Assessment of simplified methods to measure 3’-deoxy-3’-[\(^{18}\text{F}\)]fluorothymidine uptake changes in EGFR mutated non-small cell lung cancer patients undergoing EGFR tyrosine kinase inhibitor treatment

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ABSTRACT

3'-deoxy-3'-[18F]fluorothymidine ([18F]FLT) positron emission tomography – computed tomography (PET-CT) provides a non-invasive assessment of proliferation and, as such, could be a valuable imaging biomarker in oncology. The aim of the present study was to assess the validity of simplified quantitative parameters of [18F] FLT uptake in non-small cell lung cancer (NSCLC) patients before and after start of treatment with a tyrosine kinase inhibitor (TKI).

METHODS
Ten patients with metastatic NSCLC, harboring an activating EGFR mutation, were included in this prospective observational study. Patients underwent [15O]H2O and [18F]FLT PET-CT scans on three separate occasions: within 7 days prior to treatment, and 7 and 28 days after the first therapeutic dose of a TKI (gefitinib or erlotinib). Dynamic scans were acquired and venous blood samples were collected during the [18F]FLT scan to measure parent fraction and plasma and whole blood radioactivity concentrations. Simplified measures (standardized uptake value (SUV) and tumor-to-blood ratio (TBR)) were correlated with fully quantitative measures derived from kinetic modeling.

RESULTS
Twenty-nine out 30 [18F]FLT PET-CT scans were evaluable. According to the Akaike criterion, a reversible two tissue model with four rate constants and a blood volume parameter was preferred in 84% of cases. Relative therapy induced changes in SUV and TBR correlated with those derived from kinetic analyses ($r^2 = 0.83-0.97$, $P < 0.001$, slope $= 0.72-1.12$). [18F]FLT uptake significantly decreased at 7 and 28 days after start of treatment compared to baseline ($P < 0.01$). Changes in [18F] FLT uptake were not correlated with changes in perfusion, as measured using [15O]H2O.

CONCLUSION
SUV and TBR could both be used as surrogate simplified measures to
assess changes in $[^{18}\text{F}]\text{FLT}$ uptake in NSCLC patients treated with a TKI, at the cost of a small underestimation in uptake changes or the need for a blood sample and metabolite measurement, respectively.
4.1 INTRODUCTION

Lung cancer is the leading cause of cancer related death worldwide and 85% of all lung cancers are non-small cell lung cancer (NSCLC) [1]. Moreover, the majority of patients present in an advanced stage when treatment options are limited (stage III-IV, based on the 7th edition of international association for the study of lung cancer (IASLC) [2]). Novel treatments with targeted drugs have been developed for stage IV NSCLC based on, amongst others, molecular alterations in the epidermal growth factor receptor (EGFR) [3]. EGFR is a transmembrane receptor that is involved in cellular processes such as proliferation, angiogenesis, invasion and resistance to apoptosis. Activating mutations in the EGFR domain result in continuous downstream effects. The intracellular tyrosine kinase part of EGFR can be inhibited reversibly by the EGFR tyrosine kinase inhibitors (TKI) gefitinib and erlotinib. Both drugs have shown efficacy in tumors harboring an activating mutation in the EGFR gene, but limited efficacy in EGFR wildtype [4-6]. Compared with cytotoxic treatment, TKI toxicity profiles are mild and treatment results in a benefit with respect to progression-free survival. Consequently, in this subgroup of patients with an activating EGFR mutation, quality of life is improved with TKI treatment compared with cytotoxic chemotherapy [7].

