

Can 3'-deoxy-3'-¹⁸F-fluorothymidine or 2'-deoxy-2'-¹⁸F-fluoro-d-glucose PET/CT better assess response after 3-weeks treatment by epidermal growth factor receptor kinase inhibitor, in non-small lung cancer patients? Preliminary results

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Abstract

The objectives of this study was to study the diagnostic efficacy of 3'-deoxy-3'-fluorine-18-fluorothymidine (¹⁸F-FLT) and of 2'-deoxy-2'-¹⁸F-fluoro-d-glucose (¹⁸F-FDG) positron emission tomography/computed tomography (PET/CT) for response evaluation following three weeks treatment by epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) in non small cell lung cancer (NSCLC) patients. Fifteen patients of advanced stage (IIIB-IV) NSCLC planned for oral 1st or 2nd/3rd line EGFR-TKI treatment were enrolled in the study. Baseline, prior to treatment, and follow-up after three weeks, ¹⁸F-FLT and ¹⁸F-FDG PET/CT imaging was performed in all patients. The standard uptake lean body mass (SUL_{peak}) and total lesion glycolysis (TLG) values of the hottest lesions were calculated in all patients using semi-quantitative analysis. Statistical analysis on PET semi-quantitative data was used to evaluate the overall survival (OS) and progression free survival (PFS). The patients were either classified as responders or non-responders or at a steady state according to the PET response criteria in solid tumors (PERCIST). The receiver operating characteristic curve (ROC) analysis was done on the ¹⁸F-FDG PET/CT clinical responders, to derive the cut-off values on the corresponding data sets between responders and non responders. Results showed that in responders ¹⁸F-FDG SUL_{peak} values better predicted OS and PFS values when compared to ¹⁸F-FLT SUL_{peak} values and also were a better predictor of OS as compared to the TLG values. In responders, the ROC analysis carried out on ¹⁸F-FLT PET/CT imaging data in responders indicated a decrease of $\geq 22\%$ in SUL_{peak} and a decrease of ≥ 0.7 in absolute values. Three (3/15) patients developed resistance to EGFR-TKI treatment at 3 months of follow-up. In conclusion, in both responders and in non responders, patients with NSCLC treated for 3 weeks by EGFR-TKI, both OS and PFS were better predicted by ¹⁸F-FDG SUL_{peak} than by ¹⁸F-FLT SUL_{peak}. Although, the difference was only borderline, yet, ¹⁸F-FDG SUL_{peak} was a better predictor of OS compared to TLG values. However, to validate these findings, studies need to be carried in a larger number of patients.

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Introduction

Positron emission tomography using 2-deoxy-2-¹⁸F-fluoro-d-glucose (¹⁸F-FDG) is the most commonly used imaging modality in cancer diagnosis, staging and treatment response. The thymidine analog 3'-¹⁸F-fluoro-3'-deoxythymidine (¹⁸F-FLT)-PET which has been used as a marker of cell proliferation, accumulates in cells by thymidine kinase-1 (TK-1) which is the key enzyme of the pyrimidine salvage pathway of DNA synthesis and its activity is 3-4 times higher in the malignant than in the benign cells [1, 2]. Since this enzyme is functional only during the S-phase of the cell cycle, thus the uptake of ¹⁸F-FLT is related to cell proliferation [3]. More recently, the monoclonal antibody (MIB-1) has been developed using recombinant portion of the nuclear antigen Ki-67 as an immunogen. This antibody recognizes the Ki-67 nuclear antigen, which is associated with cell proliferation and is found throughout the cell cycle. A positive correlation between the TK-1 enzyme activity and the degree of cell proliferation as measured by Ki-67 (MIB-1) and by the expression of the proliferating cell nuclear antigen (PCNA) has been reported [4].

Pre-clinical molecular studies have suggested that ¹⁸F-FLT PET/CT is a superior technique compared to ¹⁸F-FDG PET/CT for treatments response to chemotherapy, anti-proliferative agents and kinase inhibitors [5, 6]. Combined ¹⁸F-FLT and ¹⁸F-FDG PET

imaging have been reported to have an incremental value in staging non-small cell lung cancer (NSCLC) [7]. Further, the advent of newer anticancer therapeutic agents warrants the need for accurate diagnostic markers for the evaluation of treatment response monitoring and for predicting the disease progression especially in inoperable NSCLC patients.

