scientific reports

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Presence of liver metastasis correlated with high tumor abundance and indicated adverse prognostic feature in *EGFR* mutation non-small-cell lung cancer patients

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EGFR-TKIs are effective therapies for non–small cell lung cancer (NSCLC) patients with *EGFR*-activating mutations. However, responses vary within individuals and resistant disease inevitably emerges. A prospective cohort of 130 patients with advanced *EGFR* mutation NSCLC were enrolled. Pre-and post-treatment plasma from subjects treated with EGFR-TKIs were obtained. The correlation between *EGFR* mutation abundance using the Idylla™ ct*EGFR* mutation assay, radiographic assessment, and clinical outcomes were analyzed. Eighty-nine patients with retrieved blood collection were analyzed. Undetectable ct*EGFR* (49.5%), detectable ct*EGFR* CqMut-high (23.5%), and detectable ct*EGFR* CqMut low (27%) using CqMut cutoff at 28.1, represented consecutive incremental tumor burden by radiographic assessment and outcome of treatment. Median PFS was 13.4 months [95% CI 12.0-14.8] in undetectable ct*EGFR*, 10.4 months [95% CI 9.9–10.9] in ct*EGFR* CqMut-high, and 5.9 months [95% CI 3.8–7.4] in ct*EGFR* CqMut-low. Number of metastasis sites > 3 was found in 22.7%, 23.8%, and 58.3% of the 3-tier ct*EGFR* tumor burden levels, respectively (*p*-value 0.01). Presence of liver metastasis was significantly correlated with number of metastasis sites > 3 and ct*EGFR* CqMut-low (45.8%). Liver metastasis was an independent factor of reduced PFS and OS by multivariate analysis with an HR = 2.41 [95% CI 1.27–4.60, *p*-value 0.007]) and HR = 2.96 [95% CI 1.35–6.51, *p*-value 0.007], respectively. The pretreatment ct*EGFR* detection using the Idylla™ ct*EGFR* mutation assay served as a surrogate marker for tumor abundance and tumor burden. Presence of liver metastasis was found to be a clinical predictor associated with high tumor abundance and worsening treatment outcomes.

Keywords Non-small cell lung cancer, Epidermal growth factor receptor mutation, Tyrosine kinase inhibitor, Circulating *EGFR* mutation testing

Abbreviations

EGFR	Epidermal growth factor receptor
TKI	Tyrosine kinase inhibitor

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Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are effective therapies for non–small cell lung cancer (NSCLC) patients with *EGFR*-activating mutations. However, responses vary within individuals with progression-free survival (PFS) ranging from a few months to several years and resistant disease inevitably emerges. Recent studies have shown that *EGFR* mutation-positive NSCLC is highly heterogeneous at the cellular level, facilitating the clonal expansion of resistant tumors via multiple molecular mechanisms[1,2]. Intratumor heterogeneity was correlated with poor prognostic outcomes in stage I-III resectable lung cancer[3]. Intratumor heterogeneity was also a characteristic of genomic complexity in more advanced diseases[4]. High tumor burden is also correlated with high intratumor heterogeneity[5,6]. The detectable pretreatment ct*EGFR*[7–12] and the presence of liver metastasis[13–15] had been reported as prognostic values. The relative abundance of *EGFR* mutations and tumor burden was also reported[16] .

As molecular genotyping has been traditionally performed using tissue biopsies that represent only a small sample from a single tumor site, it is thought not to reflect the actual intra-tumoral heterogeneity status of tumors. Liquid biopsy is a non-invasive method to identify somatic mutations from circulating tumors (ctDNA) for molecular profiling. Theoretically, it offers a real-time assessment of total-body molecular tumor genotypes, assuming all tumor deposits shed DNA into the bloodstream. Ease of sampling also allows frequent evaluation and monitoring of mutational load over time.

In this study, we will explore the association between tumor burden, semi-quantitative *EGFR*-mutant abundance using the Idylla™ ct*EGFR* mutation assay, and depth of response in patients with advanced NSCLC treated with EGFR-TKI. To fill the gap by exploring the *EGFR* abundance, the tumor burden and clinical factors might let the clinician define risk stratification and adopt precision medicine for individual patients.

Materials and methods

Study population

A prospective cohort of 130 patients who were diagnosed with recurrent or metastatic NSCLC and *EGFR*-activating mutation at The King Chulalongkorn Memorial Hospital (KCMH) over a period of 2 years (January 1, 2020, to December 31, 2021) was enrolled. All patient tumors harbored sensitizing *EGFR*-activating mutations. All patients had been treated with EGFR-TKIs (gefitinib, erlotinib, afatinib, and osimertinib). Pretreatment plasma from 89 patients were obtained (Figure S1). Clinicopathologic features including gender, age, Eastern Cooperative Oncology Group (ECOG) performance status (PS) is assessed on a scale from 0 to 5, with higher scores indicating greater levels of disability (0 indicates the individual is fully active and able to perform all pre-disease activities without restriction, 1 reflects some limitation in strenuous activity but the ability to perform light or sedentary work, 2 signifies the ability to perform self-care but not work, 3 indicates limited ability to perform self-care and confinement to a bed or chair for more than half of the day, 4 represents complete disability, with an inability to perform self-care and confinement to a bed or chair, and 5 indicates death), histological type, smoking status, *EGFR* mutation status, and type of EGFR-TKIs were retrieved. Tumor response and follow-up were assessed every two to three months as the standard protocol for lung cancer treatment. Objective response rate (ORR) and PFS were determined according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) and classified as progressive disease (PD), complete response (CR), partial response (PR), or stable disease (SD) by our investigators (TT, WC, II) who were blinded to patient treatment outcomes. All patients provided written informed consent. This study was approved by the Institutional Review Board of the Faculty of Medicine at Chulalongkorn University, Bangkok, Thailand (No. 894/63) and performed in accordance with the Health Insurance Portability and Accountability Act and the Declaration of Helsinki (as revised in 2013).