To evaluate treatment response in patients an objective non-invasive (imaging) biomarker that can be used early after start of treatment would be useful, as it provides a means to identify ineffective treatment at an early stage. By discontinuing such treatment unnecessary toxicities and costs can be prevented. Moreover, an imaging biomarker could provide an early read-out of treatment efficacy in drug development (e.g. phase 2 and 3 trials) [8]. Positron emission tomography – computed tomography (PET-CT) using 3’-deoxy-3’-[18F]fluorothymidine ([18F]FLT) might be a good candidate, as [18F]FLT is a proliferation marker and uptake of [18F]FLT correlates with immunohistochemistry for proliferation in lung, brain and breast cancer [9]. In addition, changes in maximum standardized uptake values (SUV) 7 days after start of treatment with erlotinib correlated with response measured on CT 6 weeks after start of treatment in NSCLC patients [10]. [18F]FLT follows the salvage pathway of endogenous thymidine in the cell, but is not incorporated into deoxyribonucleic acid (DNA) [11]. Published data on response evaluation using [18F]FLT PET, however, are contradictory [12-14] and it is not clear to what extent this heterogeneity is related to different pharmacokinetic characteristics, biological changes, image resolution, or PET quantification methods. The reference method for quantification of PET studies is full
kinetic modeling, which requires arterial blood sampling and dynamic scanning [15]. This procedure is not suited for daily clinical practice where whole body acquisitions are needed and it limits the number of centers that can take part in multicenter studies. Therefore, accurate simplified protocols and analytical methods are needed. These methods, should be validated against full kinetic modeling both before and after start of treatment, as tumor blood flow, fractional blood volume, or plasma clearance of $[^{18}\text{F}]$FLT may change due to treatment. Treatment induced changes in kinetics are accounted for in kinetic modeling, but not in simplified measures such as SUV or tumor-to-blood ratios (TBR) [16], and these simplified measures should therefore be validated. The aim of this project is to perform such a technical validation study and to facilitate future biological validation studies.

This prospective clinical study investigated whether simplified quantitative methods could be used as alternative measures to evaluate changes in $[^{18}\text{F}]$FLT uptake in NSCLC patients after start of treatment with TKIs.

4.2 MATERIALS AND METHODS

4.2.1 PATIENTS
Patients with stage IV NSCLC and activating EGFR mutations were recruited in six medical centers in The Netherlands. The institutional review board of the VU University Medical Center approved this study and all subjects signed a written informed consent. The study was registered in the Dutch trial register (trialregister.nl, ID number NTR3557). Patients were scheduled for three dynamic $[^{18}\text{F}]$FLT PET-CT scans; prior to treatment, and 7 and 28 days after the first therapeutic dose of an EGFR TKI. The early response assessment time point was chosen at 7 days after start of treatment, as erlotinib and gefitinib reach a steady state at day 7. The selection for erlotinib or gefitinib treatment was made by the treating pulmonary physician and consisted of 250 mg gefitinib or 150 mg erlotinib orally OD. All scans were performed in the VU University Medical Center, Amsterdam, The Netherlands.

4.2.2 PET IMAGING
PET scans were performed using a Gemini TF-64 PET-CT scanner (Philips Medical Systems, Cleveland, Ohio, USA [17]), with an axial field of view (FOV) of 18 cm. The scan field was determined by a nuclear physician such that the lung tumor
was positioned centrally in the FOV based on a diagnostic CT thorax. Patients had been fasting for 4 hours prior to the start of the scan to avoid possible food induced thymidine changes. Tracer injection and venous blood sampling was performed through a venous cannula in the fore-arm. A bolus injection of 370 MBq $[^{15}\text{O}]\text{H}_2\text{O}$ in 5 mL saline was performed at a rate of 0.8 mL/s, followed by 35 mL saline flush at 2.0 mL/s. At start of the $[^{15}\text{O}]\text{H}_2\text{O}$ injection, a dynamic emission scan was started with a total duration of 10 minutes, binned in 26 frames with the following frame length: 1x10, 8x5, 4x10, 2x15, 3x20, 2x30 and 6x60 s. Next, a low dose CT with 120 kV and 50 mAs was performed to correct the former emission scan for attenuation. After an interval of at least 15 minutes following injection of $[^{15}\text{O}]\text{H}_2\text{O}$ to allow for decay of $^{15}\text{O}$, an $[^{18}\text{F}]\text{FLT}$ scan was performed. This dynamic emission scan was started at the time of a bolus injection of 370 MBq of $[^{18}\text{F}]\text{FLT}$ in 5 mL saline [18] at a rate of 0.8 mL/s followed by a flush of 35 mL saline at 2.0 mL/s. Immediately after injection, rest activity in the syringe was measured to calculate the net injected $[^{18}\text{F}]\text{FLT}$ dose. The $[^{18}\text{F}]\text{FLT}$ scan was binned in 36 frames (1x10, 8x5, 4x10, 3x20, 5x30, 5x60, 4x150, 4x300 and 2x600 s) with a total duration of 60 minutes. Afterwards, a second low dose CT scan was acquired to correct the $[^{18}\text{F}]\text{FLT}$ scan for attenuation. All dynamic scan data were corrected for dead time, decay, scatter and randoms, and reconstructed using the 3D row-action maximum-likelihood algorithm (RAMLA), resulting in a transaxial spatial resolution of ~5 mm full width at half maximum in the center of the FOV [17]. Venous blood samples were drawn during the dynamic $[^{18}\text{F}]\text{FLT}$ PET scan at 5, 10, 20, 30, 40 and 60 minutes after injection. First 3-5 mL blood was drawn prior to each sample, followed by a blood sample of 7 mL and a flush of 2.5 mL saline after sampling. Whole blood activity concentration, plasma activity concentration and parent fraction of $[^{18}\text{F}]\text{FLT}$ were measured for all samples [19].

