 (81.8%)/4 (18.2%)	20 (87%)/3 (13%)	0.70
Charlson Comorbidity Index	Median 6 (range 6-7)	Median 6 (range 6-7)	1.0
Treatment			
EGFR-TKI generation (1 st /2 nd vs. 3 rd)	22 (100%)	21 (91.3%) / 2 (8.7%)	0.49
Prior chemotherapy or radiotherapy	5 (22.7%)	10 (43.5%)	0.21
Molecular features			
EGFR mutation type (ex19del/L858R/other)	14 (63.6%)/6 (27.3%)/2 (9.1%)	12 (52.2%)/9 (39.1%)/2 (8.7%)	0.69
CEA at baseline, ng/mL	16.8 (0.8->1000)	11.7 (0.65-645)	0.84
CEA ≥ 5 ng/mL	12 (54.5%)	18 (78.3%)	0.12
Adjusted EGFR VAF, %	37.5 (6.5-60.0)	110.0 (64.4-720.0)	<0.01
Comutation (<i>TP53</i> , <i>MET</i> , PI3K)	5 (22.7%)	15 (65.2%)	<0.01

aVAF: Adjusted variant allele frequency; EGFR: Epidermal growth factor receptor; ECOG PS: Eastern Cooperative Oncology Group performance status; TKI: Tyrosine kinase inhibitor; CEA: Carcinoembryonic antigen; *TP53*: Tumor protein p53; *MET*: MET proto-oncogene, receptor tyrosine kinase; PI3K: Phosphatidylinositol 3-kinase.

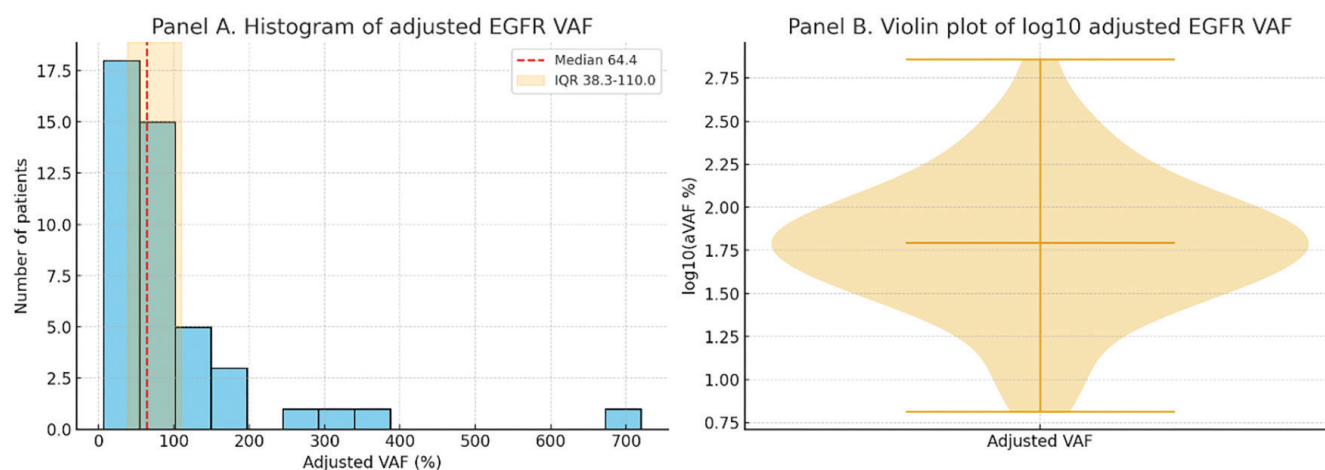


FIGURE 1: Distribution of adjusted EGFR variant allele frequency (aVAF) at baseline. Panel A shows the histogram of aVAF values with the median (red dashed line) and IQR (shaded area) indicated. Panel B displays the violin plot of log-transformed aVAF values, illustrating the overall spread and density of the data. Adjusted VAF was derived by normalizing the raw VAF to the estimated percentage of neoplastic cells (aVAF=VAF/TC% $\times$ 100).

TC%: Tumor cell percentage; IQR: Interquartile range; EGFR: Epidermal growth factor receptor

with patients carrying L858R substitution in exon 21 (12.8 and 13.1 months, respectively). Overall, 44.4% of patients carried at least one additional alteration besides EGFR. The most frequent co-mutation involved *TP53* (15.6%), followed by *MET* amplification (11.1%) and *PIK3CA* mutations (6.7%). In the OncoPrint plot, the majority of patients had no additional alterations, while a minority carried one or more co-mutations, most commonly in *TP53*. The bar plot highlights the relative contribution of each gene, with *TP53* predominating, while *MET* and *PIK3CA* events were less frequent (Figure 2).