The oral EGFR-TKI, inhibitor treatment with erlotinib and gefitinib have been investigated as 1st and 2nd/3rd line treatments in advanced stage NSCLC patients using clinical follow and laboratory investigations including immunohistochemistry [8, 9]. Erlotinib has shown good efficacy in NSCLC patients regardless of age, sex, ethnicity or histological variations [10]. A significant improvement in the progression free survival (PFS) and the overall survival (OS) was noted with erlotinib in patients with advanced stage NSCLC by using response evaluation criteria in solid tumors [11].

Biomarkers which have been assessed to predict response to oral EGFR-TKI are; the presence of mutations in the EGFR gene, the EGFR gene copy number and the EGFR protein expression [12]. As compared to ¹⁸F-FDG uptake, the ¹⁸F-FLT uptake has been reported to correlate better with the Ki-67 proliferating activity [13]. Further, ¹⁸F-FLT PET has been reported to predict response to 7-days of gefitinib treatment in patients with advanced adenocarcinoma of the lung. A decrease of >10.9% in SUV_{max} was used as the cut-off for predicting response to gefitinib treatment in this study [14]. The direct comparison of ¹⁸F-FLT PET and ¹⁸F-FDG PET for response evaluation to erlotinib in NSCLC patients has been reported previously [15]. However, no study describes the comparative diagnostic utility of the two PET tracers for response evaluation to erlotinib and gefitinib in NSCLC patients. Moreover, no definite cut-off value for response evaluation has been described in the literature using ¹⁸F-FLT PET imaging in NSCLC patients receiving either of these drugs. However, a range of 10%-30% decrease in SUV_{max} value on ¹⁸F-FLT PET imaging has been used previously for response evaluation to EGFR TK1 treatment.

The present prospective study aims at comparing ¹⁸F-FLT PET with the conventional ¹⁸F-FDG PET imaging in assessing therapeutic response after three weeks of oral EGFR-TKI treatment using either gefitinib or erlotinib (both drugs are EGFR inhibitors) and also to observe the utility of PET semi-quantification data in predicting the PFS and OS. For response assessment, we used PERCIST criteria of 20% and 30% decrease in SUV_{max} on ¹⁸F-FLT PET imaging.

Materials and methods

Patients

Fifteen patients 7 male and 8 female, with mean age of 56.6y; range 28-80y and histologically proven NSCLC disease (stages IIIB and IV) who attended the Lung Cancer Clinic run by the Department of Pulmonary Medicine at the Postgraduate Institute of Medical Education and Research (PGIMER, Chandigarh, India) over a period of 15 months, from July 2010 to September 2011, were prospectively included in the study. All these patients had some indications for initiation of EGFR-TKI as 1st, 2nd/3rd line of treatment [16, 17]. EGFR TKI has been indicated as monotherapy for the

treatment of patients with locally advanced or metastatic NSCLC with or without EGFR mutations and after failure of both platinum-based and docetaxel chemotherapy or who are refractory or intolerant to chemotherapeutic agents.

Prior to treatment initiation, all patients underwent baseline investigations which included complete physical examination, histopathological examination of the primary lung tumor, ¹⁸F-FDG and ¹⁸F-FLT PET/CT imaging. Other inclusion criteria included Eastern Cooperation Oncology Group (ECOG) performance status of 0 to 2 [17], The ECOG status is commonly used to assess the disease status in terms of patients' quality of life and prognosis following an appropriate treatment. Patients who had received prior treatment with oral EGFR-TKI or were allergic and/or intolerant to these drugs were excluded from the study. The PFS and OS of metabolic responders and non-responders were taken as the end point of the study. Baseline patient characteristics are presented in Table 1.

