

VU Research Portal

Tumor characterization using radiolabeled anti-cancer drugs in NSCLC patients

Bahce, I.

2017

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Bahce, I. (2017). *Tumor characterization using radiolabeled anti-cancer drugs in NSCLC patients*.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

Discussion

The studies described in this thesis aimed to investigate the potential of PET, using radiolabeled targeting agents directed against NSCLC tumors, as a modality to characterize these tumors and determine their sensitivity to the corresponding targeting agents. For these proof of concept studies [^{11}C]erlotinib was used as a TKI tracer and [^{89}Zr]bevacizumab as a labeled mAb. The selection of [^{11}C]erlotinib as a TKI PET tracer was based on the fact that erlotinib is one of the most commonly used TKI in NSCLC. Similarly, [^{89}Zr]bevacizumab was selected as a immunoPET tracer as it is commonly used in clinical practice.

Chapter 2

Chapter 2 comprises a review of the current literature on TKI PET and immunoPET in NSCLC. This review also includes all studies described in this thesis.

This review shows that in NSCLC patients, so far only EGFR TKIs were used in published TKI PET studies. The two most studied radiolabeled EGFR TKI are [^{11}C]PD153035 and [^{11}C]erlotinib. Preclinically, these tracers have consistently demonstrated high uptake in tumors with activating EGFR mutations, indicating that increased affinity of mutated EGFR to TKIs can be imaged. In clinical pilot studies, visualizing and quantifying tumor uptake was possible with both tracers. In addition, significant correlations were found between high tumor tracer uptake and improved therapeutic outcome on erlotinib treatment. However, as these tracers are labeled with the short-lived carbon-11 isotope, they are not suitable for widespread clinical use. To circumvent this limitation, fluorine-18 labeled second generation irreversibly binding EGFR TKI, e.g. [^{18}F]F-PEG6-IPQA and [^{18}F]afatinib, are being studied. In preclinical studies, these tracers did show better tumor-to-background ratios as compared to [^{11}C]PD153035 and [^{11}C]erlotinib, respectively. Clinical studies using these fluorine-18 labeled tracers are ongoing.

Furthermore, this review shows that preclinical immunoPET studies were performed using radiolabeled mAbs directed against all currently actionable mAb targets in NSCLC, i.e. EGFR, VEGF-A, VEGFR2 and PD-1. For all these targets, immunoPET showed its capacity to image target expression. Nevertheless, only a very limited number of clinical studies have been published using radiolabeled mAbs that are of interest in the treatment of NSCLC. In fact, nearly all clinical studies used [^{89}Zr]bevacizumab and demonstrated that tumor uptake could be visualized and quantified. Although various interesting correlations were found between tumor [^{89}Zr]bevacizumab uptake and clinical outcome, the predictive and prognostic value of imaging tumor VEGF-A with immunoPET is still unclear.

Chapter 3

The clinical study in chapter 3 describes the first-in-human TKI PET study that aimed to investigate the effect of activating EGFR mutations on tumor uptake of [^{11}C]erlotinib, by using full tracer kinetic modeling with continuous and discrete arterial sampling, and metabolite analysis. This elaborate analysis of the PET data was needed to establish the best tracer kinetic model and subsequently calculate the most accurate tracer accumulation value. The experimental design of this study included 10 patients with NSCLC, i.e. 5 with an EGFR exon 19 deletion and 5 without. Patients were scanned twice (to evaluate test-retest variability) on the same day. Each scanning procedure included a low dose CT scan, a dynamic [^{15}O]H₂O PET scan and a dynamic [^{11}C]erlotinib PET scan.

Tracer kinetic modeling showed a preference for the reversible two tissue compartment model with metabolite corrected arterial plasma input function (2T4k), with volume of distribution (V_T) as the quantitative measure of [^{11}C]erlotinib uptake. In this study, tumor V_T was significantly higher in tumors with activating EGFR mutations than in those without. Reproducibility of tumor V_T values was good. The higher tumor uptake in the mutated group is in accordance with preclinical studies, and was attributed to increased specific binding due to the increased affinity of the mutated EGFR molecule for erlotinib. Potential confounding variables, such as EGFR expression levels or tumor blood flow as measured with [^{15}O]H₂O, did not contribute to this effect, as they were not correlated with the intergroup V_T difference. This study highlights the potential capacity of TKI PET for imaging the affinity of target molecules for TKI.