Patients with higher adjusted VAF demonstrated significantly better treatment outcomes. ORR was 78.3% in the high aVAF

group compared with 45.5% in the low aVAF group ($p=0.033$). Similarly, DCR reached 100% in the high aVAF group compared with 72.7% in the low aVAF group ($p=0.009$). Patients with high aVAF had a markedly longer median PFS compared with those in the low aVAF group (median 31.0 months, 95% CI: 8.6-53.5 vs. 8.6 months, 95% CI: 6.9-14.9). The difference was statistically significant (log-rank $p=0.032$). Similarly, OS was prolonged in the high aVAF group compared with the low aVAF group (median 47.6 months, 95% CI: 24.9-70.3 vs. 14.2 months, 95% CI: 0-29.9). This difference also reached statistical significance (log-rank $p=0.025$) (Figure 3).

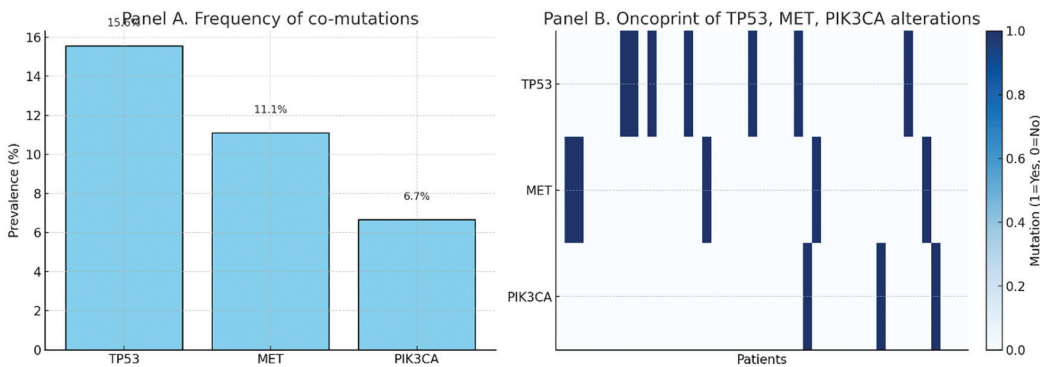


FIGURE 2: Frequency and distribution of co-mutations in the study cohort. The most common alteration was *TP53* (15.6%), followed by *MET* (11.1%) and *PIK3CA* (6.7%).