Table 1. Patients' characteristics prior to treatment with ECOG pre treatment status and during the follow-up period

Age	Range	28-84y
	Median	57y
Gender	Male	7
	Female	8
Histology	Adenocarcinoma	13
	Squamous cell	1
	Undifferentiated	1
Smoking Status (current/former)	Smokers	6
	Never smokers	9
Staging	IIIB	4
	IV	11
Tyrosine kinase Inhibitor	Gefitinib (250mg)	9
	Erlotinib (150mg)	6
Indication for treatment initiation	1 st line	3
	2 nd and 3 rd line	12
Patients' survival	Living	9
	Dead	6
*ECOG status	Number of patients	
	Pre-treatment	Post-treatment, 3m
0	4	1
1	4	5
2	5	3
3	2	1
4	0	5

*ECOG: Eastern Cooperation Oncology Group. Grade 0: Fully active, able to carry on all pre-disease performance without restriction. Grade 1: Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. Grade 2: Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours. Grade 3: Capable of only limited self care, confined to bed or chair more than 50% of waking hours. Grade 4: Completely disabled and cannot carry on any self care, totally confined to bed or chair

The study was approved by our Institute's Ethics Committee and a written informed consent was obtained from all patients for their participation in the study.

Treatment

Patients received an oral dose of either gefitinib (250mg) or erlotinib (150mg) daily as per the established Institute's protocol [18]. Epidermal GFR TKI is indicated as monotherapy for patients with locally advanced or metastatic NSCLC who have: a) Poor performance status or are otherwise unfit for systemic chemotherapy (1st line treatment with EGFR-TKI), b) Presence of sensitizing EGFR gene mutations like (1st line treatment with EGFR-TKI). These data were however not available for our patients since testing facilities were not available in our institute. Prior to availability of EGFR gene mutation status, clinical criteria like female gender, non-smoking status and East Asian descent were deemed to be predictive of response to EGFR-TKI treatment. c) Progressed refractory disease, during or relapsed subsequent 1st line and/or 2nd line chemotherapy (2nd/3rd line EGFR-TKI treatment). The first line chemotherapy is generally a platinum based doublet while the second line chemotherapy can either be a single non-platinum agent or a platinum doublet. Tablets of erlotinib, of 150mg or gefitinib, of 250mg once a day are the worldwide recommended dosage schedules for these drugs when used in patients with NSCLC and the same was followed in the present study. If disease progressed, treatment was discontinued. In case of drug toxicity the dose was reduced. Since, neither the gefitinib nor erlotinib tablet can be split in two, for patients with intolerance to treatment, the dose was reduced from one tablet per day to one tablet every alternate day and was stopped in case of severe toxicity like intolerable (grade 3/4) side effects as papulopustular skin rash, and diarrhoea. Treatment was resumed only if the patient fully recovered from drug toxicity in less than 2 weeks.

PET/CT acquisition protocol and image analysis

At baseline and after 3 weeks of treatment with oral EGFR-TKI (gefitinib/erlotinib), all patients were kept fasting for at least 6h before the ¹⁸F-FDG injection and blood glucose levels were always kept below 150mg/dL. Imaging by PET/CT was performed in 3-D mode using a dedicated PET/CT scanner (Discovery STE-16, GE, Milwaukee, USA) at a median uptake time of 77.0min (range 61-101min) following an intravenous injection (i.v.) of ¹⁸F-FDG with mean administered activity of 366.0MBq (range 261.59-475.82). Similarly, ¹⁸F-FLT PET/CT imaging was performed in all patients using a mean radioactivity of 239.0MBq (range 204.98-326.71) and median uptake time of 56.0min, (range: 51-82min). The ¹⁸F-FLT kits were procured from ABX advanced biochemical compounds (Radeberg, Germany). Using this kit's constituents, ¹⁸F-FLT was synthesized from the precursor-3-N-Boc-5'-o-dimethoxytrityl-3'-o-nosylthymidine, using a common approach for introducing the label with the nucleophilic ¹⁸F-fluoride. The detailed methodology for labelling of ¹⁸F with FLT is reported elsewhere [19, 20].

Imaging by the sequence of either ¹⁸F-FDG or ¹⁸F-FLT, PET/CT was randomly chosen and both these examinations were performed within one week. Whole-body scans were acquired in overlapped bed positions from skull to mid thigh and 1-2min acquisition was performed for each bed posi-

tion. All patients were imaged without sedation. Computed tomography was performed after injection of contrast media using a tube current of 115mAs and a voltage of 130kVp. After transmission scan, 3-D PET acquisitions were done for 1-2min per bed position. Image reconstruction was done using iterative reconstruction (ordered subset expectation maximization) algorithm. Transaxial, coronal, and sagittal images were obtained after reconstruction. The study protocol, image acquisition and image reconstruction remained identical for both baseline and follow-up PET imaging. All images were interpreted by two experienced nuclear medicine physicians.