4.2.3 PET DATA ANALYSIS
The outer 5 planes (~2 cm) of the FOV were not used for quantification. Tumors were delineated on an averaged image of the last three frames of the $[^{18}\text{F}]\text{FLT}$ scan using a 50% threshold of SUV peak corrected for local contrast as described previously [20, 21]. In addition, healthy lung volumes of interest (VOI) were drawn centrally in the contralateral lung using a cylindrical VOI with a diameter of 1.2 cm and an axial length of 2.0 cm. A bone marrow VOI of 1.2 cm diameter and 2.4 cm axial length was drawn over a corpus vertebra. Time-activity curves (TAC) were generated by projecting VOIs onto all frames of the dynamic $[^{15}\text{O}]\text{H}_2\text{O}$ and $[^{18}\text{F}]\text{FLT}$ scans. An image derived input function (IDIF) was extracted from the ascending aorta with
2x2 voxels in 5 planes of the early frames (frames 4-6) of both $[^{15}O]H_2O$ and $[^{18}F]FLT$ scans, and corresponding TACs were generated. Tails of the $[^{18}F]FLT$ IDIF TACs (time interval 500-3600 s) were calibrated with measured radioactivity concentrations in the venous blood samples and the entire IDIF TAC was rescaled with this calibration factor. In addition, IDIFs were corrected for both plasma-to-blood ratios and metabolites to obtain calibrated parent $[^{18}F]FLT$ plasma input functions [11].

Outcome measures of full kinetic modeling and simplified approaches were obtained using an in-house developed software tool in MATLAB (MATLAB 7.10, The MathWorks Inc, Natick, MA). $[^{15}O]H_2O$ data were analysed using the standard single tissue reversible plasma input model with additional blood volume parameter [22]. For $[^{18}F]FLT$, the compartmental model underlying the biology of $[^{18}F]FLT$ uptake has been described previously [11]. Reversible and irreversible two tissue plasma input model were investigated for $[^{18}F]FLT$ uptake with blood volume parameter. Individual data points of time activity curves were weighted based on frame length and whole scanner true counts per frame [23]. These weighting factors were included in the figure of merit (cost function) during curve fitting. Dual input models for both reversible and irreversible models with blood volume parameter were evaluated to assess the effects of possible cellular uptake of labelled metabolites [24]. Net influx rate ($K_i$) and volume of distribution ($V_T$) were derived from kinetic rate constants:

\[
K_i = \frac{K_1 \times k_3}{(k_2 + k_3)} \quad \text{Eq. 4.1}
\]

\[
V_T = \frac{K_1}{k_2} \left( 1 + \frac{k_3}{k_4} \right) \quad \text{Eq. 4.2}
\]

In addition, binding potential ($BP_{ND}$) was calculated for the reversible two tissue model:

\[
BP_{ND} = \frac{k_3}{k_4} \quad \text{Eq. 4.3}
\]
Simplified measures, SUV and TBR, were calculated for two time intervals; 40-60 and 50-60 minutes:

\[
\text{SUV} = \frac{\text{Activity Concentration VOI (kBq/mL)}}{[^{18}\text{F}]\text{FLT Dose (MBq)}/\text{Normalization factor}}
\]

Eq. 4.4

\[
\text{TBR} = \frac{\text{Activity Concentration Tumor (kBq/mL)}}{\text{Activity Concentration Blood (kBq/mL)}}
\]