TP53: Tumor protein p53; *MET*: MET proto-oncogene, receptor tyrosine kinase

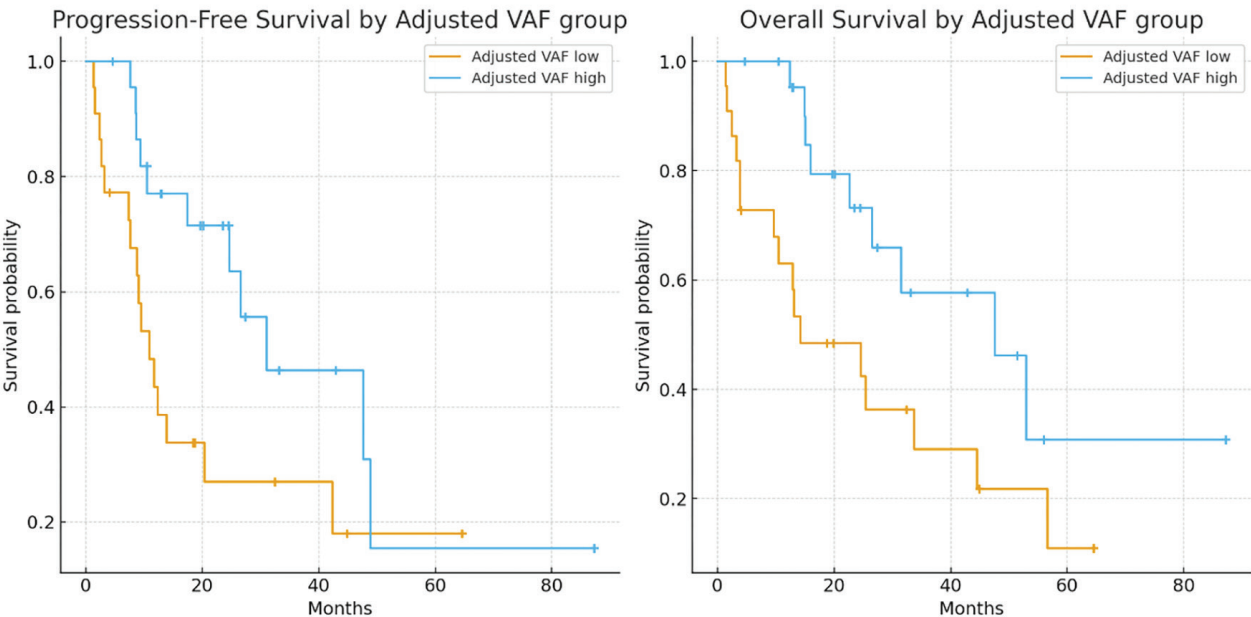


FIGURE 3: Kaplan-Meier curves for progression-free survival (left) and overall survival (right) according to adjusted EGFR variant allele frequency (aVAF) groups. Patients were dichotomized at the cohort median aVAF into low and high groups. Censored observations are indicated by plus symbols. aVAF was derived by normalizing the raw VAF to the estimated percentage of neoplastic cells ($aVAF = VAF/TC\% \times 100$).

TC%: Tumor cell percentage; *IQR*: Interquartile range; *EGFR*: Epidermal growth factor receptor

In univariable analyses, Charlson Comorbidity Index ≥ 7 , poor performance status, and adjusted VAF were significantly associated with both PFS and OS. Specifically, patients with higher comorbidity burden (HR: 3.39 for PFS; HR: 3.42 for OS) and ECOG ≥ 2 (HR: 4.00 for PFS; HR: 5.02 for OS) had worse outcomes. Conversely, high adjusted VAF was protective, being associated with a reduced risk of progression (HR: 0.43; $p=0.032$) and death (HR: 0.40; $p=0.025$). Other factors, including age, number of metastatic organs, TKI generation, *EGFR* mutation subtype, and baseline CEA, were not statistically significant predictors (Table 3).

In the multivariable Cox regression analysis, adjusted VAF remained a significant independent predictor of PFS. Patients in the high aVAF group had a significantly lower risk of progression (HR: 0.36; 95% CI: 0.16-0.80; $p=0.012$). The Charlson Comorbidity Index was independently associated with PFS, and higher CCI predicted worse outcome (HR: 3.54; 95% CI: 1.41-8.93; $p=0.007$). The CEA group lost statistical significance in the final model ($p=0.14$). Other variables,

including age, performance status, number of metastatic organs, and *EGFR* mutation subtype, were not retained as independent predictors after adjustment. In multivariate Cox analysis for OS, poor performance status (HR: 5.01; 95% CI: 1.65-15.25; $p=0.005$), non-ex19 *EGFR* mutation subtype (HR: 22.65; 95% CI: 3.29-155.78; $p=0.002$), and elevated baseline CEA (HR: 4.40; 95% CI: 1.28-15.11; $p=0.019$) emerged as independent predictors of worse outcome. Conversely, high adjusted VAF was significantly associated with a reduced risk of death (HR: 0.27; 95% CI: 0.10-0.74; $p=0.011$) (Table 4).

Among patients with available post-progression molecular testing ($n=18$), the acquired *EGFR* T790M mutation was detected in 7 patients, whereas 11 were T790M-negative. When aVAF, treated as a continuous variable, was compared between the two groups, the median aVAF was numerically higher in the T790M-positive group (median 75.8%; IQR: 56.0-192.5; range 24.0-305.0) than in the T790M-negative group (median 70.0%; IQR: 41.3-99.0; range 27.5-340.0). However, this difference did not reach statistical significance ($p=0.68$).

TABLE 3: Univariable Cox proportional hazards analyses for PFS and OS.