For calculation of standardized uptake value (SUV), fixed size (1.2cm), volumetric spherical regions of interest were drawn over the lesions containing the area with focally increased uptake. These uptake values were then normalized to lean body mass (SUL) to derive SUL_{peak} values. The lesions with highest SUL_{peak} were identified on the baseline PET images and compared with the corresponding lesions on the follow-up PET images for the purpose of response evaluation as a function of change in the SUL_{peak} values.

The tumor lesion glycolysis (TLG) of the lesions for the ¹⁸F-FDG PET/CT scans were analyzed using PET volume computer assisted reading (VCAR) software on advantage workstation version adv 4.6; GE, Milwaukee, USA. We defined TLG as (SUV_{avg})x(tumor volume) with a fixed automated threshold of SUV_{max} of 3 in the volume of interest (VOI). In case of the ¹⁸F-FLT-PET study, SUL_{peak} values over the ¹⁸F-FLT avid lung lesions were calculated by considering liver uptake as background. However, in case of liver involvement of the disease, the aortic arch was taken as the background activity.

Positron emission tomography response criteria in solid tumors 1.0 for ¹⁸F-FDG PET [21] were used for the response evaluation of the lesions. Subsequently, we also tried to optimize the percentage change for SUL_{peak} for the ¹⁸F-FLT PET/CT study between responders and non responders through the receiver operating characteristic (ROC) curves on the ¹⁸F-FDG PET/CT study.

For response assessment, we used PERCIST 1.0 criteria of more than 30% decline in the SUL_{peak} as the cut-off value in responders to treatment, by both ¹⁸F-FDG and ¹⁸F-FLT, PET/CT studies. Additionally, responders were also identified if had a decline of more than 20% of ¹⁸F-FLT PET/CT SUL_{peak} as part for further analysis.

Table 2. Response evaluation to EGFR-TSI treatment based on the SUV_{peak} and the TLG values

Response criteria	Number of patients showing:			
	Complete response	Partial response	Stable disease	Progressive disease
¹⁸ F-FDG PET (PERCIST \geq 30% decrease in SUV _{peak})	0	03	09	03
¹⁸ F-FLT PET (\geq 30% decrease in SUV _{peak})	0	02	09	04
¹⁸ F-FDG PET (\geq 45% decrease in TLG values)	0	03	10	02

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The grouping as per PERCIST 1.0 criteria has been elaborated in Table 2. All patients who showed complete, partial response or stable disease were considered to have disease control (DC). Patients with progressive disease were categorized under no disease control (NDC).

Response assessment on clinical follow-up

Patients' response assessed by the treating oncologist was based upon the clinical status, anatomical imaging (radiography, CT or MRI) and the laboratory investigations performed every 3 months. The time to progression was calculated from initiation of EGFR-TKI treatment to the first clinical/laboratory evidence of any disease progression. Response to treatment was objectively assessed according to response evaluation criteria in solid tumors (RECIST 1.0) [21]. The median period (not the longest period) is the commonly used parameter to describe the follow-up and the same is presented in Table 3 along with an appropriate statistical analysis.

Statistical analysis

Statistical analysis was carried out by using the statistical package for social sciences software (SPSS Inc., Chicago, IL, version 15.0). Statistically significant was considered a P value of <0.05. All quantitative variables were expressed as median, mean and range. Standard deviation (SD) was also calculated. Median overall survival and progression free survival for DC and NDC groups and the significance of SUL_{peak} for both ¹⁸F-FDG, ¹⁸F-FLT and TLG for the prediction of PFS and OS were estimated using Kaplan-Meier analysis.

The time to progression and death served as endpoints. The PFS and OS were compared by log-rank test. This test is a non-parametric test for comparing survival distributions of two samples.