Quantitative circulating DNA assays and semi-quantitative plasma *EGFR* mutation assay

Blood samples were collected in EDTA containers and centrifuged at 1600 g for 10 min. The plasma was stored at − 80 °C until used. Two milliliters of plasma were extracted using DNA blood Mini Kit (QIAGEN) according to the manufacturer's protocol. Quantitative circulating DNA measurement was performed with primer and Taqman probe specific to reference gene *RPP30* using ABI PRISM® 7500 system (Applied Biosystems, Carlsbad, CA, USA) and standard curve as previous published[17]. Total amount of circulating DNA, measured by amplified *RPP30*, was calculated per 1 ml of plasma.

For semi-quantitative plasma *EGFR* mutations, two milliliters of plasma were loaded into a fully-automated Idylla™ ct*EGFR* Mutation Assay cartridge (Biocartis NV, Mechelen, Belgium) as per protocol recommendation. The cycle of quantification (Cq) was analyzed by the Idylla console software. The Idylla™ ct*EGFR* Mutation Assay covered 49 mutations of the *EGFR* gene (including G719X in exon 18, 36 deletions in exon 19, T790M/S768I in exon 20, 5 insertions in exon 20, and L858R/L861Q in exon 21). The control signal corresponded to the amplification of the *EGFR* wild-type. The predefined range of the difference between the *EGFR*-mutant Cq (CqMut) and sample processing control (SPC) Cq signal is defined as "mutation detected" results. Out of this range, a "no mutation detected" result is reported. Higher CqMut values indicate a lower mutation abundance[18].

Statistical analysis

Categorical variables were summarized by frequencies and percentages while continuous variables were reported by median and interquartile range (IQR). Variables include age, gender, ECOG PS, smoking status, histology, stage at diagnosis, presence of liver or brain metastasis, number of metastatic sites, *EGFR* mutation subtypes, EGFR TKI, and treatment outcome were analyzed using Chi-square or Fisher exact test for categorical data. The Mann-Whitney test was used for continuous data, as appropriate. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff value of CqMut to define high abundance vs. low abundance of plasma *EGFR* mutation. Progression-free survival (PFS) was calculated from the first day of treatment with EGFR TKI to disease progression or death from any cause, whichever occurred first. Overall

survival (OS) was calculated from the date of diagnosis of recurrent or metastatic disease until the date of death or last follow-up. Patients who did not develop the event (progression or death) at the end of the study were censored at the date of June 30, 2022. Comparative PFS and OS were analyzed by the Kaplan-Meier method and log-rank test. Multivariate analysis was performed by binary logistic regression or Cox's proportional hazards regression model, as appropriate. The level of statistical significance was determined as a *p-value* less than 0.05. All statistical analyses were conducted using SPSS 23.0 (SPSS Inc, Chicago, Illinois, USA) or GraphPad Prism 9.4 (GraphPad Software, San Diego, CA, USA).

Results

Patient characteristics

Eighty-nine *EGFR-mutated advanced-stage* NSCLC patients were included in the analysis. At the data cut-off, the median follow-up time was 19.1 months (95% confidence interval [CI], 18.1–20.0). Sixty-two patients (70%) had disease progression, and 39 patients (44%) had died. The median PFS and OS of the overall study cohort were 10.4 months (95% CI 7.1–13.8) and 21.6 months (95% CI NE-NE), respectively. Patient baseline characteristics are summarized in Table 1. The median age was 67 years (interquartile range [IQR] 59 to 74 years), with 65% women. Most patients were never smokers (74%) and had a 0–1 score of ECOG PS (85%). The majority had adenocarcinoma (93%), metastatic disease at presentation (87%), and 0–2 metastatic sites (66%). Baseline brain