Chapter 4

Chapter 4 provides an extensive tracer kinetic modeling study of [^{11}C]erlotinib. Compared with the tracer kinetic modeling aspect of the previous study, this methodological study included more patients, fitted the PET data using a larger number of kinetic models, and additionally evaluated simplified models to assess whether they could be used in future clinical whole body [^{11}C]erlotinib scans. Dynamic [^{15}O]H₂O and [^{11}C]erlotinib scans were available for 17 NSCLC patients, 8 with and 9 without an activating EGFR mutation. For 10 of the 17 patients, a retest scan on the same day was available. Kinetic modeling included single tissue and two tissue irreversible and reversible plasma input models. In addition, several advanced models that account for uptake of radiolabeled metabolites were evaluated, including a model where no correction for radiolabeled metabolites in plasma was performed. Simplified methods consisted of standardized uptake value (SUV) and tumor-to-blood ratio (TBR) for several scan intervals.

This study demonstrated that tumor kinetics were best described using the reversible two tissue compartment model without correcting the arterial plasma input function for radiolabeled metabolites (2T4k-WP), yielding optimal fits to the data, acceptable test–retest variability, no dependence on perfusion changes, and differentiating between the two clinical groups. V_T values, estimated using 2T4k-WP and 2T4k (used in Chapter 3), were highly correlated, and similar test–retest variability and separation between clinical groups were obtained. The 2T4k model did not perform better than the uncorrected model (2T4k-WP), which was probably caused by uncertainty in the estimation of true metabolite fractions. Investigation of simplified approaches showed that SUV curves did not reach equilibrium within the time of the scan. In contrast, TBR normalized to whole blood (TBR-WB) for the 40–60 min interval best correlated with V_T derived from 2T4k-WP. This indicates that static [^{11}C]erlotinib scans are best performed 40–60 minutes post injection using TBR-WB as semi-quantitative measure of uptake.

Chapter 5

Chapter 5 describes a study investigating the effects of oral erlotinib therapy on [^{11}C]erlotinib kinetics and tumor uptake. This study also looked at the effects of erlotinib therapy on tumor blood flow and its correlation with tumor [^{11}C]erlotinib uptake. In addition, the simplified uptake parameters SUV and TBR were investigated again. This time, however, also TBR values based on venous blood samples were compared with corresponding values based on arterial samples in order to investigate whether arterial cannulation can be omitted from the scanning procedure.

In this study, 10 out of the 13 patients included were scanned twice within a 1 to 2 weeks interval, i.e. on and off erlotinib therapy. Each procedure consisted of a low-dose CT scan, a dynamic [^{15}O]H₂O PET scan, and a dynamic [^{11}C]erlotinib PET scan with arterial and venous sampling at six time points. On therapy, tumor tracer kinetics again were best fitted using the 2T4k model, with V_T as measure of uptake. In all patients, tumor V_T was lower on therapy than off therapy. This finding, which is consistent with preclinical "blocking" studies, probably was due to a decrease in available binding sites under erlotinib therapy, because abundantly present non-labeled erlotinib occupies the ATP binding pockets of the EGFR molecules. This finding was independent of tumor perfusion, as perfusion measured by [^{15}O]H₂O remained unchanged during erlotinib therapy.

Both arterial and venous TBR values in the 40–50 and 50–60 min p.i. intervals were found to be strongly correlated with tumor V_T , whilst SUV did not correlate with V_T , confirming and extending the results of the previous chapter to a data set with

reduced V_T . Although there was a strong correlation between venous and arterial TBR, venous TBR was consistently lower than arterial TBR, indicating that arterial and venous values should not be interchanged.