Curve analysis of ROC was used to optimize the percentage change for SUL_{peak} for the ¹⁸F-FLT PET/CT study between responders and non responders. Characteristic analysis by ROC was subsequently performed on ¹⁸F-FDG PET/CT data in clinical responders to evaluate the cut-off values of the responders on the ¹⁸F-FLT PET/CT images.

Logistic regression analysis was applied to see if PFS and OS correlated with various parameters viz. sex, history of smoking, histopathology and the treatment regimen. Based upon ¹⁸F-FDG and ¹⁸F-FLT PET findings, the patients were characterized as having complete or partial response and/or stable disease.

Results

No statistically significant difference was noted between the injected dose and the uptake time in the baseline and the follow-up ¹⁸F-FDG and ¹⁸F-FLT PET/CT scans [18].

Representative baseline and follow-up ¹⁸F-FDG PET/CT and ¹⁸F-FLT PET/CT scans indicating a partial response to EGFR-TKI treatment in a NSCLC patient are presented in Figures 1 and 2, respectively. Likewise representative baseline and follow-up ¹⁸F-FDG-PET/CT and ¹⁸F-FLT PET/CT scans indicating disease progression to EGFR-TKI treatment in another NSCLC patient are presented in Figures 3 and 4, respectively.

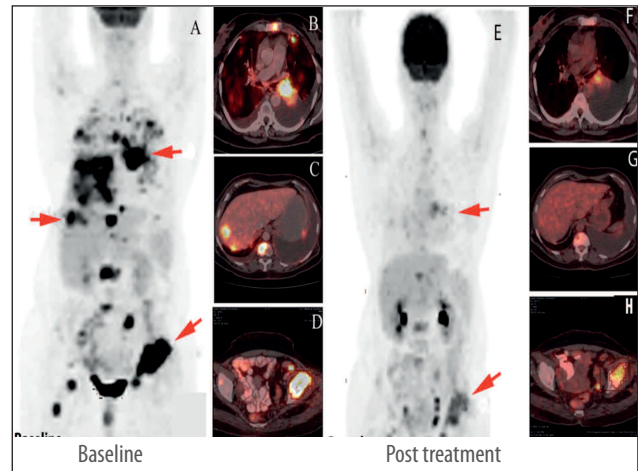


Figure 1. ¹⁸F-FDG PET/CT baseline (A, B, C, D) and post treatment (E, F, G, H) images showing partial response on transaxial fused images in soft tissue lesions in the left lower lobe of the lung (B and F), in segment VII of the liver and the D-12 vertebra (C and G) and a lytic lesion in the left iliac bone (D and H). A and E are baseline and post-treatment maximum intensity projection (MIP) PET images demonstrating overall partial response.

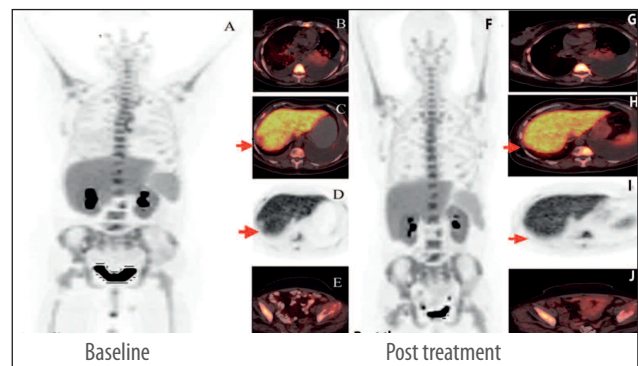


Figure 2. ¹⁸F-FLT PET/CT baseline (A, B, C, D, E) and post treatment (F, G, H, I, J) images showing partial response on trans-axial fused images in soft tissue lesion in the left lower lobe of the lung (B and G), segment VII of the liver and D-12 vertebra (C and H) and lytic lesion in the left iliac bone (E and J). A and F are baseline and post-treatment MIP-PET images demonstrating overall partial response.