Characteristics	All ($N=89$)	Undetectable ctEGFR ($N=44$)	Detectable ctEGFR CqMut-high ($N=21$)	Detectable ctEGFR CqMut-low ($N=24$)	*P*-values
Age at diagnosis, n (%)					
Median (IQR)	67 (59–74)	68 (60–75)	67 (58.5–72.5)	66 (56–72)	0.64
<60 years	24 (27%)	10 (22.7%)	7 (33.3%)	7 (29.2%)	
≥ 60 years	65 (73%)	34 (77.3%)	14 (66.7%)	17 (70.8%)	
Gender, n (%)					
Male	31 (34.8%)	13 (29.5%)	7 (33.3%)	11 (45.8%)	0.40
Female	58 (65.2%)	31 (70.5%)	14 (66.7%)	13 (54.2%)	
ECOG PS, n (%)					
0–1	76 (85.4%)	38 (86.4%)	20 (95.2%)	18 (75%)	0.15
≥ 2	13 (14.6%)	6 (13.6%)	1 (4.8%)	6 (25%)	
Smoking status, n (%)					
Never	66 (74.2%)	33 (75%)	14 (66.7%)	19 (79.2%)	0.62
Current/former	23 (25.8%)	11 (25%)	7 (33.3%)	5 (20.8%)	
Histology, n (%)					
Adenocarcinoma	83 (93.3%)	42 (95.5%)	20 (95.2%)	21 (87.5%)	0.42
NSCLC NOS	6 (6.7%)	2 (4.5%)	1 (4.8%)	3 (12.5%)	
Number of metastatic sites, n (%)					
<3 sites	59 (66.3%)	34 (77.3%)	15 (71.4%)	10 (41.7%)	**0.01***
≥ 3 sites	30 (33.7%)	10 (22.7%)	6 (28.6%)	14 (58.3%)	
Brain metastasis, n (%)	22 (24.7%)	10 (22.7%)	5 (23.8%)	7 (29.2%)	0.84
Liver metastasis, n (%)	22 (24.7%)	8 (18.2%)	3 (14.3%)	11 (45.8%)	**0.02***
EGFR mutation subtypes, n (%)					
Del19	53 (59.6%)	24 (54.5%)	15 (71.4%)	14 (60.9%)	0.68
L858R	30 (33.7%)	16 (36.4%)	5 (23.8%)	9 (37.5%)	
Uncommon[a]	6 (6.7%)	4 (9.1%)	1 (4.8%)	1 (4.2%)	
Line of EGFR TKIs, n (%)					
First line	85 (95.5%)	42 (95.5%)	20 (95.2%)	23 (95.8%)	0.99
Later line	4 (4.5%)	2 (4.5%)	1 (4.8%)	1 (4.2%)	
Type of EGFR TKIs, n (%)					
Gefitinib or Erlotinib	83 (93.3%)	40 (90.9%)	21 (100%)	22 (91.7%)	0.37
Afatinib	2 (2.2%)	1 (2.3%)	0	1 (4.2%)	
Osimertinib	4 (4.5%)	3 (6.8%)	0	1 (4.2%)	
Duration of TKI treatment, median (IQR, months)	10.5 (6.6–14.1)	12.8 (7.0-15.9)	11.0 (7.9–13.5)	6.8 (3.6–11.6)	0.48
ORR, n (%)	46 (67%)	17 (55%)	13 (81%)	16 (73%)	0.09

Table 1. Patient characteristics in the overall population and according to baseline plasma *EGFR* mutation status. [a]Uncommon *EGFR* mutations, including G719X in exon 18 ($n=2$), de novo T790M in exon 20 ($n=1$), L861G or Q in exon 21 ($n=2$) and complex mutation ($n=1$). Del19: exon 19 deletion, ECOG PS: Eastern Cooperative Oncology Group performance status, EGFR: epidermal growth factor receptor, IQR: interquartile range, ORR: overall response rate.

and liver metastases were present in 24.7% and 24.7% of the overall population, respectively. Our prospective cohort revealed a slightly higher prevalence of liver metastasis than the others, which revealed the prevalence of liver metastasis around 10–20%[13–15]. This might reflect the advancement at the time of diagnosis of Thai NSCLC patients. Regarding *EGFR* mutation status, 53 patients (60%) harbored exon 19 deletion, 30 patients (34%) harbored L858R, and six patients (6%) had uncommon mutations including G719X in exon 18 ($N=2$), de novo T790M in exon 20 ($N=1$), L861G or Q in exon 21 ($N=2$) and one patient had complex mutations including L861Q with G719X.

A total of 85 patients (96%) received EGFR TKIs as first-line treatment, and the remaining four patients (4%) were treated subsequently with chemotherapy. Gefitinib, erlotinib, afatinib, and osimertinib were administered in 29 (33%), 54 (61%), 2 (2%), and four patients (4%), respectively. The objective response rate (ORR) of EGFR TKIs in our cohort was 69.7%. The remaining were SD 13% ($n=12$), and PD 9% ($n=8$). The response was not available in 7 patients. At the data cutoff, 26 (29%) patients were still receiving treatment. The median duration of EGFR TKI treatment was 10.5 months (IQR 6.6 to 14.1 months).

Impact of quantitative circulating DNA and semi-quantitative ct*EGFR* detection

The median total amount of pretreatment circulating DNA (cirDNA) was 12 [range 0.9–144] ngml^{-1} of plasma. Using the Idylla™ ct*EGFR* mutation assay, 45 patients (50.5%) had detectable pretreatment plasma *EGFR* mutation (ct*EGFR*). Pretreatment cirDNA in detectable ctEGFR by the Idylla™ ct*EGFR* mutation assay was not significantly higher than undetectable ct*EGFR* (median 14.7 [range 1.7–85.9] and 9.7 [range 0.9–144.9] ngml^{-1}, respectively, p-value 0.16). There was a very modest correlation between cirDNA and the sum of target lesions (Spearman's $r=0.25$, p-value 0.04, Figure S2D). The total amount of cirDNA, which included tumor-derived and non-tumor-derived[19] might not correctly reflect tumor-derived genomic abundance.

