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Early Change in FDG-PET Signal and Plasma Cell-Free DNA Level Predicts Erlotinib Response in *EGFR* Wild-Type NSCLC Patients[1]

Anne Winther-Larsen*, Joan Fledelius†, Christina Demuth*, Catharina M Bylov‡, Peter Meldgaard§ and Boe S Sorensen*

*Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; †Department of Nuclear Medicine, Herning Regional Hospital, Herning, Denmark; ‡Department of Radiology, Aarhus University Hospital, Aarhus, Denmark; §Department of Oncology, Aarhus University Hospital, Aarhus, Denmark

Abstract

INTRODUCTION: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are a treatment option in the second- or third-line palliative setting in *EGFR* wild-type (wt) non–small cell lung cancer (NSCLC) patients. However, response rates are low, and only approximately 25% will achieve disease control. Early prediction of treatment resistance could accelerate discontinuation of ineffective treatment and reduce unnecessary toxicity. In this study, we evaluated early changes on 18F-fluoro-D-glucose (F-18-FDG) positron emission tomography/computed tomography (PET/CT) and in total plasma cell-free DNA (cfDNA) as markers of erlotinib response in *EGFR*-wt patients. *METHODS*: F-18-FDG-PET/CT scans and blood samples were obtained prior to erlotinib initiation and were repeated after 1 week (PET/CT) and 1 to 4 weeks (blood sample) of treatment. Level of cfDNA was measured by droplet digital polymerase chain reaction. Percentage change (%) in SUL_{peak} and total lesion glycolysis (TLG) on FDG-PET/CT and in plasma cfDNA was correlated to radiological response, progression-free survival (PFS), and overall survival (OS). *RESULTS*: Fifty patients were prospectively enrolled. A significant correlation was found between CT response and % TLG ($P = .003$). All patients with early metabolic progression showed radiological progression. Increased % TLG and % cfDNA were significantly correlated with shorter PFS ($P = .002$ and $P = .004$, respectively) and OS ($P = .009$ and $P = .009$, respectively). Multivariate analysis indicated % cfDNA to be the strongest predictor of OS. *CONCLUSION*: Early increase in TLG on F-18-FDG-PET/CT correlates with radiological progression, and shorter PFS and OS. Early increase in cfDNA predicts shorter PFS and OS. Both assessments are promising tools for early detection of nonresponders and reduced OS in TKI-treated *EGFR*-wt NSCLC patients.

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Introduction

Tyrosine kinase inhibitors (TKIs) targeting the epidermal growth factor receptor (EGFR) have emerged as important treatment options in non–small cell lung cancer (NSCLC) patients. Activating mutations in the *EGFR* gene have proven to be crucial predictors of treatment response [1]. However, mutation status cannot solely predict outcome because a fraction of *EGFR* wild-type (wt) patients also benefits from the treatment. Hence, EGFR-TKIs are a treatment option in the second- or third-line palliative setting in these patients [2,3]. However, additional clinical tools are needed to distinguish nonresponders from responders and thereby increase the ability to

Address all correspondence to Anne Winther-Larsen, MD, PhD, Department of Clinical Biochemistry, Aarhus University Hospital, Palle Juul-Jensens Blvd. 99, 8200 Aarhus N, Denmark.
E-mail: anlarsen@rm.dk

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end an ineffective EGFR-TKI treatment earlier so that a more effective treatment can be offered to the patient.