Variable	PFS HR (95% CI)	p-value	OS HR (95% CI)	p-value
Age (<65 vs. ≥ 65)	0.90 (0.41-1.94)	0.793	0.96 (0.43-2.13)	0.924
Charlson Comorbidity Index (≥ 7 vs. <7)	3.39 (1.41-8.13)	0.006	3.42 (1.39-8.44)	0.007
Performance status (0-1 vs. ≥ 2)	4.00 (1.59-10.07)	0.003	5.02 (1.83-13.73)	0.002
Brain metastasis (yes vs. no)	1.35 (0.61-2.98)	0.446	2.23 (0.99-5.01)	0.051
Number of metastatic organs (≤ 2 vs. >2)	1.37 (0.51-3.67)	0.529	2.25 (0.88-5.71)	0.087
TKI generation (1 st /2 nd vs. 3 rd)	0.51 (0.67-4.03)	0.530	0.68 (0.09-5.19)	0.715
<i>EGFR</i> mutation subtype (ex19 vs. L858R)	0.58 (0.23-1.49)	0.265	0.61 (0.22-1.67)	0.341
<i>EGFR</i> mutation subtype (ex19 vs. other)	0.95 (0.27-3.31)	0.942	1.32 (0.38-4.59)	0.656
Baseline CEA (<5 vs. ≥ 5 ng/mL)	1.57 (0.63-3.92)	0.330	1.59 (0.59-4.28)	0.356
Adjusted VAF (low vs. high)	0.43 (0.20-0.95)	0.032	0.40 (0.17-0.91)	0.025

VAF: Variant allele frequency; *EGFR*: Epidermal growth factor receptor; PFS: Progression-free survival; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval; TKI: Tyrosine kinase inhibitor; CEA: Carcinoembryonic antigen.

TABLE 4: Multivariable Cox proportional hazards analyses.

Variable	PFS HR (95% CI)	p-value	OS HR (95% CI)	p-value
Age (<65 vs. ≥ 65)	1.77 (0.66-4.77)	0.254	1.92 (0.66-5.63)	0.234
Charlson Comorbidity Index (>7 vs. ≤ 7)	3.54 (1.41-8.93)	0.007	1.43 (0.41-4.94)	0.572
Performance status (0-1 vs. ≥ 2)	2.47 (0.69-8.90)	0.166	5.01 (1.65-15.25)	0.005
Brain metastasis (yes vs. no)	0.86 (0.21-3.57)	0.838	1.51 (0.34-6.75)	0.584
Number of metastatic organs (≤ 2 vs. >2)	0.23 (0.05-1.05)	0.058	0.60 (0.12-2.83)	0.519
TKI generation (1 st /2 nd vs. 3 rd)	0.48 (0.14-1.66)	0.425	0.09 (0.01-0.58)	0.012
<i>EGFR</i> mutation subtype (ex19 vs. L858R/other)	7.02 (1.32-37.31)	0.068	22.65 (3.29-155.78)	0.002
Adjusted VAF (low vs. high)	0.36 (0.16-0.80)	0.012	0.27 (0.10-0.74)	0.011
Baseline CEA (<5 vs. ≥ 5 ng/mL)	2.09 (0.78-5.58)	0.140	4.40 (1.28-15.11)	0.019

VAF: Variant allele frequency; *EGFR*: Epidermal growth factor receptor; PFS: Progression-free survival; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval; TKI: Tyrosine kinase inhibitor; CEA: Carcinoembryonic antigen.

DISCUSSION

In this study, we evaluated the prognostic significance of baseline *EGFR* aVAF in patients with metastatic *EGFR*-mutant lung adenocarcinoma. Higher aVAF levels were associated with significantly prolonged PFS and OS, and aVAF remained an independent prognostic factor in multivariable Cox regression analyses. These findings suggest that aVAF is not merely a biological measurement but a clinically meaningful indicator for risk stratification in this patient population. Across the literature, the prognostic relevance of VAF in *EGFR*-mutant NSCLC appears to vary according to sample type (tissue versus plasma) and computational methodology.^{15-17,19,20} Early studies in patients with advanced lung adenocarcinoma harboring the *EGFR* L858R mutation suggested that mutant allele frequency could predict response to *EGFR*-TKI therapy. In the study by Ono et al.¹⁶, involving 29 patients with advanced L858R-mutant lung adenocarcinoma, the median VAF was approximately 18%, and a 9% cut-off was proposed to stratify clinical outcomes; patients with lower VAF derived less benefit from *EGFR*-TKIs, whereas higher VAF levels were associated with improved treatment response and outcomes. Similarly, Friedlaender et al.¹⁷ demonstrated in a cohort of 42 *EGFR*-mutant NSCLC patients that higher *EGFR* allelic frequency was significantly associated with prolonged PFS under targeted therapy (HR: 0.27; 95% CI: 0.09-0.79; $p=0.017$). Furthermore, in a multicenter study by Gieszer et al.¹⁵, conducted largely in the pre-osimertinib era and including 89 advanced *EGFR*-mutant NSCLC patients predominantly treated with erlotinib or gefitinib, high tumor tissue *EGFR*-aVAF ($\geq 70\%$) was associated with significantly longer PFS compared with low aVAF ($< 70\%$) (median PFS: 52 vs. 26 weeks; $p<0.001$). Consistent with our findings, high *EGFR*-aVAF was also linked to improved OS ($p=0.011$) and remained an independent predictor of PFS in multivariable analyses. Taken together, these data support the concept that quantitative assessment of *EGFR* mutational burden, beyond simple mutation presence or subtype, may refine prognostic stratification and help identify patient subgroups more likely to benefit from *EGFR*-TKI therapy.