Baseline characteristics of patients with undetectable vs. detectable ct*EGFR* were reported. However, detectable ct*EGFR* was further classified into low vs. high abundance (next session) (Table 1). Patients with a high number of metastatic sites (≥ 3 sites) were significantly associated with detected ct*EGFR* (44.4% vs. 22.7%). Presence of liver metastasis was also correlated with detected ct*EGFR* (31.1% vs. 18.2%). Seventy-one patients (80%) had measurable lesions at baseline. The sum of target lesions was not different between detectable and non-detectable ct*EGFR*, with a median of 30 [IQR 16–43] and 23 [IQR 9.5–38], p-value 0.3, respectively. There were no differences in the objective response rate by ct*EGFR* status at baseline. The ORR between undetectable and detectable ct*EGFR* was 64% and 71%, respectively (p-value 0.12). The association between depth of response and pretreatment ct*EGFR* status was explored. A waterfall plot revealed that the maximum percent change from baseline in the sum of the longest diameters of target lesions among the undetectable and detectable ct*EGFR* was similar (− 33% [IQR-51.6, -21.7] and − 41.9% [IQR − 52.9, -30.4], respectively) (Figure S3A).

At the data cut-off, patients with pretreatment undetectable ct*EGFR* mutation had a significantly longer PFS and a higher though non-significant difference in OS than patients with detectable ct*EGFR*. Median PFS was 13.4 months [95% CI 12.0-14.8], and 8.7 months [95% CI 6.7–10.7], respectively; p-value 0.03; Fig. 1A. Uncommon *EGFR* mutations and the presence of liver metastasis were independent prognostic factors for PFS in multivariate analysis (HR 7.71 [95% CI 2.97–19.99], p-value < 0.001 and HR 2.41 [95% CI 1.27–4.60], p-value 0.007, respectively) (Table 2). Median OS was shorter in detectable than undetectable ct*EGFR*; 14.6 months vs. not reached (NR) respectively [95% CI 10.9–18.3, p-value 0.009; Fig. 1B). Presence of liver metastasis, poor performance status (ECOG ≥ 2), and detectable ct*EGFR* were significant independent prognostic factors for OS with HRs of 2.96 [95% CI 1.35–6.51, p-value 0.007], 2.21 [95%CI 1.03–4.73, p-value 0.04] and 1.99 [95% CI 1-3.98, p-value 0.05], respectively) (Table 3).

Impact of semi-quantitative ct*EGFR* mutation and clinical outcomes to EGFR-TKIs

Based on a previous study demonstrating the concordance between the quantitative droplet digital PCR (ddPCR) *KRAS* mutation allele frequency (MAF) values and Idylla™ ct*KRAS* Mutation Assay CqMut values[18], we completed a semi-quantitative analysis of *EGFR* mutation by using the Cq of mutant *EGFR* values (CqMut) to compare high vs. low abundance. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cutoff value of CqMut. Using the cutoff value of 28.1, the sensitivity and specificity were 61.3% and 92.3%, respectively, with an AUC of 0.74 (95% CI 0.60–0.89).

Patients were divided into detectable ct*EGFR* CqMut-high (low abundance) and CqMut-low (high abundance) categories based on the cutoff value (≥ 28.1 or < 28.1). Baseline characteristics between the ct*EGFR* CqMut-high and ct*EGFR* CqMut-low are shown in Table 1. Patients with ct*EGFR* CqMut-high (low abundance) had a substantially lower number of metastatic sites (< 3 metastatic sites; 71.4%) and an absence of liver metastases (85.7%). There was no significant difference in age, gender, ECOG PS status, smoking status, histology, baseline brain metastasis, lines of treatment, and generation of EGFR TKI (1st, 2nd and 3rd) between the high and low abundance groups. The association between the depth of response and pretreatment ctEGFR CqMut status was also analyzed. A waterfall plot demonstrated that the maximum percent change from baseline in the sum of the longest diameters of target lesions was similar between the ctEGFR CqMut-high and ctEGFR CqMut-low groups (-34.4% [IQR: -48.9, -30.8] and − 48.4% [IQR: -58.3, -31.5], respectively) (Figure S3B).

Patients with ct*EGFR* CqMut-high revealed a higher though non-significance PFS (Table S1) and a statistically significant better OS than patients who had ct*EGFR* CqMut-low with a median PFS of 10.4 months [95% CI 9.9–10.9] and 5.9 months [95% CI 3.8–7.4], respectively; p-value 0.09 (Fig. 1C). Median OS was not reached (NR) in ct*EGFR* CqMut-high and 10.6 months [95% CI 8.3–12.9] in ct*EGFR* CqMut-low, p-value < 0.001 (Fig. 1D). The multivariate Cox proportional hazards regression model performed on the clinical prognostic factors revealed that ct*EGFR* CqMut values were significantly associated with OS (HR 3.20; 95% CI 1.22–8.39, p-value 0.02) (Table S2).

