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Identifying advanced stage NSCLC patients who benefit from afatinib therapy using ^{18}F-afatinib PET/CT imaging

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ABSTRACT

Objectives: Non-small cell lung cancer (NSCLC) tumors harboring common (exon19del, L858R) and uncommon (e.g. G719X, L861Q) activating epidermal growth factor receptor (EGFR) mutations are best treated with EGFR tyrosine kinase inhibitors (TKI) such as the first-generation EGFR TKI erlotinib, second-generation afatinib or third-generation osimertinib. However, identifying these patients through biopsy is not always possible. Therefore, our aim was to evaluate whether ^{18}F-afatinib PET/CT could identify patients with common and uncommon EGFR mutations. Furthermore, we evaluated the relation between tumor ^{18}F-afatinib uptake and response to afatinib therapy.

Materials and methods: ^{18}F-afatinib PET/CT was performed in 12 patients: 6 EGFR wild type (WT), 3 EGFR common and 3 EGFR uncommon mutations. Tumor uptake of ^{18}F-afatinib was quantified using TBR_WB_{60-90} (tumor-to-whole blood activity ratio 60−90 min post-injection) for each tumor. Response was quantified per lesion using percentage of change (PC): [(response measurement (RM)–baseline measurement (BM))/BM]×100. Statistical analyses were performed using t-tests, correlation plots and sensitivity/specificity analysis.

Results: Twenty-one tumors were identified. Injected dose was 348 ± 31 MBq. Group differences were significant between WT versus EGFR (common and uncommon) activating mutations ($p = 0.03$). There was no significant difference between EGFR common versus uncommon mutations ($p = 0.94$). A TBR_WB_{60-90} cut-off value of 6 showed the best relationship with response with a sensitivity of 70 %, a specificity of 100 % and a positive predictive value of 100 %.

Conclusion: ^{18}F-afatinib uptake was higher in tumors with EGFR mutations (common and uncommon) compared to WT. Furthermore, a TBR_WB_{60-90} cut-off of 6 was found to best predict response to therapy. ^{18}F-afatinib PET/CT could provide a means to identify EGFR mutation positive patients who benefit from afatinib therapy.

1. Introduction

With over 2 million new cases each year, lung cancer is one of the most prevalent cancer types worldwide, and is associated with the highest cancer-related mortality, accounting for over 22 % of cancer-related deaths [1–3]. Historically, advanced stage non-small cell lung cancer (NSCLC) was treated with chemotherapy. However, over the past decade, targeted therapies directed against oncogenic driver pathways (i.e. pathways promoting cell growth) have revolutionized the treatment of NSCLC tumors. One such targetable oncogenic driver is the epidermal growth factor receptor (EGFR) pathway. Mutations in the kinase domain of this EGFR pathway can lead to a constitutive, ligand-independent activation of the EGF receptor [4]. Examples of such activating EGFR mutations are exon19 deletions or the exon 21 L858R point mutation.

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Patients with such an activating EGFR mutation are best treated with EGFR tyrosine kinase inhibitors (TKI), as these TKI achieve higher response rates and longer durations of response as compared to chemotherapy or immunotherapy [5–10]. Therefore, EGFR TKI are the standard of care in patients with activated EGFR mutations. In approximately 85 % of patients, the EGFR mutation found in the tumor DNA is either an exon19 deletion or a L858R point mutation, the so-called common mutations [4,11,12]. Approximately 10 % of patients harbor uncommon mutations, such as G719X, L861Q and S768I mutations [4,12–15]. Because these mutations are uncommon, high-level evidence on treatment efficacy is lacking. The available data suggests that afatinib provides favorable treatment responses, especially in the G719X, L861Q and S768I variant subgroups when compared to other EGFR TKI [14,16,17].

Molecular analysis of the tumor DNA is the gold standard to assess the tumor EGFR mutational status. This is typically determined through a representative histological tumor biopsy. However, this may not always be feasible due to difficult to reach tumor localizations or low yields of tumor cells. Furthermore, tumors within a single patient can show intra- and interlesional differences in EGFR mutational expression [16], which could result in tumor biopsies that are not representative, highlighting the need for an alternative non-invasive means to identify these.