Chapter 6

Chapter 6 presents a case report of a patient with a right upper lobe tumor, harboring an activating EGFR mutation, who underwent 2 dynamic [^{11}C]erlotinib scans over the course of her disease history. Her first PET scan, prior to initiation of erlotinib therapy, showed homogeneously elevated tracer uptake in the tumor. She responded well to erlotinib therapy and remained free of disease progression for 18 months, supporting the notion that elevated tumor [^{11}C]erlotinib uptake was associated with erlotinib efficacy. Then, she developed progression at the site of the primary tumor and a second [^{11}C]erlotinib scan was performed after discontinuation of erlotinib therapy. This latter scan showed an overall decrease in tumor [^{11}C]erlotinib uptake. In addition, the uptake pattern was heterogeneous. A biopsy from a low uptake region of the tumor revealed the presence of the secondary resistance mutation T790M. These findings support the concept that TKI PET can image (regional) tumor sensitivity and resistance to a TKI due to differences in EGFR affinity for that TKI.

Chapter 7

Chapter 7 describes the first-in-human immunoPET study using [^{89}Zr]bevacizumab in NSCLC patients who were scheduled to be treated with a bevacizumab containing regimen. This study aimed to assess whether [^{89}Zr]bevacizumab tumor uptake could be visualized and quantified, and to explore whether uptake was correlated with clinical outcome. Seven NSCLC patients underwent 2 static PET scans, i.e. at days 4 and 7 after injection of [^{89}Zr]bevacizumab. Tumor tracer uptake, quantified using peak standardized uptake values (SUV_{peak}), was found to be approximately 4 times higher than in non-tumorous background tissues, both on days 4 and 7. This means that tumors could be clearly visualized within the non-tumorous background. Although VEGF-A, the target for [^{89}Zr]bevacizumab, is considered to be a soluble and freely circulating ligand, the elevated tumor uptake supports the notion that VEGF-A concentrations are higher in tumor areas due to high paracrine expression and subsequent binding to extracellular matrix glycoproteins, such as heparan sulfate proteoglycans and neuropilins. These glycoproteins act as non-signaling co-receptors that facilitate binding of VEGF-A to VEGFR molecules. Another possible mechanism may be the internalization of [^{89}Zr]bevacizumab into cells within the tumor. After internalization, the ^{89}Zr label may become trapped in the lysosomes and show up on the PET scan. A positive trend but no significant correlation could be found between SUV_{peak} and overall

survival or progression free survival to bevacizumab containing chemotherapy. This indicates that more research is needed to clarify whether [⁸⁹Zr]bevacizumab uptake is predictive for bevacizumab efficacy.

Future aspects

[¹¹C]erlotinib and TKI PET

The studies in this thesis added new information to the existing literature on [¹¹C]erlotinib PET. Briefly, NSCLC tumors with EGFR mutations were shown to have a higher [¹¹C]erlotinib uptake as compared to those without. Also, tumor [¹¹C]erlotinib uptake was shown to decrease when patients were on erlotinib therapy. Furthermore, analysis of simplified uptake models have shown that TBR (arterial or venous) 40 to 60 min p.i. correlated best with V_T , measured with the gold standard method.

The advantage of using a simplified uptake measure such as TBR as opposed to V_T is the possibility of performing whole body PET scans. However, at present, it is unclear whether TBR is sufficiently accurate to discriminate between patient groups with different sensitivities to erlotinib. Another unclear item is whether TBR is able to identify heterogeneity in uptake between and within tumor lesions.

To address these items, a clinical study is currently ongoing at the VUmc. This study is including 3 groups of EGFR-mutated NSCLC patients with different sensitivities to erlotinib, i.e. (1) TKI-naïves prior to start of TKI, (2) after disease progression under TKI, and (3) after a TKI-holiday and prior to re-treatment with TKI. Only patients with at least 2 separate tumor lesions are included, the response to erlotinib therapy of each lesion is monitored. All patients undergo a static whole body scan using TBR 40-88 min p.i. as a measure of uptake. This study will allow to assess whether TBR can discriminate differences in erlotinib sensitivity between patient groups, and within patients.