Eq. 4.5

Normalization factors for SUV were bodyweight (BW), lean body mass (LBM) and body surface area (BSA) [25]. For TBR, whole blood (TBR WB) and parent plasma (TBR PP) were used as denominator. In addition, SUV and TBR were calculated per time frame to generate SUV and TBR curves over time. Relative differences at 7 and 28 days after start of treatment compared to baseline were calculated for all parameters:

\[
\% \text{ difference} = \frac{\text{response} - \text{baseline}}{\text{baseline}} \times 100
\]

Eq. 4.6

4.2.4 STATISTICAL ANALYSIS
Data was tested for normality by evaluating histograms of all parameters. Mean and standard deviation (SD) were used when normal distributed, and median and interquartile range (IQR) otherwise. The optimal pharmacokinetic model was selected based on the Akaike criterion [26]. Statistical analysis comprised linear regression to assess the correlation between simplified and full kinetic outcome measures. R-square (\(r^2\)), slope and intercept of linear regressions were derived, together with 95% confidence interval (CI). Friedman and Wilcoxon Signed-Rank test were used to test for statistical differences between pre- and post-treatment scans. \(P\)-values <0.05 were considered significant. Statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corp, Armonk, NY, 2010).
4.3 RESULTS

Between September 2012 and December 2013, 10 patients were included (demographics and EGFR mutation types in Table 4.1). Baseline scans were performed within a median of 1 day (range 0-3) before start of treatment with exception of one patient whose baseline scan was performed 26 days prior to start of treatment with gefitinib. This patient started erlotinib treatment after the baseline scan but stopped five days later due to gastrointestinal toxicities. Three weeks later, when symptoms recovered, gefitinib treatment was initiated and follow-up [18F]FLT scans were performed after start of treatment with gefitinib without performing a new baseline scan (as the patient had already been pretreated with erlotinib). Follow-up scans were performed with a median of 7 (IQR 6-9) and 28 (IQR 27-29) days after start of treatment. Body weight of patients was not significantly different between scans, with 67 (IQR 67-89), 68 (IQR 66-89) and 67 (IQR 64-89) kg for baseline, and 7 and 28 days after start of treatment respectively (Friedman test, $P = 0.88$).

All patients completed the three study visits. One [18F]FLT scan was not evaluable due to a scanner failure. Four [15O]H$_2$O scans were not performed because of technical or logistical problems. Median net injected [18F]FLT doses were 383 (IQR 352-394), 376 (IQR 364-390) and 376 (IQR 365-385) MBq for scans one, two and three, respectively (Friedman test, $P = 0.50$). Injected [15O]H$_2$O was 370 MBq for every scan. Median parent fractions of [18F]FLT at 60 minutes after injection were 76% (IQR 73-84), 79% (IQR 74-82) and 81% (IQR 76-82) for scans one, two and three, respectively (Friedman test, $P = 0.90$). Median plasma-to-blood ratios at 60 minutes after injection were 1.19 (IQR 1.13-1.27), 1.19 (IQR 1.15-1.24) and 1.19 (IQR 1.15-1.23) for scans one, two and three, respectively (Friedman test, $P = 0.64$). Median IDIF calibration factor was 0.86 (IQR 0.80-0.94).

Twenty-four [18F]FLT avid lesions were detected at baseline, with 22 and 17 lesions evaluable (visually detected above background) at 7 and 28 days after start of treatment, respectively (Figure 4.1). Overall, based on Akaike, a two tissue reversible model was preferred over a two tissue irreversible model in 84% (88% at baseline, 82% and 82% at 7 and 28 days after start of treatment, respectively, Friedman test, $P = 0.14$). Dual input pharmacokinetic modeling did not improve kinetic analyses according to the Akaike criterion. No differences in modeling of [18F]FLT kinetics between patients treated with erlotinib or gefitinib were observed and therefore data were pooled. Results of full kinetic modeling, and SUV and TBR analyses per scan are shown in Table 4.2.

$V_T$
TABLE 4.1 Patient demographics.