Positron emission tomography (PET) could provide an answer. A number of EGFR-directed PET tracers have been developed to evaluate the presence of EGFR mutations or to assess whether tumor tracer uptake could serve as a predictive biomarker for response to TKI treatment [17]. These tracers vary from radiolabeled EGFR TKI such as the first-generation EGFR inhibitor ^{11}C-erlotinib to specifically developed tracers without therapeutic analog such as ^{18}F-MPG [17,18]. For example, our group showed that tumor ^{11}C-erlotinib uptake was significantly higher in patients with activating EGFR mutation positive NSCLC patients (EGFR exon 19 deletions) [18,19]. However, to our knowledge, no studies using a radiolabeled EGFR TKI have assessed both the presence of EGFR mutations as well as the treatment response to treatment by the same EGFR TKI [20]. Furthermore, no other studies have specifically looked at uncommon activating EGFR mutations.

Radiolabeled afatinib is of interest as a PET tracer, as afatinib has shown activity against both common and uncommon activating EGFR mutations [14,21]. Afatinib can be labeled with fluorine-18 ($t_{1/2}$ 110 min), which, as opposed to labeling with the shorter-lived carbon-11 ($t_{1/2}$ 20 min), makes ^{18}F-afatinib more apt for wider spread clinical use. Due to its longer half-life, ^{18}F-labeled drugs can be transported more easily to other PET centers. Our group previously showed that tumor accumulation of ^{18}F-afatinib can be quantified in NSCLC patients [22]. Pharmacokinetic modeling showed that tumor tracer uptake was best fitted with a two-tissue, irreversible compartment model (2T3K) and the simplified measure TBR_WB_{60-90} (tumor-to-(whole) blood activity ratio and a scan interval of 60−90 min post-injection) was most appropriate to quantify tumor tracer uptake. However, no data on the correlation between tumor ^{18}F-afatinib uptake and EGFR mutational status or tumor afatinib response was reported.

In this study, we aimed to assess whether tumor ^{18}F-afatinib uptake could differentiate between tumors harboring wild-type EGFR, common activating EGFR mutations (exon19 deletion or L858R mutation) and uncommon activating EGFR mutations. We also evaluated whether ^{18}F-afatinib uptake could predict response to afatinib therapy.

2. Methods

2.1. Study design and patient inclusion

We performed a prospective PET imaging study using ^{18}F-afatinib in NSCLC patients, planned for afatinib therapy. All patients underwent a PET scanning procedure using the novel tracer ^{18}F-afatinib. The primary endpoint of the study was to investigate the tracer tumor pharmacokinetic model, the results of which have already been previously published by van de Stadt et al. Here, we present the correlation of the tumor ^{18}F-afatinib uptake and the clinical outcomes of afatinib therapy, which was a secondary endpoint of this study. All evaluable patients who underwent a dynamic ^{18}F-afatinib PET and afatinib therapy were included in the study. Only 1 patient, who underwent static scanning for the purpose of dosimetry, was excluded, as quantification of uptake was not possible for this analysis.

A total of 12 consecutive patients were therefore evaluable for this present study. Exon 19 deletions and the L858R point mutation (exon 21) were considered EGFR common mutations. Other mutations that were reported in literature to be sensitive to afatinib treatment were considered uncommon mutations. Prior systemic therapies (i.e. chemotherapy or immunotherapy) were allowed, but all patients were afatinib-naïve. Only tumors of >1.5 cm were assessed. A full list of inclusion and exclusion criteria can be found in the Supplementary data.

2.2. Review medical ethics committee

This study was approved by the medical ethics committee of the Amsterdam University Medical Center, location VUmc (Amsterdam, The Netherlands). Each patient gave written informed consent prior to inclusion.