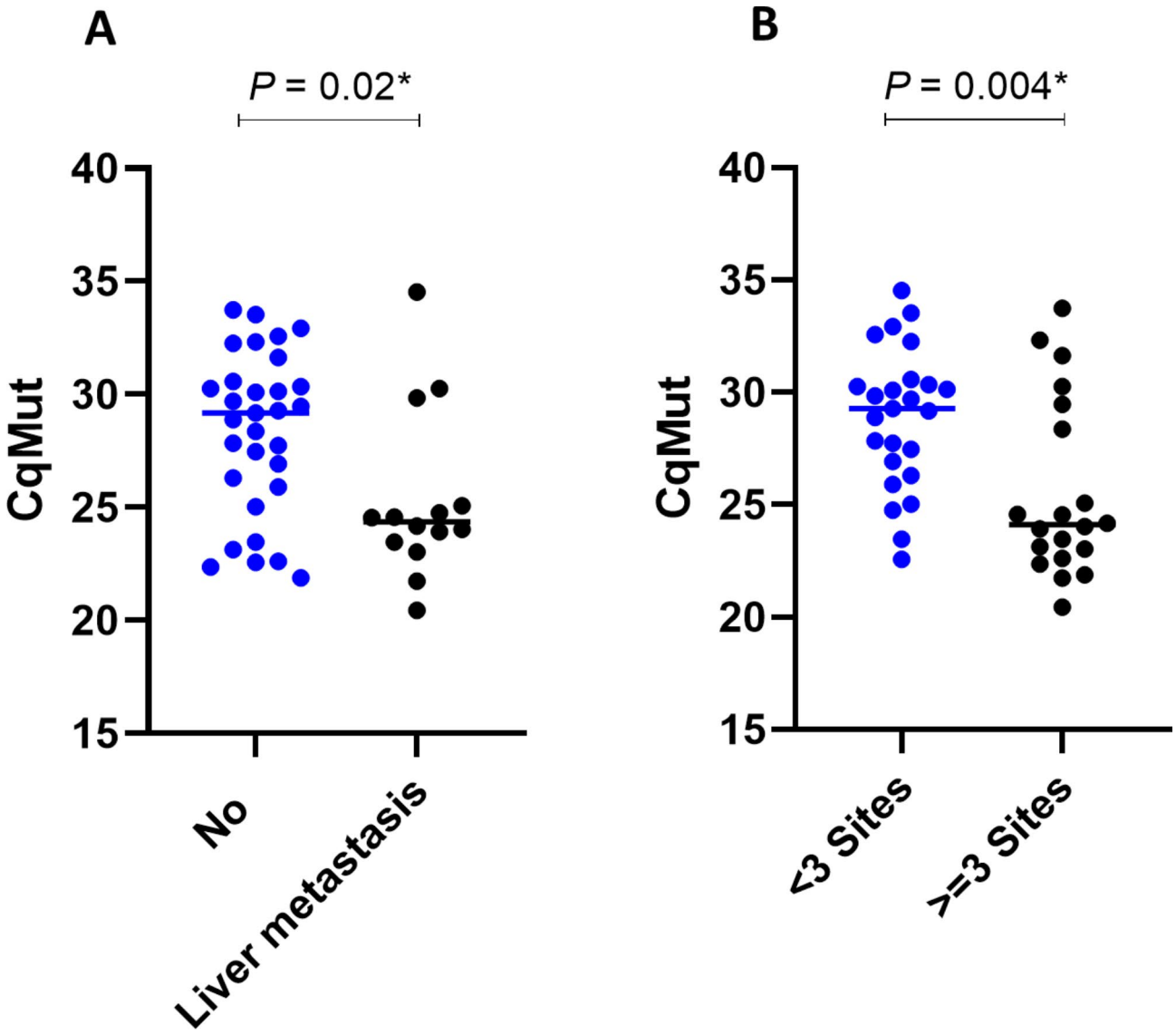

Fig. 1. Progression-free survival (PFS) and overall survival (OS) in patients with *EGFR*-mutant NSCLC according to detectable ct*EGFR* mutation status and Cq of mutant *EGFR* values at baseline. (**A**) PFS (13.4 months for undetectable ct*EGFR* mutation, and 8.7 months for detectable ct*EGFR* mutation; $p = 0.03$). (**B**) OS (not reached for undetectable ct*EGFR* mutation, and 14.6 months for detectable ct*EGFR* mutation; $p = 0.009$). (**C**) PFS (10.4 months for CqMut-high, and 5.9 months for CqMut-low; $p = 0.09$). (**D**) OS (not reached for CqMut-high, and 10.6 months for CqMut-low; $p < 0.001$). Statistical significance (*, $p < 0.05$).

Association between detectable ct*EGFR* mutation and tumor burden

We investigated whether pretreatment of ct*EGFR* CqMut values was associated with tumor burden in terms of the number of metastatic sites and the presence of liver metastases at the time of diagnosis. The patients with liver metastases or a higher number of metastatic sites (more than three sites) had significantly lower ct*EGFR* CqMut values (median of 24.3[IQR 23.5–24.9] and 24.1 [IQR 22.9–28.6], respectively) than those without liver metastases or < 3 number of metastatic sites (median of 29.1 [IQR 26.1–30.4] and 29.2 [IQR 26.9–30.3], *p*-value 0.02 and 0.004, respectively) (Fig. 2A-B). Correlations were found between ct*EGFR* CqMut and the total amount of cirDNA with the number of metastatic sites (Spearman's $r = -0.38$, *p*-value 0.01 and Spearman's r 0.32, *p*-value 0.004) (Figure S2A & C). However, the sum of the longest diameters of all target lesions that were measurable was not significantly different in patients who had low ct*EGFR* CqMut-low as compared to high ct*EGFR* CqMut-patients (85.23 mm vs. 66.81 mm, *p*-value 0.24). Collectively, these results suggested that patients with low ct*EGFR* CqMut values, indicating high tumor abundance, were associated with a high tumor burden, which may contribute to worse clinical outcomes.