An early response assessment on a 2′-deoxy-2′-[18F] fluoro-D-glucose (F-18-FDG) positron emission tomography/computed tomography (PET/CT) scan performed during the first 2 weeks of treatment is a promising new tool for treatment response prediction. A change in FDG uptake has been visualized as early as after 2 days of TKI treatment [4,5], and studies have shown an association between the early metabolic response and treatment outcome [4–8]. However, these studies have evaluated patients with either unselected or mixed *EGFR* mutation status. Patients with *EGFR* activating mutations have a considerably better effect on EGFR-TKIs, and whether early FDG-PET response assessment is predictive in a cohort consisting exclusively of *EGFR*-wt patients is still unknown. Furthermore, in prior studies, standardized uptake value (SUV) metrics have been used for FDG-PET response assessment; yet, these parameters only represent a change in FDG uptake in a single voxel of the tumor or a small region of interest in the tumor. A more informative parameter could be the volume-based parameter total lesion glycolysis (TLG) because it reflects the entire metabolic tumor burden by combining volumetric data of tumors with the metabolic activity. In chemotherapy-treated NSCLC patients, two studies have reported TLG to be superior to SUV_{max} and SUV_{peak} for early response prediction [9,10], and this could also apply for TKI-treated patients.

Total plasma cell-free deoxyribonucleic acid (cfDNA) is appearing as a new potential biomarker in cancer. cfDNA is believed to be shed by both normal cells and tumor cells. The amount found in the circulation increases when cells are undergoing apoptosis or necrosis. Higher levels have been identified in cancer patients compared with noncancer patients [11–13], and the level has been suggested to reflect the tumor burden in patients. Therefore, changes in cfDNA concentration could be associated with treatment response; however, the predictive value of an early change in cfDNA value during TKI treatment has not yet been investigated.

Thus, changes in 18-F-FDG-PET signals as well as changes in cfDNA levels are two promising methods for early response assessment. Hence, the aim of this study was to evaluate the predictive value of each of these methods in a cohort of advanced-stage *EGFR*-wt NSCLC patients treated with erlotinib in second or third line. Moreover, we compared the value of the two FDG-PET–derived parameters, TLG and SUL_{peak}, for the early metabolic response prediction.

Material and Methods

Patients and Study Design

In this prospective, single-center study, 67 patients with stage III or IV NSCLC were enrolled from April 2013 until August 2015 at the Department of Oncology, Aarhus University Hospital, Denmark. Patients were candidates for enrolment if they were eligible to initiate treatment with erlotinib in a palliative setting. Details on inclusion criteria and study treatment have been described previously [14]. The study was approved by the Central Denmark Region Committees on Biomedical Research Ethics (no. 1-10-72-19-12) and reported to ClinicalTrial.gov (NCT02043002). Each patient gave written informed consent before inclusion. For the purpose of this work, we included patients from the enrolled cohort who were *EGFR*-wt, were treated in second- or third-line, and had undergone paired scans and/or paired blood samples.

18-FDG-PET/CT scans were performed pretreatment and after 7 to 10 days of erlotinib treatment. Blood samples were collected prior to erlotinib initiation and after 1 to 4 weeks of treatment. CT scans of the chest and abdomen were conducted before and after 9 to 11 weeks of treatment or earlier on clinical indication. Further evaluation CT scans were performed every 12 weeks during the treatment period. Neuroimaging was performed on clinical indication. Routine clinical and biochemical evaluation was performed every fourth week in the first 12 weeks and subsequently every sixth week.

Data on clinical characteristics and response were collected from medical files. Testing for *EGFR* mutations and *anaplastic lymphoma kinase (ALK)* translocations had been performed as part of the diagnostic workup and is described in detail in Supplementary File 1.

Response Assessment on FDG-PET/CT and CT Imaging

All F-18-FDG-PET/CT scans were performed on a combined PET/CT scanner (Siemens Biograph TruePoint 40) at the Department of Nuclear Medicine and PET-Centre, Aarhus University Hospital, Denmark. The imaging protocol is described in Supplementary File 1. Same scanner model, protocol for acquisition, and reconstruction software were used in all patients. Data on amount of injected 18-F-FDG, uptake time, and plasma glucose concentration are shown in Supplementary Table 1.