2.3. F-afatinib synthesis

Synthesis of ^{18}F-afatinib was performed as described by Slobbe et al. [23]. Briefly, starting from 7-chloro-quinazoline-4(3 H)-one, 3-chloro-4-trimethylammonium-nitrobenzene triflate was synthesized. Subsequently, 3-chloro-4-trimethylammonium-nitrobenzene triflate was labelled with fluorine-18 using the peptide coupling reagent benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP). Finally, synthesized ^{18}F-afatinib was purified by semipreparative HPLC chromatography.

2.4. PET/CT scanning

All patients were scanned on an Ingenuity TF PET/CT (Philips, Best, The Netherlands). This study pools data from 3 scanning protocols, see below for the description of the protocols.

In protocol 1, patients underwent low-dose CT for attenuation correction followed by an extended 90 min dynamic ^{18}F-afatinib (370 MBq, SA > 18.5 GBq/μmol) PET scan.

In protocol 2 and 3, a low-dose CT was followed by a 60-minutes dynamic ^{18}F-afatinib PET scan. Subsequently, a whole body low-dose CT and a whole body 30-minute ^{18}F-afatinib emission scan (3 min per bed position) were performed, fully overlapping from the top of the skull to mid-thighs to ensure that potential metastasis are in the field of view [24].

Four patients underwent scanning protocol 1. Five patients underwent scanning protocol 2. and 3 patients underwent scanning protocol 3.

All PET scans were acquired in list-mode. Whole body PET scans were reconstructed using BLOB-OS-TF (Philips default algorithm) and dynamic PET scans were reconstructed using a 3-dimensional row-action maximum-likelihood algorithm into time frames with progressive increase in frame duration [25]. For the 90 min dynamic ^{18}F-afatinib scan (protocol 1), 22 frames were used (1 × 15, 3 × 5, 3 × 10, 4 × 60, 2 × 150, 2 × 300, 7 × 600 s). For the 60 min dynamic ^{18}F-afatinib scan (protocol 2) 19 frames were used (1 × 15, 3 × 5, 3 × 10, 4 × 60, 2 × 150, 2 × 300 and 4 × 600 s). Dynamic and whole body reconstructions included all usual corrections, such as detector normalization, and decay, dead time, attenuation, randoms, and scatter corrections. Resulting images consisted of 4 × 4 × 4 mm voxels with a resolution of about 5 mm FWHM (full with at half maximum).

2.5. Parameter acquisition

All volumes of interest (VOIs) were defined manually for each individual tumor using software developed in-house. VOI definitions of tumors were based primarily on acquired CT images with images projected in parallel, avoiding necrosis and blood vessels as much as possible. If tracer uptake was visually similar outside a 0.5 cm margin of the tumor, 0.5 cm was added to the VOI.

2.6. Uptake parameter selection

The uptake parameter for quantifying tumor ^{18}F-afatinib uptake was TBR_WB_{60-90}, i.e. tumor-to-blood ratios based on summed images obtained from 60−90 min post-injection from both dynamic and static scans. TBR_WB_{60-90} has been validated for ^{18}F-afatinib against the uptake parameter Ki as obtained through pharmacokinetic modeling by van de Stadt et al. [22]. Liver metastases were excluded because of the spill-over effect of the high-to-very-high background activity concentrations. Tracer uptake was assessed in all lesions, group comparison was performed using the calculated TBR_WB_{60-90} values of both the primary tumor lesion and the tumor lesion with the highest uptake. This was done to ensure that group difference analysis was not biased in a situation where the primary tumor did not show the highest tracer uptake.

2.7. Treatment response

Radiological tumor response to afatinib therapy was measured as a percentage of change (PC) for each tumor, where PC was defined as [(response measurement (RM) – baseline measurement (BM)) / BM] * 100 %. This is done in accordance to RECIST v1.1. BM was prior to start of afatinib therapy. For each tumor lesion in the field of view (FoV), the longest diameter was measured using the low-dose CT that was acquired as part of the ^{18}F-afatinib PET study protocol [26]. Immediately after the PET protocol, therapy was started by orally dosing 40 mg once a day. Radiological response was assessed 4–6 weeks after starting therapy, when patients underwent a contrast-enhanced (Jobitridol) CT thorax-abdomen for treatment evaluation. Results were expressed as PC, where a negative PC indicates tumor decrease (response to treatment) and a positive value indicates tumor increase (no response to treatment). Progression-free survival (PFS) was determined from the date of start of afatinib treatment to the date of disease progression, according to RECIST v1.1.