Variables[a]	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (< 60/≥60)	1.16 (0.67–1.98)	0.60		
Gender (male/female)	1.03 (0.60–1.75)	0.92		
ECOG PS (≥ 2/0–1)	1.71 (0.89–3.29)	0.11		
Smoking (ever/never)	0.98 (0.55–1.76)	0.95		
Histology (non-ADC/ADC)	0.68 (0.21–2.17)	0.52		
Number of metastatic sites (≥ 3/<3)	2.15 (1.30–3.58)	**0.003***	1.73 (0.92–3.25)	0.09
Brain metastasis (yes/no)	1.31 (0.75–2.29)	0.35	0.88 (0.49–1.61)	0.69
Liver metastasis (yes/no)	2.79 (1.61–4.83)	**< 0.001***	2.41 (1.27–4.60)	**0.007***
EGFR subtype (uncommon/common)	4.09 (1.69–9.86)	**0.002***	7.71 (2.97–19.99)	**< 0.001***
Common *EGFR* subtype (Del19/L858R)	1.13 (0.64–1.98)	0.68		
Line of TKI (first/later)	0.80 (0.25–2.55)	0.70		
Generation of TKI (first/second-third)	4.57 (0.63–33.13)	0.13		
Detectable ct*EGFR* (yes/no)	1.74 (1.05–2.89)	**0.03***	1.57 (0.93–2.64)	0.09

Table 2. Univariate and multivariate Cox regression analyses of PFS in the overall population. [a]Category after the slash (/) was set as a reference category. ADC: adenocarcinoma, ctDNA: circulating tumor DNA, Del19: exon 19 deletions, ECOG PS: Eastern Cooperative Oncology Group performance status, EGFR: epidermal growth factor receptor, TKI: tyrosine kinase inhibitor.

Variables[a]	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (< 60/≥60)	0.82 (0.40–1.68)	0.58		
Gender (male/female)	1.09 (0.56–2.13)	0.81		
ECOG PS (≥ 2/0–1)	2.34 (1.11–4.95)	**0.03***	2.21 (1.03–4.73)	**0.04***
Smoking (ever/never)	0.76 (0.35–1.66)	0.50		
Histology (non-ADC/ADC)	1.28 (0.39–4.15)	0.69		
Number of metastatic sites (≥ 3/<3)	1.92 (1.01–3.67)	**0.05***	1.01 (0.47–2.18)	0.97
Brain metastasis (yes/no)	1.26 (0.62–2.53)	0.52		
Liver metastasis (yes/no)	3.34 (1.70–6.53)	**< 0.001***	2.96 (1.35–6.51)	**0.007***
EGFR subtype (uncommon/common)	2.34 (0.83–6.62)	0.11		
Common *EGFR* subtype (Del19/L858R)	0.78 (0.39–1.56)	0.49		
Line of TKI (first/later)	2.16 (0.30–15.77)	0.45		
Generation of TKI (first/second)	2.24 (0.30–16.50)	0.43		
Detectable ct*EGFR* (yes/no)	2.38 (1.22–4.64)	**0.01***	1.99 (1.00–3.98)	**0.05***

Table 3. Univariate and multivariate Cox regression analyses of OS in the overall population. [a]Category after the slash (/) was set as a reference category. ADC: adenocarcinoma, ctDNA: circulating tumor DNA, Del19: exon 19 deletions, ECOG PS: Eastern Cooperative Oncology Group performance status, EGFR: epidermal growth factor receptor, TKI: tyrosine kinase inhibitor.

Dynamic change of ct*EGFR* status during EGFR-TKI treatment

Eight to 12-week post-treatment plasma samples were obtained from 65 patients. Of the 45 patients who had detectable ct*EGFR* at baseline, 37 patients (82%) completed a follow-up blood sample. Among this group, ct*EGFR* switched from detectable to undetectable during treatment in 28 patients, while nine patients continued to have detectable ct*EGFR*., 4 of which showed decreasing ct*EGFR* in plasma after EGFR TKI treatment. 81% of patients who showed a subsequent reduction or undetectable in ct*EGFR* during treatment (ct*EGFR* clearance) achieved a radiologic response by RECIST criteria. Among those with persistent detectable ct*EGFR* during treatment (ct*EGFR* non-clearance), 40% had radiologic progressive disease. The mean percentage change of tumor shrinkage was 41.4 ± 3.1 in the ct*EGFR* clearance group and 18.1 ± 9.8 in the ct*EGFR* non-clearance group (*p*-value 0.015).

Patients who achieved ct*EGFR* clearance during treatment exhibited a longer PFS. However, the difference was not statistically significant compared to patients with ct*EGFR* non-clearance (median PFS 10.4 months [95% CI: 6.9–13.9] vs. 7.3 months [95% CI: 5.8–8.8]; *p*-value 0.06) (Fig. 3A). However, ct*EGFR* clearance was associated with a significantly improved OS compared to ct*EGFR* non-clearance. The median OS was 19 months [95% CI: 13.1–24.9] for patients with ct*EGFR* clearance versus 9.7 months [95% CI: 6.7–12.8] for those without clearance (*p*-value < 0.001) (Fig. 3B).