<table>
<thead>
<tr>
<th></th>
<th>no. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>64</td>
</tr>
<tr>
<td>Range</td>
<td>52-75</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>3</td>
</tr>
<tr>
<td>History of smoking</td>
<td>4</td>
</tr>
<tr>
<td>Never</td>
<td>3</td>
</tr>
<tr>
<td><strong>EGFR mutation</strong></td>
<td></td>
</tr>
<tr>
<td>Exon 18 G719X</td>
<td>2</td>
</tr>
<tr>
<td>Exon 19 del E746-A750</td>
<td>2</td>
</tr>
<tr>
<td>Exon 21 L585R</td>
<td>6</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Gefitinib</td>
<td>7</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>3</td>
</tr>
</tbody>
</table>

decreased significantly relative to baseline at both 7 and 28 days after start of treatment (Friedman test, \(P < 0.001\)). Within the evaluable lesions, no significant difference between 7 and 28 days after start of treatment was observed (Wilcoxon Signed Rank test, \(P = 0.46\)). SUV and TBR showed the same absolute trend as \(V_T\). Relative changes in SUV and TBR correlated strongly with relative changes in \(V_T\), with an \(r^2\) of 0.83-0.97 (\(P < 0.001\)) and a slope of 0.72-1.12 (Figure 4.2, Table 4.3). The intercept ranged from -5 to 12%, which was not significantly different from zero, except for TBR WB showing a positive bias of 7-12% (Table 4.3). SUV reached equilibrium at 30 minutes after injection, whereas TBR was still increasing at 60 minutes after injection (Figure 4.3).

\(^{18}\text{F}\)FLT kinetics in bone marrow and normal lung were best fitted using a two tissue irreversible model with blood volume parameter in 56, 90 and 80% (bone marrow), and 78, 80 and 70% (lung) for scans one, two and three, respectively (Friedman test \(P = 0.10\) and 0.72 for bone marrow and lung, respectively). \(^{18}\text{F}\)FLT influx in bone marrow
and lung, obtained from $K_i$ of the two tissue irreversible model, was not significantly different at 7 or 28 days after start of treatment compared to baseline (Friedman test $P = 0.24$ and 0.46 for bone marrow and lung, respectively).

Tumor perfusion did not significantly change after start of treatment, with a median $K_i$ of $[^{15}\text{O}]\text{H}_2\text{O}$ of 0.41 (IQR 0.31-0.61), 0.35 (IQR 0.25-0.59) and 0.39 (IQR 0.29-0.77) mL/cm$^3$/min for scans one, two and three, respectively (Friedman test $P = 0.29$). Relative changes in tumor perfusion were not correlated with relative changes in $[^{18}\text{F}]\text{FLT}$ uptake, with $r^2$ of 0.05 and 0.06 at 7 and 28 days after start of treatment, respectively ($P = 0.38$ and 0.42, Figure 4.4).
FIGURE 4.1 Fused $[^{18}F]$FLT PET-CT image of a patient with NSCLC stage IV, with primary tumor in the right lung and a contralateral bone metastasis, at baseline (A) and 7 (B) and 26 (C) days after start of treatment with erlotinib.
TABLE 4.2 [18F]FLT uptake at baseline and 7 and 28 days after start treatment.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>7 days after start of treatment</th>
<th>28 days after start of treatment</th>
<th>Friedman test P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmacokinetic model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_t$</td>
<td>0.294 (0.242-0.389)</td>
<td>0.255 (0.190-0.312)</td>
<td>0.260 (0.193-0.424)</td>
<td>0.11</td>
</tr>
<tr>
<td>$k_b$</td>
<td>0.129 (0.080-0.175)</td>
<td>0.083 (0.069-0.123)</td>
<td>0.088 (0.073-0.135)</td>
<td>0.05</td>
</tr>
<tr>
<td>$BP_{ND}$</td>
<td>5.66 (3.27-8.21)</td>
<td>3.12 (2.45-5.35)</td>
<td>3.64 (2.61-6.06)</td>
<td>0.02</td>
</tr>
<tr>
<td>$V_b$</td>
<td>0.066 (0.050-0.128)</td>
<td>0.082 (0.054-0.170)</td>
<td>0.101 (0.065-0.171)</td>
<td>0.001</td>
</tr>
<tr>
<td>$V_T$</td>
<td>4.53 (3.12-5.52)</td>
<td>3.41 (1.68-3.81)</td>
<td>2.99 (2.60-3.79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$K_t$</td>
<td>0.063 (0.051-0.082)</td>
<td>0.050 (0.031-0.058)</td>
<td>0.048 (0.040-0.063)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Simplified models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBR PP 40-60 min</td>
<td>5.0 (3.3-5.8)</td>
<td>3.4 (1.8-4.6)</td>
<td>3.5 (3.0-4.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>TBR PP 50-60 min</td>
<td>5.3 (3.3-5.8)</td>
<td>3.5 (1.9-4.9)</td>
<td>3.8 (3.2-4.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>TBR WB 40-60 min</td>
<td>4.3 (3.3-5.4)</td>
<td>3.3 (2.0-4.4)</td>
<td>3.4 (2.9-3.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>TBR WB 50-60 min</td>
<td>4.5 (3.3-5.8)</td>
<td>3.5 (2.2-4.6)</td>
<td>3.6 (3.1-4.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>SUV LBM 40-60 min</td>
<td>2.5 (2.0-3.3)</td>
<td>1.9 (1.2-2.5)</td>
<td>1.9 (1.6-2.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SUV LBM 50-60 min</td>
<td>2.4 (2.0-3.2)</td>
<td>1.9 (1.2-2.5)</td>
<td>1.9 (1.6-2.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: $V_b$, blood volume fraction; $V_T$, volume of distribution; $BP_{ND}$, binding potential; SUV, standardized uptake value; TBR, tumor-to-blood ratio; PP, parent plasma; WB, whole blood; LBM, lean body mass