2.8. EGFR mutational status

All biopsies were assessed for activating mutations of the EGFR gene using next-generation sequencing (NGS). Briefly, the EGFR gene samples were amplified using polymerase chain reactions (PCR). Next, the PCR products were purified and sequenced. Lastly, the PCR products were analyzed by a DNA sequencer for mutations [27].

2.9. Statistical analysis

All kinetic analyses were performed using software developed in-house. Descriptive statistical analyses were performed to assess and report endpoints. Tests to assess normality of the uptake parameter were performed using Q-Q plots. Normal distribution of the uptake parameter was confirmed and therefore T-tests were performed to assess group differences. Group difference analysis was performed twice: once using the primary tumor of each patient, and also by using the tumor with the highest uptake within each patient. A TBR_WB_{60-90} cut-off value to correlate whether tumors would respond to afatinib therapy or not was assessed both visually and statistically, using the Fisher's exact test. Sensitivity, specificity, positive predictive values and negative predictive values were obtained. To optimize clinical usability, we determined the best cut-off value for TBR_WB_{60-90} with a high specificity and positive predictive value. All analyses were performed using IBM SPSS (version 26).

3. Results

3.1. Baseline patient characteristics

Twelve consecutive evaluable patients were included in this study. 6 patients were EGFR-wild type (WT), 3 patients had an EGFR common mutation (EGFR com) and 3 patients had an EGFR uncommon mutation (EGFR uncom). No resistance mutations were found. Patient characteristics are provided in Table 1. Unintentionally, only exon 19 deletions were included in the EGFR common group. Tracer injection showed no adverse or clinically detectable effects in any of the subjects. Typical images are shown in Fig. 1, both of a wild type patient (patient 11) and a mutated patient (patient 8).

3.2. Baseline tumor characteristics

In total twenty-one tumor VOIs were included in this analysis. Characteristics of each VOI are described in Table 2, including TBR_WB_{60-90} values and PC. PC is missing in patients 7 and 10 because patients died before we were able to measure a response. Furthermore, 2 tumor VOIs were bone metastases within the vertebrae (VOIs 0802 and 0803, VOI number 2 and 3 respectively from patient 8). Response measurements could not be performed on those lesions as the soft tissue parts of these metastases within the vertebrae could not be delineated and were therefore left out of the response measurement analysis.

3.3. Group differences

TBR_WB_{60-90} values were significantly different between the WT group (median 3.25, interquartile range (IQR) 2.1–5.2) and the EGFR common group (median 7.6, IQR 5.1–9.3) for the primary lesions ($p = 0.03$) and highest tumor lesions ($p = 0.04$), respectively; and between the WT versus all mutations (median 6.2, IQR 3.8–8.4) for the primary lesions ($p = 0.03$) and highest tumor lesions ($p = 0.04$), respectively. No significant difference was found between the mutation groups ($p = 0.93$ and 0.94). The difference between the WT versus the EGFR uncommon group showed a positive trend, but was not significant using the primary tumor ($p = 0.08$) and highest uptake tumor ($p = 0.11$).

Table 1
Baseline characteristics.

Patient nr	Gender	Age (y)	EGFR group	EGFR mutation	Afatinib line of treatment
1	F	68	1	N/A	1
2	M	51	2	Exon 19 deletion	1
3	M	69	2	Exon 19 deletion	1
4	F	57	3	Exon 19 L747P insertion	1
5	F	54	3	Exon 18 G719A point mutation	1
6	M	69	1	N/A	3
7	M	61	1	N/A	4
8	F	47	2	Exon 19 deletion	1
9	F	70	3	Exon 18 G709 T deletion	1
10	M	80	1	N/A	3
11	M	78	1	N/A	4
12	M	54	1	N/A	4

Notes: EGFR group: 1 is the wild type group, 2 is the common activating EGFR mutations group and 3 is the uncommon activating EGFR mutations group.