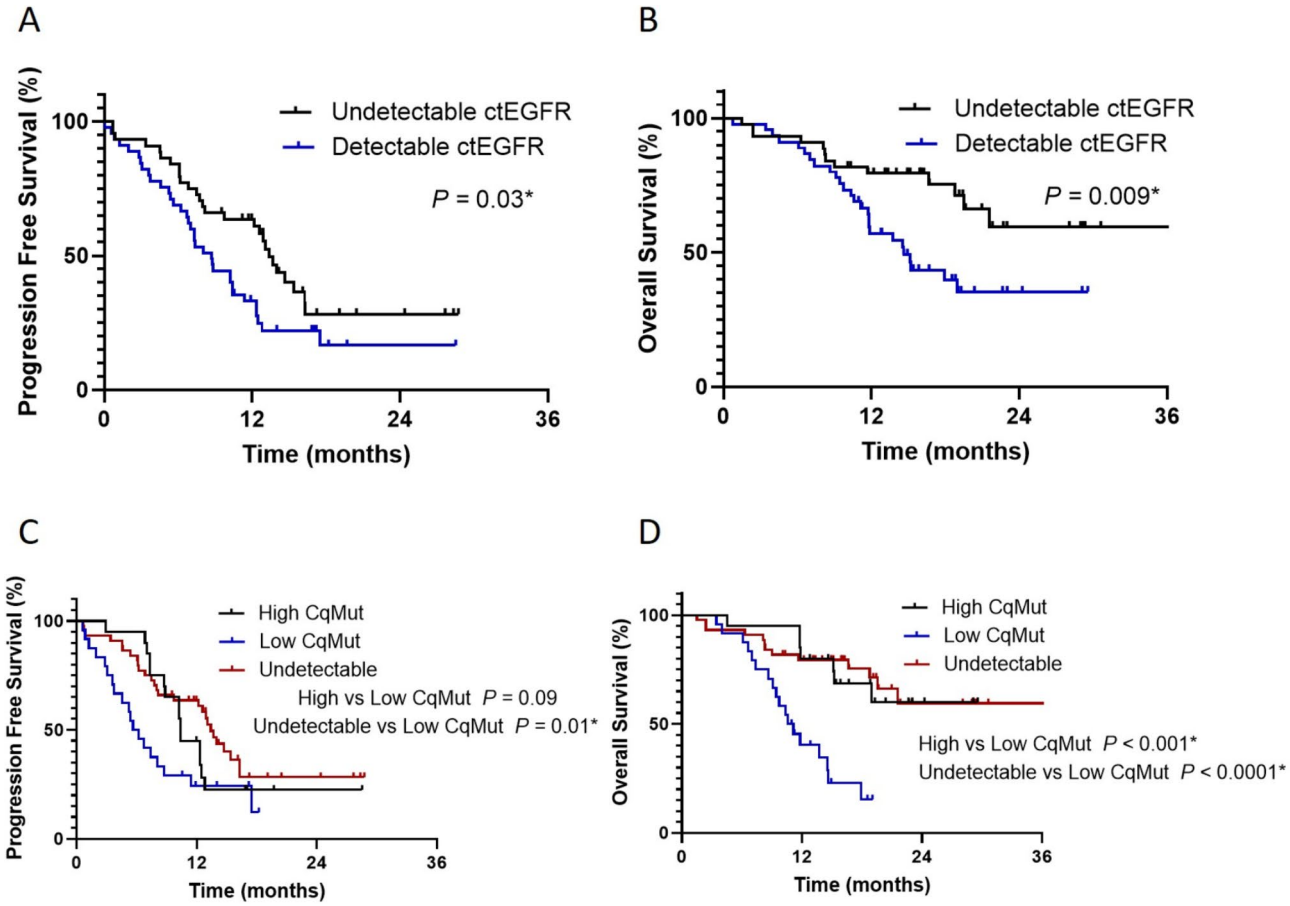

Fig. 2. Association between Cq of mutant *EGFR* values, the total amount of circulating DNA (cirDNA), and tumor burden. (**A**) the presence of liver metastases. (**B**) the number of metastatic sites. Statistical significance (*, $p < 0.05$).