An experienced nuclear medicine physician blinded to the patient outcome analyzed all PET/CT scans using Siemens Syngo.via software. All SUV values were normalized to lean body mass (SUL). SUL_{peak} and whole-body TLG were calculated according to the Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST) 1.0 guideline [15] (described in Supplementary File 1). TLG could not be evaluated in two patients: in one patient due to carcinomatosis of the lung and in one patient due to multiple small lesions on the follow-up scan making tumor-volume assessment impossible. Percentage change (%Δ) in SUL_{peak} and whole-body TLG between pretreatment and follow-up scan was calculated as: (follow-up value − pretreatment value)/pretreatment value × 100. Metabolic response based on $\%\Delta SUL_{peak}$ was classified according to the PERCIST 1.0 guideline, whereas %ΔTLG was classified using a cutoff value of 25% based on observations by Kahraman et al. [16] (see Supplementary File 1).

Radiological response was evaluated on the first CT scan performed after initiation of erlotinib and quantified as %Δ in sum of longest diameter (SLD) of target lesions according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria [17].

Quantification of Total Plasma cfDNA

A peripheral blood sample of 10 ml was collected at each time point. The samples were centrifuged (1400*g* for 15 minutes), and plasma was isolated. Plasma was subsequently frozen at −80°C until further analysis. Total cfDNA was purified from 2 ml of plasma by use of the QIAamp circulating Nucleic Acid kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and eluted in a volume of 100 μl of TE buffer. To quantify the amount of cfDNA, the *beta-2-microglobulin* (*B2M*) gene was measured as previously described [18]. To account for a possible overestimation of the total cfDNA by accidental leukocyte contamination in the preanalytical phase, a unique *B-cell immunoglobulin DNA rearrangement* (*PBC*) was quantified [18]. The two genes were quantified in duplicates by droplet digital polymerase chain reaction (ddPCR, Bio-Rad QX200)

in a multiplex reaction using 5 μl of sample. Primers (Eurofins Genomics), probes (Integrated DNA technology), and ddPCR conditions are described in Supplementary File 1. Samples with a *PBC*/*B2M* ratio larger than 0.1% were excluded from further analysis as previously described [18]. As the level of cfDNA varies between patients, %Δ in cfDNA level from pretreatment to follow-up was calculated.

Statistical Analysis

Correlations between metabolic response, change in total plasma cfDNA, and radiological response were calculated using Fisher's exact test (categorical variables), Spearman's rank correlation coefficient (continuous variables), and Mann-Whitney *U* test (median values). In calculation of correlation between metabolic and radiological response, patients classified with stable disease (SD) or partial response (both metabolic and radiologic) were combined because of the low number of patients classified with partial response. Predictive accuracy of PET and cfDNA with respect to nonprogression on the CT scan was evaluated by using receiver operating characteristics (ROC) analysis (area under the curve [AUC]). Overall survival (OS) was measured from start of erlotinib treatment until death of any cause or last follow-up date (November 30, 2015). Progression-free survival (PFS) was defined as the time from initiation of erlotinib to first documentation of either clinical or radiological progression or death. If erlotinib treatment was ended without occurrence of progression or death, patients were censored at the time of discontinuation. Patients still undergoing treatment with erlotinib on the last follow-up date were censored at that day. Estimates of median PFS and OS were calculated by the Kaplan-Meier method and compared by the log-rank test. The Cox proportional-hazards model was used to calculate crude and adjusted hazard ratios (HRs). Clinical variables were dichotomized except for age (continuous). FDG-PET parameters and level of cfDNA were tested as continuous variables to avoid bias created by the cutoff values selected for classification of the variables. All tests were two-sided, and *P* values less than .05 were considered to be statistically significant. Statistical analyses were performed using SPSS statistics version 20.0 for Windows (IBM SPSS Statistics, Chicago, IL). STATA version 13 (Stata Corporation, College Station, TX) was used for preparation of Kaplan-Meier curves.

Results

Patients

A total of 50 patients were included in the final analysis. A flow diagram of inclusion is shown in Figure 1. Patient characteristics are shown in Table 1. Follow-up data were available for all patients. At the last follow-up date, one patient was still undergoing erlotinib treatment and nine patients were still alive. Erlotinib was discontinued in patients because of either radiological or clinical progression of disease (n = 41), toxicity (n = 7), or death (n = 1).