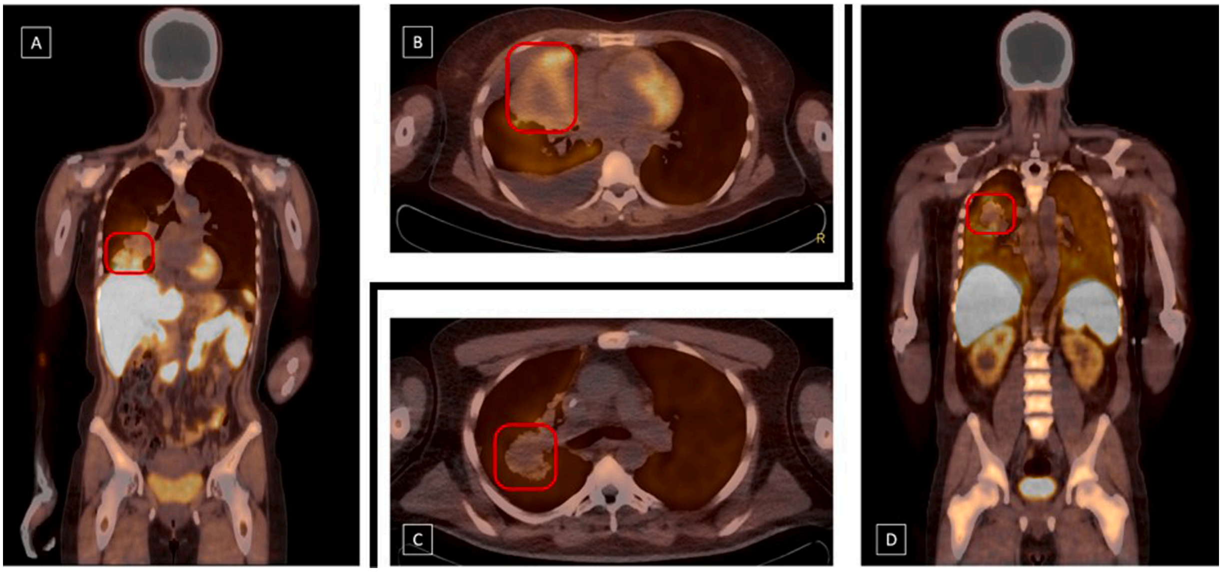

Fig. 1. Typical PET/CT images of 2 NSCLC patients are shown. The coronal (A) and axial (B) PET/CT images of a 48-year-old female patient with an EGFR exon 19 deletion show an increased tracer tumor uptake, corresponding with a tumor $TBR\_WB_{60\text{-}90}$ of 6.15. The tracer uptake is higher near the edges of the tumor, possibly due to central necrosis. This tumor responded well to afatinib therapy with a 60 % decrease of the largest tumor diameter. The axial (C) and coronal (D) PET/CT images of a 54-year old male patient with an EGFR wild type tumor. The tumor tracer uptake, as measured by $TBR\_WB_{60\text{-}90}$ was 5.0. This tumor did not respond to afatinib treatment with 52 % increase of tumor diameter after 6 weeks.

Table 2
Tumor characteristics and results.