FIGURE 4.2 Correlation of percentage change of SUV and TBR for time interval 50-60 minutes and $V_T$ derived using a two tissue reversible model with blood volume parameter at 7 (A) and 28 (B) days after start of treatment. The solid line represents the line of identity.
TABLE 4.3 Correlation of relative changes in simplified measures versus $V_r$.

<table>
<thead>
<tr>
<th></th>
<th>7 days after start of treatment</th>
<th>28 days after start of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>Slope (95% CI)</td>
</tr>
<tr>
<td>TBR PP 40-60 min</td>
<td>0.90</td>
<td>0.99 (0.83-1.14)</td>
</tr>
<tr>
<td>TBR PP 50-60 min</td>
<td>0.89</td>
<td>1.04 (0.86-1.21)</td>
</tr>
<tr>
<td>TBR WB 40-60 min</td>
<td>0.92</td>
<td>0.96 (0.83-1.10)</td>
</tr>
<tr>
<td>TBR WB 50-60 min</td>
<td>0.90</td>
<td>1.01 (0.84-1.17)</td>
</tr>
<tr>
<td>SUV LBM 40-60 min</td>
<td>0.90</td>
<td>0.78 (0.66-0.91)</td>
</tr>
<tr>
<td>SUV LBM 50-60 min</td>
<td>0.91</td>
<td>0.80 (0.68-0.92)</td>
</tr>
</tbody>
</table>

FIGURE 4.3 SUV LBM and TBR PP as function of time at baseline for all patients, showing equilibrium of SUV LBM being reached at 30 minutes, whilst TBR PP still increases at 60 minutes. Solid lines represent TBR PP and dashed lines represent SUV LBM.
This study investigated the correlation of relative changes in simplified and full kinetic \(^{18}\text{F}\)FLT measures in NSCLC patients after start of TKI treatment to assess whether simplified measures could be used as surrogate markers of treatment response. A two tissue reversible model with blood volume parameter was the preferred model for tumor \(^{18}\text{F}\)FLT kinetics. Relative changes in SUV and TBR correlated well with relative changes in \(V_T\) \((r^2 = 0.83\text{-}0.97)\) and, therefore, these simplified measures can be used as alternative parameters to evaluate changes in \(^{18}\text{F}\)FLT uptake due to TKI treatment.
Parent fractions and plasma-to-blood ratios were not significantly different at baseline and after start of treatment. This suggests that plasma kinetics of [18F]FLT is stable after start of treatment with TKI, without inter- and intrasubject variability. Therefore, a population based input function might be a valid alternative for kinetic analysis of response evaluation studies in patients who are treated with a TKI, however this requires further evaluation in a larger patient group [27].

[18F]FLT uptake showed reversible kinetics in the majority of the lesions. Although preference for the reversible model showed a decreasing trend after start of treatment, this was not statistically significant. Extended scan duration might provide better fits and a more robust estimates of \( k_4 \) with a sustainable preference for a reversible model after start of treatment [28]. Apart from single input function models, also dual input function models were investigated, to assess whether in- and efflux of metabolites plays a role. However, these models did not provide better fits and therefore results from the two tissue reversible model with blood volume parameter was used. From this model, the macorparameter \( V_T \) represents the most robust result and was used as reference to assess simplified uptake measures. \( V_T \) decreased significantly after start of treatment, but was not significantly different between 7 and 28 days after start of treatment (Table 4.2). Microparameter \( K_1 \), which represents [18F]FLT influx, did not change after start of treatment \( (P = 0.11) \), suggesting that expression of nucleoside transporters to the cell membrane are not up- or downregulated after start of treatment, as has been described in mice [29]. In addition, microparameter \( k_3 \) decreased after start of treatment \( (P = 0.05) \), indicating decreased thymidine kinase activity and thereby a decreased proliferation rate. Therefore, measured changes in [18F]FLT uptake could be attributed to changes in phosphorylation rate (\( k_j \)) instead of cell membrane transport (\( K_1 \)).