Correlation between FDG-PET/CT and CT Response

A significant correlation was found between radiological response and metabolic response on FDG-PET when TLG was used for response assessment (P = .003) (Table 2). Twelve patients were classified with metabolic progression, and all showed radiological progression. Thereby, 44% (12/27) of patients showing progression on CT could be identified by early FDG-PET. The %ΔTLGs found in the 12 patients classified with metabolic progression are shown in Supplementary Table 2. Likewise, assessment of the $\%\Delta SUL_{peak}$ showed a high predictive value of early metabolic progression (Table 2). However, only 21% (6/28) of patients demonstrating radiological progression could be identified by this measure. When continuous %Δ in early PET response was correlated to %ΔSLD measured on the CT scan, a correlation was found using both PET parameters (%ΔTLG: Spearman's correlation coefficient = 0.356, P = .024; $\%\Delta SUL_{peak}$: Spearman's correlation coefficient = 0.327, P = .034). The ROC analyses showed that the prediction of nonprogression by %ΔTLG was 0.93 (95% CI, 0.84-1.00; $P < .001$) and by $\%\Delta SUL_{peak}$ was 0.85 (95% CI, 0.74-0.96; $P < .001$) (Supplementary Figure 1).

Correlation Between Total Plasma cfDNA Level and CT Response

Leukocyte DNA contamination was found in samples from nine patients, and these patients were excluded from further analysis. The overall median %ΔcfDNA was 49% (range, −91 to 6249). A median increase of 58% was seen in patients classified with radiological progression, whereas only a median increase of 5% was found in patients classified with SD (Table 2); however, the difference was not statistically significant. Of the 18 patients showing radiological progression, 13 patients (72%) showed an increase in cfDNA, whereas the same fraction was 50% in patients with SD. No significant correlation was found when the continuous %ΔSLD was correlated to continuous %ΔcfDNA (Spearman's correlation coefficient = 0.206, P = .284). In line with this, the ROC analysis showed a relative poor AUC of 0.62 (95% CI, 0.45-0.86; P = .162) (Supplementary Figure 1).

Correlation Between FDG-PET/CT Scans, cfDNA Level, and Survival

The overall median PFS of all patients was 2.7 months (95% CI, 2.5-2.9), and the median OS was 6.0 months (95% CI, 3.7-8.3). Kaplan-Meier curves of PFS and OS according to FDG-PET response are shown in Supplementary Figure 2. Patients classified with progression by any of the two assessments had a significantly shorter PFS than patients classified with nonprogression (P = .014 [SUL_{peak}] and P = .024 [TLG]). Univariate Cox regression analyses showed that an increase in %ΔTLG and %ΔcfDNA was significantly correlated to shorter PFS (Table 3) and shorter OS (Table 4). A trend toward an association with PFS was found for $\%\Delta SUL_{peak}$, whereas there was no correlation to OS. To evaluate the independent impact of %ΔTLG and %ΔcfDNA, multivariate Cox regression analyses were performed. Increase in %ΔTLG and %ΔcfDNA both remained independent predictors of shorter PFS (%ΔTLG: adjusted HR = 1.02 [95% CI, 1.00-1.03], P = .045; % ΔcfDNA: adjusted HR = 1.001 [95% CI, 1.00-1.002], P = .017) (Table 3). Furthermore, increase in %ΔcfDNA remained an independent predictor of shorter OS (Table 4), whereas %ΔTLG did not show an independent correlation.