EGFR group	Patient number	VOI number	Tumor type	Tumor size baseline (mm)	TBR_WB_{60-90}	PC (%)
1	01	01	Primary	28	2.1	+ 4
1	06	01	Primary	58	3.9	+ 5
1	07	01	Primary	49	2.6	N/A
1	10	01	Primary	72	1.6	N/A
1	11	01	Primary	48	2.0	+ 6
1	12	01	Primary	46	5.0	+ 52
1	12	02	Metastasis	11	5.7	+ 132
1	12	03	Metastasis	15	5.95	+ 124
2	02	01	Primary	21	4.1	−49
2	03	01	Metastasis	31	10.7	−34
2	03	02	Metastasis	36	8.3	−26
2	03	03	Primary	45	10.3	−24
2	08	01	Primary	79	6.15	−60
2	08	02	Metastasis	18	3.8	N/A
2	08	03	Metastasis	18	7.6	N/A
3	04	01	Metastasis	22	8.4	−30
3	04	02	Metastasis	20	3.3	−5
3	04	03	Primary	64	12.7	−16
3	05	01	Primary	47	2.6	−7
3	09	01	Primary	19	6.2	−20
3	09	02	Metastasis	42	1.9	−16

Notes: EGFR group: 1 is the wild type group, 2 is the common activating EGFR mutations group and 3 is the uncommon activating EGFR mutations group.

3.4. Tumor tracer uptake versus response to afatinib therapy

Correlation of tumor ^{18}F-afatinib uptake using TBR_WB_{60-90} and radiological tumor response, as PC, is shown in Fig. 2 for all tumor VOIs of patients that underwent response measurement. Two patients (7 and 10) died before response measurement could take place. WT tumors showed tumor growth under afatinib therapy whereas the common and uncommon activating mutation positive tumors showed a decrease in tumor diameter. WT tumors showed TBR_WB_{60-90} values lower than 6, and no treatment response. EGFR mutation positive tumors showed TBR values above and below 6, with a median value of 6.2, with treatment response. We explored a cut-off value of 6, but also 5 and 7, to discriminate between responding and non-responding tumors. This analysis confirmed that 6 was the preferred cut-off value with a p-value of 0.01, whereas a cut-off of 5 and 7 show p values of 0.30 and 0.09, respectively (see Supplement II, Tables 1–3).

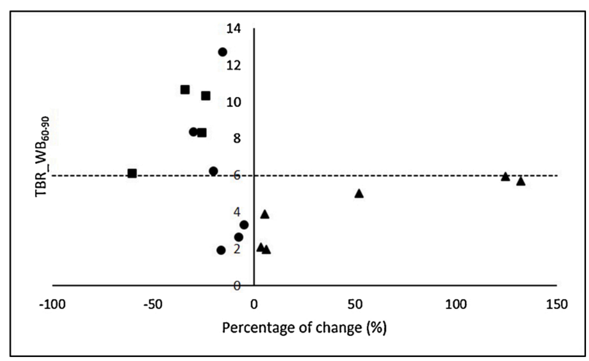

Fig. 2. PC < 0 represents tumor decrease, indicative for treatment response. PC > 0 represents tumor growth. **Triangles** represent EGRF wild type tumors, **squares** represent common activating EGFR mutations and **circles** represent uncommon activating EGFR mutations. The **dotted line** represents the cut-off TBR value of 6.

The cut-off value of 6 showed a sensitivity of 70 % and a specificity of 100 % (see Table 2 Supplement II). Positive predictive value was 100 %, indicating that no false-positives occurred.

The negative predictive value is 67 %, which means that in 67 % percent of the tumors with a TBR < 6, no response was seen. Therefore, a TBR of < 6 was not indicative for either response or no response. Different cut-off values have also been evaluated (see Tables 1–3 in Supplement II). In case of a cut-off value of 7, specificity remained 100 % but sensitivity dropped to 50 %. With a cut-off value of 5, sensitivity was 70 % but specificity dropped to 50 % (see Table 1 Supplement II). Tables containing the evaluations of all values are provided in Supplement II.

PFS is shown in Fig. 3. Both the presence of activating EGFR mutations and a TBR below 6 were predictive for PFS. This indicates that not only radiological response on the level of individual lesions can be predicted by the TBR cut-off value of 6 but also the prediction of response on patient level as measured by PFS is comparable to EGFR mutational status.