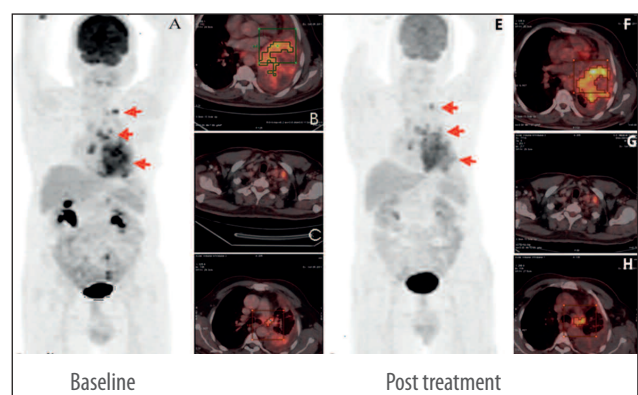


Figure 3. ¹⁸F-FDG PET/CT baseline (A, B, C, D) and post treatment (E, F, G, H) images showing disease progression on trans-axial fused images in soft tissue lesions (collapsed/consolidation) in the lower lobe of the left lung (B; SUL_{max}: 6.2 and F; SUL_{max}: 8.4), the left supra-clavicular lymph node (C; SUL_{max}: 4.2 and G; SUL_{max}: 7.5) and the sub-aortic lymph nodes (D and H). A and E are baseline and post-treatment MIP-PET images.

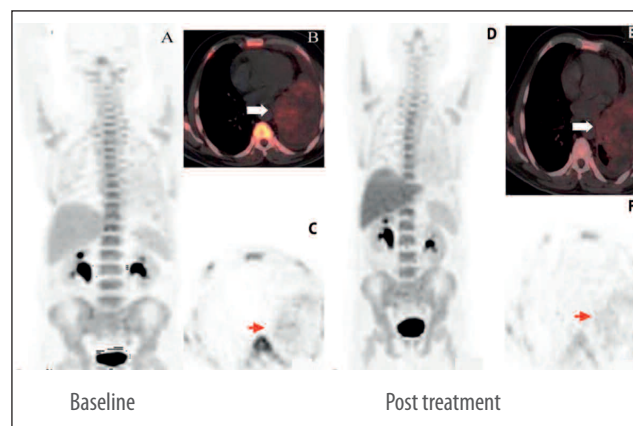


Figure 4. ^{18}F -FLT PET/CT baseline (A, B, C) and post treatment (D, E, F) images showing disease progression on trans-axial fused images in the right lung (B; SULmax: 2.5 and E; SULmax: 4.8). A and D are baseline and post-treatment MIP- PET images showing disease progression. C and F are non-fused (only PET) trans-axial images

A semi-quantitative analysis of the ^{18}F -FDG PET/CT data based upon the SULpeak, revealed a borderline significant difference in PFS (P: 0.05) and a significant difference in OS (P: 0.006) in patients with disease control when compared with the corresponding values of PFS and OS in patients having no disease control (Table 3).

A similar analysis carried out on ^{18}F -FLT PET/CT data for disease control did not show any statistically significant difference in PFS (P:0.2) and in OS (P:0.07) in the two groups of patients.

Based on TLG values derived from ^{18}F -FDG PET/CT data, we observed a statistically significant difference (P: 0.013) in the overall survival in patients with DC as compared to those having NDC. However, no statistically significant difference (P: 0.3) was noted in PFS amongst the two groups of patients (Table 3) with PFS in DC being 51 days and in NDC being 20 days. This showed that patients with DC as judged by TLG values had better survival compared to NDC, irrespective of the disease recurrence.

We further noted by the ^{18}F -FDG and the ^{18}F -FLT PET studies a disease progression in 3/15 patients who had responded to the EGFR-TKI treatment at 3 weeks, but a clinical follow-up, suggested resistance to treatment.

Discussion

Early response assessment in NSCLC patients is cost effective and may help to achieve optimal patients' care during the course of EGFR-TKI treatment as second line treatment or as maintenance treatment in NSCLC [22]. Although, we did not perform EGFR mutation studies other researchers reported that ^{18}F -FDG PET imaging is valuable for predicting tumor response to both conventional chemotherapy and erlotinib treatment in different tumours as in GIST, in sarcomas and in NSCLC [15].

Imaging by ^{18}F -FLT-PET in pre-clinical studies, has been reported to show an excellent response to erlotinib treatment in EGFR-dependent tumors and a complete lack of response in tumors expressing the T790M erlotinib resistance mutation of EGFR [23]. However, the lower uptake of ^{18}F -FLT in general is seen in all tumours and thus limits its use as a "first-line tracer", for response evaluation to chemotherapy or radiotherapy or to both. Gefitinib and erlotinib are selective EGFR-TKI inhibitors (EGFR-TKI) and have produced good results in selected NSCLC cases in terms of objective response rate and OS [24].