Discussion

In this prospective study, we evaluated two different methods for early response assessment in *EGFR*-wt NSCLC patients treated with erlotinib because such a method is highly needed in this subgroup of patients. We demonstrated that an early change in TLG, measured on an FDG-PET/CT scan performed after 1 week of erlotinib treatment,

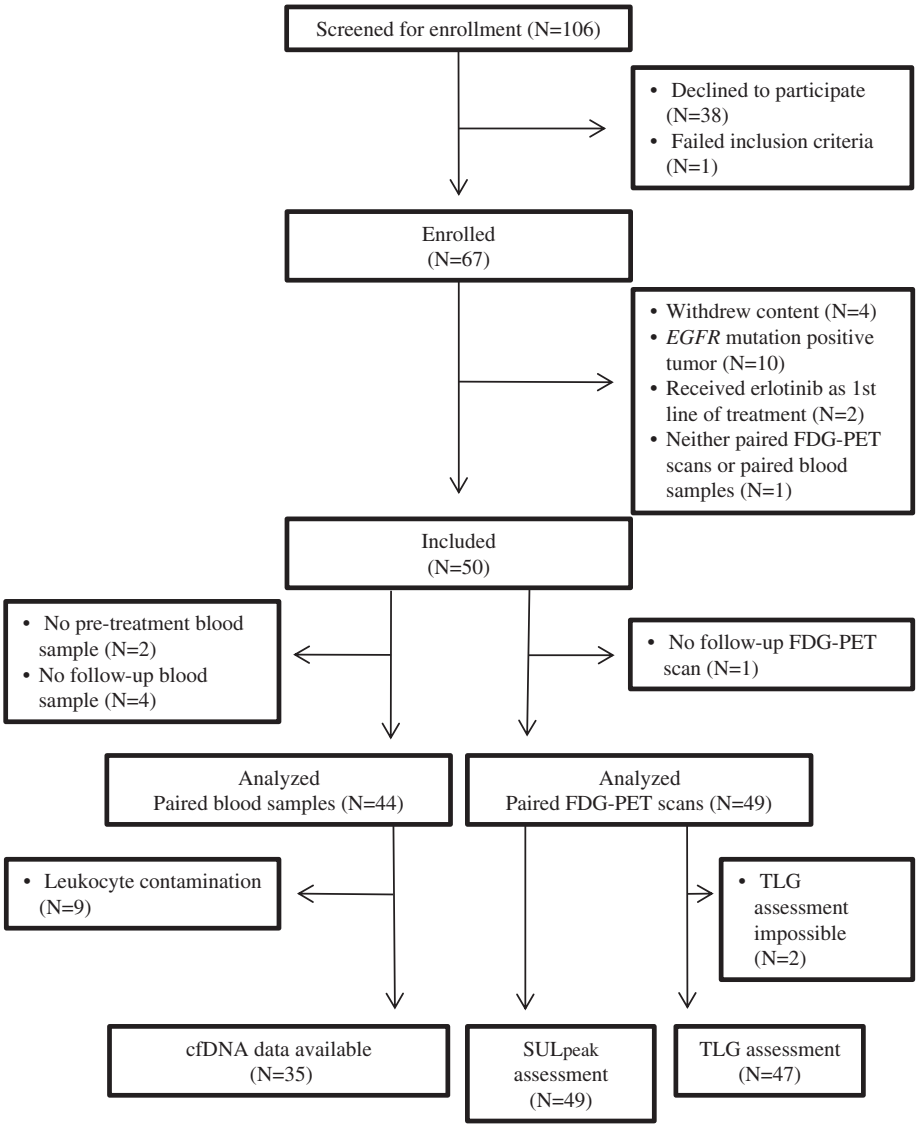

Figure 1. Consort diagram of patient inclusion.

was significantly associated with RECIST response after 10 weeks of treatment when a cutoff value of 25% was used. Most importantly, we found a high negative predictive value of early metabolic progression. All patients with progression after 1 week of treatment showed radiological progression. Thereby, 44% of all patients showing CT progression could be identified after only 1 week. Moreover, five out of six patients classified with partial metabolic response on the PET scan showed nonprogression on the CT scan. Lastly, the early metabolic change correlated with both PFS and OS.