Conceptually, TBR is a simplified model for reversible kinetics and SUV for irreversible models. However, changes in both parameters strongly correlated with changes in \( V_T \) \( (r^2 = 0.83-0.97) \) and could be used to evaluate [18F]FLT uptake responses. Relative differences in TBR WB had a significant but small positive bias, which might be caused by small differences in parent fraction per scan. Consequently, this may explain why differences in TBR PP at 50-60 minutes after injection had a high correlation with differences in \( V_T \) (0.89 and 0.95 after 7 and 28 days respectively), a slope which was close to the line of identity (1.04 and 0.99) and no bias (intercept not significantly different from zero). Therefore, this parameter appears to be a sensitive measure for detecting relative changes. Unfortunately, however, TBR PP was not stable over time and differences in acquisition time could affect outcome (higher TBR PP for
longer uptake time intervals). In addition, both a blood VOI and a blood sample are needed to measure blood activity concentration, parent fraction and plasma-to-blood ratio, limiting its feasibility in multicenter studies. Alternatively, SUV showed a plateau beyond 30 minutes after injection, and therefore measurements will be less dependent on uptake interval as long as acquisition is performed somewhere between 30-60 minutes after injection, as reported previously [30]. On the other hand, SUV underestimated relative therapy induced changes with slopes significantly <1, which is in line with previous results in locally advanced breast cancer (16). Therefore, SUV may be preferred in multicenter [18F]FLT PET studies based on feasibility, but one should be aware of possible underestimation of response assessment (18-28%) in patients treated with a TKI.

SUV normalisation can be performed using BW, LBM or BSA. For [18F]FLT, LBM might be the best normalization factor, as [18F]FLT has no specific uptake in fat or muscle. This is in agreement with the recommendation to use SUV LBM for response evaluation in [18F]FDG PET studies [31]. In the present study, bodyweight was not significantly different between successive scans and consequently SUV BW, LBM and BSA performed similarly and no definitive decision can be made with respect to the optimal SUV normalisation.

Relative changes in tumor perfusion, measured using $K_1$ of [15O]H$_2$O, did not correlate with relative changes in [18F]FLT uptake after start of treatment. This indicates that [18F]FLT uptake is independent of perfusion and that changes in [18F]FLT uptake are not caused by perfusion changes.

The treatment regimen with either gefitinib or erlotinib was determined by the treating physician. Both drugs are reversible TKI with similar mechanism of action [32, 33]. In the present study, data were pooled, as no differences in [18F]FLT kinetics were observed between the two treatment regimens.

Our study described the technical validation of simplified [18F]FLT uptake measures in NSCLC patients treated with TKI. Biological validation studies, in which relative changes in simplified measures of [18F]FLT uptake need to be correlated with pathology and/or clinical outcome, should be performed to determine the predictive value. Once this is confirmed, [18F]FLT PET could qualify as predictive biomarker of response to TKI in NSCLC. Furthermore, it is tempting to extrapolate the present positive findings of the use of simplified measures to quantify [18F]FLT uptake to other tumor and treatment types. This should, however, be validated in each case separately, as systemic therapy may alter the correlation between simplified and fully quantitative measures [34].
4.5 CONCLUSION

In NSCLC patients treated with a TKI, relative changes in SUV and TBR correlated with those in $V_T$ of $[^{18}F]$FLT. SUV LBM measured 30-60 min after injection or TBR PP at 50-60 minutes after injection could be used for future response assessment studies in NSCLC patients with activating EGFR mutation treated with a TKI, at the cost of a small underestimation in uptake changes or the need for a blood sample and metabolite measurement, respectively.

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4.7 REFERENCES


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Simplified uptake measures in $[^{18}F]FLT$ PET after EGFR TKI