In an experimental study, other researchers (2006) have shown that glucose metabolic activity closely reflected response to gefitinib and concluded that ^{18}F -FDG PET may be a valuable clinical predictor at an early phase during the course of EGFR-TKI [25]. They observed a dramatic decrease in ^{18}F -FDG uptake in gefitinib-sensitive cell lines as early as 2h following EGFR-TKI treatment.

No single response criteria or cut-off value(s) have been designated to predict the response to ^{18}F -FLT-PET imaging in various studies, considering the fact that the uptake of ^{18}F -FLT was low compared to that of ^{18}F -FDG. Other researchers have found that a threshold of >10.9% decrease of SUVmax

Table 3. Progression free survival and overall survival in sub-grouping patients with and without disease control following EGFR-TKI treatment based upon semi-quantification data obtained from the ^{18}F -FLT PET, the ^{18}F -FDG-PET and the ^{18}F -FDG TLG values

	^{18}F -FLT at 20% cut-off		^{18}F -FLT at 30% cut-off		^{18}F -FDG with SUL _{peak} values		^{18}F -FDG with TLG values	
	DC (n=12)	NDC (n=3)	DC (n=12)	NDC (n=3)	DC (n=11)	NDC (n=4)	DC (n=13)	NDC (n=2)
Median PFS in days (95% CI)	51 (51-118)	7 (0-96)	51 (51-118)	7 (0-96)	123 (61-126)	20 (0-67)	51 (47-118)	20 (0-90)
P value	0.2		0.2		0.05		0.3	
Median OS in days (95% CI)	137 (121-400)	67 (15-90)	137 (121-400)	67 (15-90)	*Not achieved (137-428)	20 (13-76)	137 (123-403)	20 (0-90)
P value	0.07		0.07		0.006		0.013	

DC: disease control, NDC: no disease control, PFS: progression free survival, OS: overall survival, CI: confidence intervals

*- median OS in days could not be calculated due to short follow-up period. Medium OS could be calculated only if 50.0% of the study subjects remained event free during the follow-up period.

from the baseline tumor ^{18}F -FLT uptake would be optimal for defining response with a positive predictive value of 92.9% [14]. Others had taken the absolute difference of 0.4 in SUV_{max} on ^{18}F -FLT-PET for defining a patient as a metabolic responder [15]. Others chose as a parameter, a lesion change of $\geq 18\%$ for SUV and of 31% for Ki index as classified for ^{18}F -FLT PET response [23]. The European Organization for Research and Treatment of Cancer (EORTC) criteria defined metabolic response/s, as a $>20\%$ reduction in SUV [21]. We compared response in our patients of ^{18}F -FLT at 20% and at 30% (PERCIST).

The use of ^{18}F -FLT PET data for deriving the change in SUL_{peak} values as a semi-quantitative measure or as a surrogate marker for treatment response assessment is still under debate. Furthermore, due to the low ^{18}F -FLT tumour uptake (and hence low SUL_{peak} values) as compared to ^{18}F -FDG tumour uptake, there are apprehensions on the use of ^{18}F -FLT PET data under the PERCIST criteria for patients' response evaluation. Other researchers have used, as we have done, the percentage of change from 10% to 30% as response evaluation factor [14, 15]. In this study in order to evaluate the appropriate cut-off percentage for defining patients' response with ^{18}F -FLT, a ROC curve was drawn with regard to the clinical responders in the ^{18}F -FDG study. The ROC analysis presented sensitivity and specificity of 100.0% while using a cut-off value of 22% decrease in SUL_{max} on the post treatment ^{18}F -FLT PET scan.

It has been reported that ^{18}F -FLT uptake can monitor the distinctive biologic responses of epithelial cancers and the highly radiosensitive normal tissue changes, during radical chemo-radiotherapy in NSCLC [26].

Other researchers [15] have reported that early ^{18}F -FDG PET scan evaluated treatment response, had a significant correlation with the longevity of PFS and OS and showed that ^{18}F -FLT PET response evaluation at one week predicted a longer PFS in advanced NSCLC patients.