Our results are consistent with findings in previous studies assessing the predictive value of an early metabolic response in TKI-treated NSCLC patients [5–8,19–21]. Despite variations in the timing of the early FDG-PET scan (2-14 days), all studies demonstrated an association between early PET response and outcome. However, these studies were performed in patients with either unselected or mixed *EGFR* mutation status. Our study is the first to show that early metabolic response monitoring is a useful predictor of outcome in a cohort consisting of only *EGFR*-wt patients. This is clinically important because biomarkers for EGFR-TKI treatment are highly needed in this patient subgroup where disease stabilization is only seen in around 25% and tumor response in approximately 8% [22]. Our findings bear important clinical significance because identification of resistant patients after just 1 week of treatment can lead to early discontinuation of ineffective treatment. This will markedly reduce the risk of unnecessary toxicity and increase the chance of receiving other potentially effective treatments before worsening of performance status.

We compared the effectiveness of two different parameters for PET response assessment. Both parameters were found to correlate with the change in SLD on the CT scan and show a high accuracy for prediction of nonprogression. However, TLG assessment was the only one of the two parameters significantly correlated to PFS and OS. Overall, our data indicate a superiority of early TLG assessment compared with SUL_{peak} for early response monitoring in TKI-treated

Table 1. Clinical Characteristics ($N = 50$)

Characteristics	*n* (%)
Age	
Median years (range)	68 (49-83)
Sex	
Female	22 (44)
Male	28 (56)
PS, ECOG	
0	4 (8)
1	36 (72)
2	10 (20)
Smoking status	
Never	1 (2)
Former *	38 (73)
Current	12 (23)
Unknown	1 (1)
Stage	
IIIa	2 (4)
IIIb	2 (4)
IV	46 (92)
Brain metastases	
Yes	7 (14)
No	45 (86)
Histology	
Adenocarcinoma	42 (84)
Squamous cell	8 (16)
EML4-ALK gene fusion †	
Positive	0
Negative	27 (54)
Unknown	23 (44)
Erlotinib treatment	
2nd line	41 (79)
3rd line	9 (17)
Prior treatment	
1st line	
Carboplatin/vinorelbine ‡	27 (54)
Carboplatin/vinorelbine /bevacizumab §	23 (46)
2nd line ¶	
Pemetrexed	5 (56)
Docetaxel	4 (44)
Timing of PET scans	
Days from pretreatment PET to erlotinib start, median (range)	1 (0-21)
Days from erlotinib start to follow-up PET, median (range) #	8 (2-23)
Timing of CT scans	
Days from pretreatment CT to erlotinib start, median (range)	14 (4-120)
Days from erlotinib start to evaluation CT, median (range)	72 (20-92)
Timing of blood samples	
Days from pretreatment sample to erlotinib start, median (range)	3 (0-24)
Days from erlotinib start to follow-up sample, median (range) ††	26 (6-58)

PS, performance status; *ECOG*, Eastern Cooperative Oncology Group; *EML4-ALK*, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase.

* Former smoker was defined as having stopped smoking at time of diagnosis.

† Only patients with adenocarcinoma were tested.

‡ Carboplatin day 1 (AUC 5) and vinorelbine day 1 and day 8 (60-80 mg/m^2 [PO]) every 3 weeks for a maximum of four cycles.

§ Bevacizumab (7.5 mg/m^2 IV day 1) was given in combination with chemotherapy. Patients with disease control received subsequent maintenance therapy every 3 weeks until progression or toxicity.

¶ Only including patients treated with erlotinib in third line.

Four patients were not scanned between 7 and 10 days after initiation of erlotinib but instead after 2, 5, 14, and 23 days, respectively.
Four patients were scanned later than 9 to 11 weeks of treatment (3 patients 12 weeks after and 1 patient 13 weeks after). Thirteen patients had their CT scan performed earlier because of suspicion of progression.

†† One patient had the follow-up sample collected before 1 week of erlotinib treatment (after 6 days) and 7 patients later than 4 weeks (30, 31, 32, 35, 40, 48, and 58 days, respectively).