4. Discussion

This EGFR TKI PET study, using ^{18}F-afatinib PET in patients who were subsequently treated with afatinib, showed that the tumor uptake of ^{18}F-afatinib was different between tumors harboring a wild type EGFR and those with activating EGFR mutations. Furthermore, high ^{18}F-afatinib uptake in tumors, defined as a tumor TBR_WB_{60-90} value above 6, enabled identifying patients that radiologically responded to afatinib therapy.

4.1. Group differences

EGFR TKI compete with ATP for binding at the ATP binding pocket of the EGF receptor. Affinity for EGFR TKI is higher when the kinase domain of the EGF receptor is in the active conformation. If an activating mutation is present, the structure of the kinase disrupts interactions that stabilize the inactive conformation, leading to activation (up to 50-fold when compared to wild-type EGFR). EGFR TKI bind tighter to active kinase domains versus inactive, leading to an affinity increase of up to 100-fold of the mutated EGF receptors for EGFR TKI than for ATP when compared to wild type EGFR [13]. This difference in affinity may explain the difference in ^{18}F-afatinib uptake between EGFR wild type and activating EGFR mutation positive tumors [4]. Accordingly, uptake of ^{18}F-afatinib and radiological response to treatment did not differ between common activating and uncommon activating EGFR mutated tumors since both tumor types are TKI-sensitive.

4.2. Correlation between tumor tracer uptake and response

Tumor EGFR mutations are the strongest predictor for response to EGFR TKI therapy [4,11,13,28]. In our study, all patients with EGFR mutations (both common and uncommon) showed radiological tumor regression on afatinib therapy, while patients with a wild type EGFR showed tumor progression. We demonstrated that a high ^{18}F-afatinib uptake (i.e. a TBR_WB_{60-90} above 6) was associated with radiological response. However, not all responding tumors had a high ^{18}F-afatinib uptake. Here, lower uptake may be due to the partial volume effect. The EGFR mutated tumors were generally smaller with a mean 35,5 versus 45,1 in the wild type group. This can explain why a low ^{18}F-afatinib can still be associated with a tumor response to afatinib, while a high ^{18}F-afatinib uptake can be a strong predictor for response.

In addition, two patients (with WT tumors) could not be accounted in the analysis as they did not undergo response measurements because they progressed clinically and died before the response CT could be performed. However, if we would consider these patients to be true negatives, as they suffered fast progression, this would result in an even larger negative predictive value of 64 % instead of 56 %, as their TBR_WB_{60-90} values was also below 6.

4.3. Limitations

In this study, we have used the simplified measure TBR_WB_{60-90} as uptake parameter, instead of the more accurate net influx rate (k_i), which is obtained through dynamic scanning and accounting for metabolites. However, k_i has limitations when used in daily clinical practice, as it requires complex dynamic scanning with complex analysis protocols, also dynamic scans are limited to only a small field of view, missing distant metastases. Previously, we have shown that TBR_WB_{60-90} has a strong correlation with k_i [22,29]. Ultimately, for routine use, TBR_WB_{60-90} appears to be a more robust and practical measure than k_i.

Only a limited number of patients were included. However, this study was designed as a proof-of-concept study and was able to show in these relatively small groups that tumor tracer uptake was significantly different in EGFR wild type tumors as compared to EGFR activating mutation positive (common and uncommon) tumors. However, it should be noted that we did not include exon 21 mutations and we therefore cannot conclude that our results are also valid in this subgroup. Larger studies are needed to validate the observed results.

4.4. Future directions

The results of our study are in line with other EGFR TKI PET studies [17,18,20,30]. However, larger studies are needed to substantiate and validate these results using ^{18}F-afatinib with TBR_WB_{60-90} as uptake parameter. Afatinib has the potential to become a PET tracer for routine clinical use, as it has the advantage to be radiolabeled inertly using a ^{18}F atom through substitution of a constitutive fluorine atom. The resulting ^{18}F-afatinib has a longer half-life as compared to tracers labeled with the short-lived ^{11}C (e.g. ^{11}C-erlotinib), allowing shipment to other scanning facilities.