Other researchers considered chest CT findings and SUV_{max} cut-off values for response assessment and reported that responders had significantly longer PFS in advanced adenocarcinoma of the lung [14]. Other researchers made similar observations in a larger series of 74 NSCLC patients treated with erlotinib [27]. They have shown that partial metabolic response as determined by ^{18}F -FDG and ^{18}F -FLT PET at day 14 and day 56 of chemotherapy was associated with improved PFS.

In the present study, a significant (P:0.006) difference was observed in OS between patients with no disease control and with disease control using SUL_{peak} values. These findings were in consonance with the observations made by others in a group of 22 patients treated with erlotinib who showed a borderline statistical difference (P:0.05) in PFS both in patients with progressive and non progressive NSCLC [7]. In the present study, ^{18}F -FLT PET imaging did not reveal any significant difference in OS and PFS amongst patients with disease control and with no disease control after 3 weeks of EGFR-TK1 inhibition treatment.

The use of total lesion glycolysis (TLG) values reported in few studies have been found to be of importance in predicting the prognosis and overall survival and risk stratification in patients with oropharyngeal, esophageal, colorectal and

brain tumours [28-31]. Other researchers [32] have recently reported that a change in TLG was not associated with any improvement in PFS. These findings supported our results as we also did not find any correlation between TLG and PFS. On the other hand, we observed that the OS was significantly associated (P:0.013) with TLG in patients with progressive and non-progressive disease. Similar observations of a strong association between functional tumor volume (FTV) and TLG for the evaluation of median survival in patients with colorectal cancer have been made in previous studies [31, 33].

Fluorine-18-FLT is a new PET tracer and its use in treatment response evaluation in different cancers of the head and neck, esophagus, lung, breast, stomach and rectus, in glioma, sarcomas and lymphomas, is still under investigation with a positive role seen in brain, lung, and breast cancers where good correlation with Ki-67 was observed in small study groups [34]. Nevertheless, few studies using this tracer have shown that a decrease of more than 20% in SUL_{peak} values in the follow-up ^{18}F -FLT PET scan, can be considered as an evidence of good response [18, 35]. In the present study, no significant difference in the patients' OS and PFS was observed while having used two different criteria for response evaluation (decline in SUL_{peak} of more than 20% and more than 30%) on ^{18}F -FLT PET/CT data.

In addition, we generated the ROC analysis for expressing the decline in SUL_{peak} values on ^{18}F -FLT images. From this analysis we observed that a decline of 22% in SUL_{peak} and a change of 0.7 in the absolute value(s) on the 3-weeks follow-up provided the possibility of using this imaging technique to differentiate responders from non-responders. However, as the study was limited by a small sample size, by low tumor uptake of ^{18}F -FLT and lack of EGFR mutations analysis, further prospective studies with head to head comparison with two PET tracers are needed in larger patients' cohorts in order to assess the diagnostic utility of ^{18}F -FLT PET imaging.

Although, EGFR-TKI offers a substantial clinical benefit to some patients with NSCLC, a significant proportion of these patients develop gene mutations and show resistance in about 6-12 months after treatment [36]. This resistance could be due to various factors such as specific TKI domain mutations, activity of downstream signaling molecules, independent of EGFR regulation, or pro-survival signals through alternate molecular pathways. A secondary mutation (T790M) may be seen at codon 790 in exon 20 of the EGFR gene in tumors treated with EGFR inhibitors and may contribute to nearly half of all the cases of acquired TKI resistance [8].

In conclusion, our study indicated that: a) in progressive and non progressive NSCLC patients treated for three weeks with EGFR-TKI treatment with gefitinib or erlotinib, ^{18}F -FDG PET/CT (SUL_{peak}) could predict overall survival and progression free survival better than ^{18}F -FLT PET/CT b) Total lesion glycolysis values of the tumor sites could not predict the overall survival. c) The ^{18}F -FLT PET/CT scan can be used for response assessment using cut-off values of 20.0 % and 30.0 % with equal confidence. This study has a limited sample size and lacks histopathology evidence of EGFR expression and gene expression.

The authors declare that they have no conflicts of interest.

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