The evidence on the prognostic value of *EGFR* VAF in *EGFR*-mutant NSCLC remains limited and inconsistent, and most reports have analyzed raw VAF values.^{16,18,21} A key challenge is that tissue-based NGS measurements are highly dependent on tumor cellularity, which reduces the comparability of VAF across specimens.²² Normalizing *EGFR* VAF to the estimated tumor cell fraction (aVAF) offers a practical means to reduce the influence of variable tumor cellularity and may provide a closer approximation of mutational burden in routine tissue specimens. In this study, aVAF was derived from tumor cellularity recorded in standard pathology reports, thereby enabling a more interpretable assessment of *EGFR* mutational

load under real-world conditions. From a biological standpoint, a higher aVAF could be consistent with an early (truncal) driver event and with a stronger dependency of the tumor on *EGFR* signaling. While this adjustment cannot replace deeper assessments of clonal structure or copy-number alterations, it represents an accessible metric that can be applied in everyday practice.

Several methodological points should also be considered when interpreting VAF-based findings. There is no widely accepted universal cutoff for VAF, and most studies—including ours—rely on cohort-specific medians or empirically selected thresholds, which limits comparability across datasets.²⁰ In addition, results may vary across centers because of differences in sequencing platforms (or PCR assays), limits of detection, bioinformatic pipelines, and panel composition.^{20,23}

When *EGFR* subtypes were evaluated within the cohort, patients with exon 19 deletions showed more favorable outcomes than those with exon 21 L858R, consistent with prior reports.^{6,24-26} We also explored whether concurrent alterations (e.g., *TP53* mutations, *MET*-axis alterations, PI3K pathway changes) influenced prognosis and whether baseline aVAF was associated with the subsequent detection of resistance mutations such as T790M. These exploratory analyses did not yield statistically significant associations, most likely reflecting limited sample size and substantial variability across small subgroups.

Study Limitations

Despite the clinical relevance of our observations, several limitations warrant emphasis. The cohort size was modest, reducing statistical power and limiting the ability to detect uncommon genomic events or small between-group differences. The retrospective design introduces the potential for selection bias, non-uniform response assessment, and incomplete documentation. In addition, the dataset was largely derived from a single center, which may constrain external generalizability. VAF analyses were primarily limited to baseline measurements, preventing the evaluation of dynamic changes during therapy and of their potential relationship with outcome. Finally, because most available evidence—including the present study—focuses on advanced-stage disease, the relevance of these findings to earlier-stage *EGFR*-mutant NSCLC remains uncertain.

CONCLUSION

In summary, our findings suggest that tissue-derived aVAF may serve as an independent prognostic biomarker in *EGFR*-mutant NSCLC. In metastatic *EGFR*-mutant lung adenocarcinoma, higher aVAF was associated with longer PFS and OS, with this association particularly evident among

patients treated with first-generation *EGFR*-TKIs. Because aVAF can be calculated from routinely reported pathology parameters, it may offer a practical prognostic tool in real-world settings. Prospective validation is needed, along with studies integrating tissue-based aVAF with liquid biopsy metrics and evaluating whether such quantitative biomarkers can support treatment-intensity decisions and therapeutic sequencing.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ankara University Human Researchs Ethics Committee (approval no: I03-359-26, date: 07.04.2026).

Informed Consent: Due to the retrospective nature of the study, the requirement for informed consent was waived.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.E., M.K., F.S.A., K.C., S.D.S., E.B.K., Concept: E.E., K.C., S.D.S., E.B.K., Design: E.E., K.C., S.D.S., E.B.K., Data Collection or Processing: E.E., M.K., F.S.A., K.C., S.D.S., E.B.K., Analysis or Interpretation: E.E., K.C., S.D.S., E.B.K., Literature Search: E.E., M.K., F.S.A., E.B.K., Writing: E.E., M.K., F.S.A., K.C., S.D.S., E.B.K.

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Supplementary Figure Link: <https://d2v96fxpocvxx.cloudfront.net/68ab204c-182b-49da-b227-bc7efe058632/content-images/6b7b5c08-2ee8-44e8-8575-2fa58176dac8.pdf>

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