This tracer could be helpful in predicting TKI sensitivity of uncommon EGFR mutations. Apart from the major uncommon mutations (i.e. G719X, S768I, L861Q), there are numerous other rare EGFR mutations with unclear TKI sensitivity. A prospective study comprising a baseline ^{18}F-afatinib PET and subsequent afatinib treatment could explore the usefulness of this tracer in these rare mutations.

Furthermore, ^{18}F-afatinib PET may be developed into a clinical tool for predicting interlesional heterogeneity for TKI sensitivity at baseline and during TKI therapy. For example, single lesions that show less tumor tracer uptake may be treated with additional local treatments such as

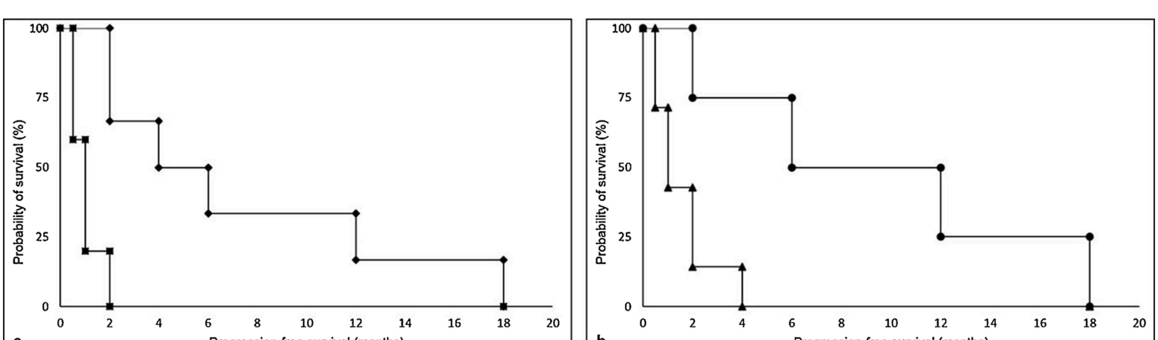

Fig. 3. **3a** depicts Kaplan-Meier survival curves stratified by EGFR mutational status. **Diamonds** represent EGFR mutation positive patients, **squares** represent EGFR wild type patients ($p = 0.01$). **3b** depicts PFS stratified by TBR value. **Circles** represent patients with tumors showing a TBR > 6, **triangles** represent patients with tumors showing TBR < 6 ($p < 0.01$).

SABR, or combinatorial systemic treatment may be envisaged if multiple lesions show lower sensitivity.

Low tracer uptake in afatinib-sensitive tumors is not well understood. Partial volume effects due to breathing motions, tumor volume effects, pathological composition of lesions, intralesional heterogeneity regarding viable tumor cells and mutation positive EGFR expression may cause low uptake in these tumors. Therefore, future studies would also need to focus on understanding the mechanisms that lead to low tracer uptake in sensitive tumors.

5. Conclusion

^{18}F-afatinib uptake was higher in tumors with an activating EGFR mutation as compared to tumors harboring a wild type EGFR. No difference was observed between common and uncommon activating EGFR mutations. High tumor ^{18}F-afatinib uptake ($TBR\_WB_{60-90} > 6$) was predictive of afatinib treatment response. ^{18}F-afatinib PET/CT is a potential means to identify patients who benefit from afatinib therapy.

Financial support

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Medical Ethics Committee of the VU university medical center.

Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

Eveline A. van de Stadt: Conceptualization, Formal analysis, Investigation, Writing - original draft, Data curation, Visualization, Project administration. **Maqsood Yaqub:** Conceptualization, Methodology, Software, Writing - review & editing, Supervision. **Adriaan A. Lammertsma:** Conceptualization, Methodology. **Alex J. Poot:** Resources. **Robert C. Schuit:** Resources, Writing - review & editing. **Sharon Remmelzwaal:** Formal analysis, Writing - review & editing. **Lothar A. Schwarte:** Resources, Writing - review & editing. **Egbert F. Smit:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Harry Hendrikse:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision. **Idris Bahce:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2021.03.016.

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