NSCLC patients. TLG is a promising parameter because it provides information of the complete metabolic tumor burden in the patient and, in addition, includes the metabolic activity of the tumor, which serves as a marker of tumor aggressiveness. One prior study has evaluated TLG assessment for early response monitoring in erlotinib-treated NSCLC patients. In 30 patients, a trend toward a correlation between metabolic response and longer PFS was found

Table 2. PET Response and Change in Level of Total Plasma cfDNA in Correlation to CT Response

	CT Response *			
	PR	SD	PD	Total
PET response using %ΔSULpeak assessment † ($P = .076$ ‡)				
PMR	0	3	2	5 (12%)
SMD	1	11	20	32 (74%)
PMD	0	0	6	6 (14%)
Total *n* (%)	1 (2%)	14 (33%)	28 (65%)	43 (100%)
PET response using %ΔTLG assessment § ($P = .003$ ‡)				
PMR	1	4	1	6 (15%)
SMD	0	9	14	23 (56%)
PMD	0	0	12	12 (29%)
Total *n* (%)	1 (2%)	13 (32%)	27 (66%)	41 (100%)
Total plasma cfDNA ($P = .172$ ¶)				
Median %ΔcfDNA (range)		5% (–91 to 401)	58% (–31 to 6249)	47% (–91 to 6249)
Total *n* (%)		12 (40%)	18 (60%)	30 (100%)

PR, partial response; *PD*, progressive disease; *PMR*, partial metabolic response; *SMD*, stable metabolic disease; *PMD*, progressive metabolic disease.

* An evaluation CT scan was performed in 44 patients. CT response was defined according to RECIST version 1.1 criteria.

† Response was defined according to PERCIST 1.0 guideline.

‡ In calculation of the *P* value, PMR and SMD as well as PR and SD were combined. *P* value was calculated by the Fisher's exact test.

§ PMR was defined as a reduction in TLG of minimum 25%, PMD as an increase in TLG of minimum 25%, and SMD as a change not classified as PMR or PMD.

¶ *P* value was calculated by the Mann-Whitney *U* test.

when a 20% or a 30% cutoff value was used for defining a response [16]. No comparison to other SUV metrics was performed in their study. However, a comparison between the value of SUV_{max}, SUV_{peak}, MTV, and TLG assessments on FDG-PET for detecting early response to chemotherapy was performed in 52 advanced-stage NSCLC patients [9]. In consistency with our data, they found TLG to be the sole parameter significantly correlated to PFS and OS.

In addition, we evaluated the predictive value of an early change in total plasma cfDNA. We demonstrated that an increase in plasma

Table 3. Univariate and Multivariate Cox Regression Analysis of PFS ($N = 50$)

Variables	HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
Age *	1.01 (0.97-1.05)	.596		
Sex				
Female	0.65 (0.35-1.22)	.180		
Male	1.00			
Histology				
Adenocarcinoma	0.95 (0.43-2.07)	.892		
Squamous cell	1.00			
Smoking				
Never or former †	0.99 (0.49-1.99)	.978		
Current	1.00			
PS, ECOG				
0-1	1.08 (0.45-2.57)	.872		
2	1.00			
Stage				
IV	0.40 (0.12-1.37)	.144		
III	1.00			
Brain metastases				
Yes	3.31 (1.34-8.21)	.010	34.54 (2.75-433.56)	.006
No	1.00		1.00	
Erlotinib treatment				
1st or 2nd line	0.85 (0.40-1.80)	.668		
3rd line	1.00			
%ΔTLG *	1.02 (1.01-1.03)	.002	1.02 (1.00-1.03)	.045
%ΔSULpeak *	1.01 (1.00-1.02)	.100		
%ΔcfDNA *	1.001 (1.00-1.001)	.004	1.001 (1.00-1.002)	.017

* Evaluated as a continuous variable.