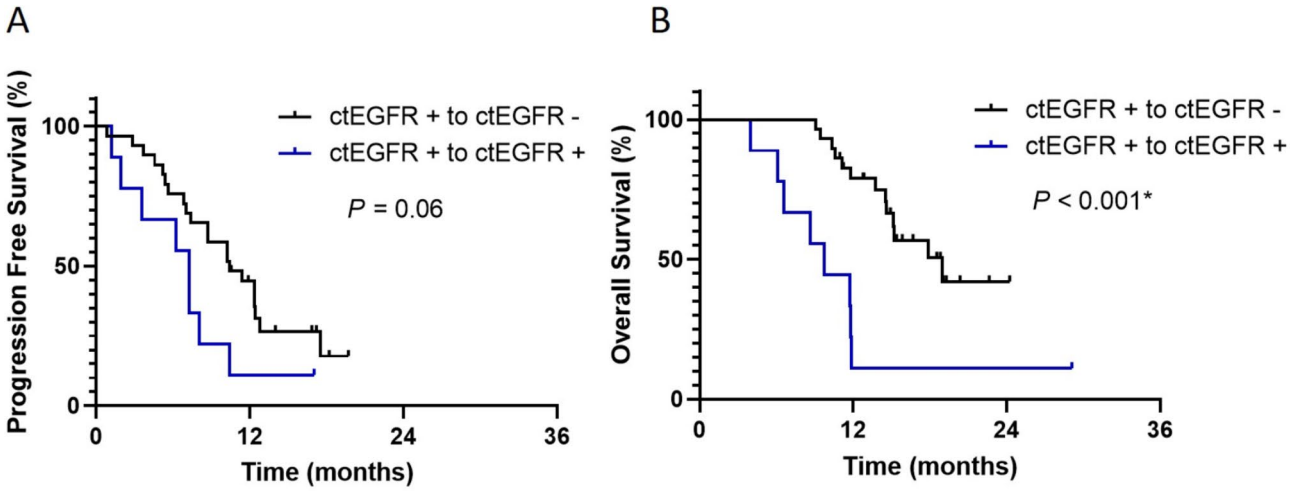

Fig. 3. Progression-free survival and overall survival in patients with *EGFR*-mutant NSCLC according to dynamic change of ctDNA status during EGFR-TKI treatment. (**A**) PFS (10.4 months for patients who had switched from detectable to undetectable ctDNA, and 7.3 months for patients who continued to have detectable ctDNA; $p = 0.06$). (**B**) OS (19 months for patients who had changed from detectable to undetectable ctDNA, and 9.7 months for patients who continued to have detectable ctDNA; $p < 0.001$). Statistical significance (*, $p < 0.05$).

Discussion

Our study demonstrated that semi-quantitative pretreatment ct*EGFR* measured by Idylla™ ct*EGFR* mutation assay testing was correlated with radiographic assessment and clinical outcomes. Consistent with previous studies, pretreatment ct*EGFR* could predict clinical outcomes in patients with *EGFR* mutation NSCLC receiving EGFR-TKIs treatment. Patients with detectable pretreatment ct*EGFR* had a significantly shorter PFS and OS than those where ctEGFR could not be detected[7,8]. Furthermore, we demonstrated an association between semi-quantitative ct*EGFR* mutation and CqMut which serves as a surrogate marker for tumor burden. Patients with detectable ct*EGFR* CqMut-low indicating high tumor abundance were associated with poorer outcomes in terms of shorter PFS/OS, consistent with other studies[6,20]. Undetectable ct*EGFR*, detectable ct*EGFR* CqMut-high, and detectable ct*EGFR* CqMut-low all represented consecutive incremental tumor abundance/tumor burdens correlated with outcomes.

We demonstrated that the presence of liver metastasis was the sole clinical factor correlated with high tumor abundance and poor prognosis. Presence of liver metastasis was significantly correlated with > 3 distant metastasis sites (77% vs. 19%, p-value < 0.001). Our multivariate analysis also found liver metastasis indicating both shorter PFS and shorter OS [PFS HR = 2.41 [95% CI 1.27–4.60, p-value 0.007; OS HR = 2.96 [95% CI 1.35–6.51, p-value 0.007]. High tumor heterogeneity, correlated with high tumor abundance, might be an underlying mechanism for poorer outcomes. Liver metastasis is employed as a surrogate marker of adverse prognosis of EGFR TKI treatment.

The ct*EGFR* clearance at initial early treatment can predict the clinical efficacy of EGFR-TKIs. It showed a correlation with radiographic partial response in 81% of patients. This finding was consistent with previous studies[7,8,21,22]. Detection of ct*EGFR* is considered complementary to tissue-based testing. A review of several commercial ct*EGFR* mutation testing machines found the Idylla™ ct*EGFR* mutation assay testing, which can be performed easily and relatively quickly (approximately 3-hr hand-on), is convenient for application in clinical practice.

Lastly, we have to state some limitations of our study. First, our cohort received first-generation EGFR TKI, which is the standard treatment reimbursed by the Civil Servant Medical Benefit Scheme (CSMBS) and universal health coverage in Thailand. Some medical oncologists think that this treatment is not a current standard option. Second, our study was conducted by using a semi-quantitative ct*EGFR* assay. It is a commercial assay, which might limit the accessibility of testing. However, we meet our goal to demonstrate a correlation between consecutive incremental tumor abundance (ct*EGFR*) and define clinical risk factors and radiographic findings using radiologists blind to ct*EGFR* mutation assay results. This correlation was demonstrated both by the baseline tumor and by response evaluation. The Presence of liver metastasis remains the best clinical predictor supporting high tumor abundance. This information might guide risk-adaptive treatment in advanced stage *EGFR*-positive lung cancer receiving EGFR TKI treatment.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Received: 6 August 2024; Accepted: 18 December 2024
Published online: 02 January 2025

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Acknowledgements

Acknowledgment: This research was supported by the Health Systems Research Institute (Thailand) (Grant number 66-153) to PS, VS and CV. The biospecimen collection was supported by Biobank, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Idylla™ reagents have been provided free of charge by Biocartis which was not involved in the study design, specimen collection, analysis, interpretation of data, report writing, and the decision to submit the article for publication.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-83930-2.

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