† Former smoker was defined as having stopped smoking at time of diagnosis.

Table 4. Univariate and Multivariate Cox Regression Analysis of OS ($N = 50$)

Variables	HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
Age *	1.00 (0.97-1.04)	.845		
Sex				
Female	0.63 (0.33-1.22)	.169		
Male	1.00			
Histology				
Adenocarcinoma	0.99 (0.43-2.26)	.977		
Squamous cell	1.00			
Smoking				
Never or former †	1.46 (0.69-3.07)	.322		
Current	1.00			
PS, ECOG				
0-1	0.59 (0.28-1.26)	.177		
2	1.00			
Stage				
IV	0.55 (0.19-1.58)	.267		
III	1.00			
Brain metastases				
Yes	3.47 (1.45-8.11)	.004	17.00 (2.94-98.44)	.002
No	1.00		1.00	
Erlotinib treatment				
1st or 2nd line	1.19 (0.52-2.72)	.676		
3rd line	1.00			
%ΔTLG *	1.02 (1.00-1.03)	.009	1.01 (1.00-1.02)	.178
%ΔSULpeak *	1.00 (0.99-1.02)	.716		
%ΔcfDNA *	1.001 (1.00-1.001)	.009	1.001 (1.00-1.001)	.043

* Evaluated as a continuous variable.

† Former smoker was defined as having stopped smoking at time of diagnosis.

cfDNA after 1 to 4 weeks of TKI treatment was independently associated with a shorter PFS and shorter OS. In the group of patients showing radiological progression, we found a higher median percentage increase in cfDNA and a higher fraction of patients with an increase in cfDNA compared with patients classified with SD. However, this difference did not reach statistical significance. We are the first to evaluate an early change in plasma cfDNA in TKI-treated NSCLC patients. The dynamics of plasma cfDNA have been evaluated in NSCLC patients treated with chemotherapy; however, studies have showed conflicting results [23–26]. Yet, none of the previous studies have accounted for a potential leukocyte DNA contamination and excluded blood samples with high contribution of leukocyte DNA as done in our study. We found contamination to be a problem in a substantial number of samples (11%), and a possible overestimation of cfDNA concentrations in prior studies could have influenced their results.

Previous studies have proposed total plasma cfDNA level to be a marker of tumor burden. Baseline plasma cfDNA values were found to correlate to both nodal stage and number of metastases in 134 NSCLC patients [27], and a significant decrease in the level of plasma cfDNA was found after tumor resection in 20 low-stage patients [12]. Our findings support these data and suggest plasma cfDNA as a promising predictor of PFS and OS in *EGFR*-wt NSCLC patients treated with erlotinib.

The strengths of our study are the prospective nature and the standardization of the FDG-PET imaging. All PET scans were performed on the same scanner model and with use of the same protocol for acquisition and reconstruction software, reducing the risk of interindividual variability of the scans. Moreover, handling of blood samples was performed in one laboratory by trained technicians, and we excluded samples with leukocyte contamination. Lastly, complete clinical data including the *EGFR* mutation status were available in all patients. In contrast, our work had some limitations to consider. Although it is one of the largest studies in the field, the impact of the study could have been increased if a higher number of patients had been included. Additionally, as the follow-up blood samples were collected in a range from 6 to 58 days after initiation of erlotinib, we are unable to define the optimal time point for early assessment of cfDNA.

Conclusion

In conclusion, our study demonstrated TLG assessment on an early FDG-PET/CT scan to be a promising predictor of response and survival in advanced-stage *EGFR*-wt NSCLC patients treated with an EGFR-TKI and to be a more robust method for response assessment than SUL_{peak}. Moreover, we showed that an early increase in the level of total plasma cfDNA predicted shorter PFS and OS, but no correlation with radiological response was seen. A combination of the two assessments could be promising for response monitoring in this patient population. Because of the low number of patients included in our study, we could not evaluate the combination of the two assessments, and future larger, prospective, randomized studies are needed to accomplish this.

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