Clinically, [¹¹C]erlotinib PET can be used in the initial diagnosis of patients with a suspicion of EGFR mutated NSCLC where no pathology proof is available. Also, whole body [¹¹C]erlotinib PET can be useful to assess residual sensitivity in different tumor lesions after disease (oligo)progression.

Although whole body [¹¹C]erlotinib PET may offer these advantages, its use will be limited to PET centers that can produce this tracer, as shipment to remote centers will not be possible due the rapid decay of carbon-11 radioactivity.

To circumvent this limitation, fluorine-18, an isotope with a longer radioactivity half-life, can be used to label EGFR TKI. Afatinib allows for the synthesis of an *in vivo* stable tracer, i.e. [¹⁸F]afatinib, by substituting its constitutive fluorine atom for the fluorine-18 isotope. A clinical study using [¹⁸F]afatinib is currently ongoing at the VUmc. Here, the optimal pharmacokinetic model, test-retest variability, the optimal simplified model for static scanning and the optimal timing for static scanning is assessed. Four groups of patients are being included, i.e. (1) TKI-naïve patients without EGFR mutations, (2) TKI-naïves with EGFR mutations, (3) TKI-resistant patients with EGFR and T790M mutations, and (4) TKI-resistant with EGFR and no T790M mutations. The accuracy of [¹⁸F]afatinib for discriminating afatinib sensitivity (using full kinetic models and simplified models) will be assessed between patient groups and within patients.

Besides EGFR, future TKI PET studies should investigate TKIs directed against other NSCLC targets such as ALK, BRAF, ROS1, RET, and MET. For example, crizotinib is a very interesting candidate. It is a multi-target TKI used in the treatment of ALK rearranged NSCLC, however, it has also activity against ROS1 and MET. Theoretically, in these patients (harboring aberrations in ALK, ROS1 and MET), radiolabeled crizotinib and PET may predict therapy response irrespective of the sensitizing DNA aberration of the tumor. This could lead to a whole new way of personalizing therapy without necessarily performing tumor molecular analysis.

[⁸⁹Zr]bevacizumab and immunPET

The [⁸⁹Zr]bevacizumab study in this thesis showed that uptake of [⁸⁹Zr]bevacizumab in NSCLC tumors could be visualized and quantified. Although there was an encouraging positive trend between tumor [⁸⁹Zr]bevacizumab uptake and therapy outcome using a bevacizumab containing regimen, the predictive value of tumor uptake is still unclear.

In this study, patients were scanned at 2 time points, for future studies that intend to scan at a single time point, the optimal timing needs to be determined. Also, whether additional blood sampling may help quantification is unclear. Furthermore, the level of tumor VEGF-A was not measured, so, this could not be correlated to tumor tracer uptake.

To assess the above raised items, particularly, to assess the prognostic and predictive value of tumor [⁸⁹Zr]bevacizumab uptake, a larger clinical trial is needed.

In routine clinical practice, [⁸⁹Zr]bevacizumab PET could provide for a predictive biomarker to identify patient subgroups or lesions within patients that are sensitive to bevacizumab therapy.

If validated as a predictive biomarker, its many practical advantages, such as easy to produce stable and shippable tracers, and the possibility for whole body scanning protocols using simplified parameters for easy uptake measurement, can facilitate a broad clinical application of this technique.

However, the clinical success of any immunoPET tracer will depend on how tracer uptake will relate to target expression and affinity, and how predictive target expression will be for therapy efficacy of the studied antibody. This seems very promising for cancer immuno therapy. For example, the expression levels of PD1 and PD-L1 on tumor infiltrating T-cells or tumor cells, respectively, although not exclusionary, are indicative of an improved immunotherapy outcome in patients with non-squamous NSCLC. ImmunoPET could provide a noninvasive means for repeated whole-body scanning to guide treatment decision management, hereby overcoming some of the limitations associated with the use of the current pathology-based PD1 and PD-L1 expression markers, such as variabilities in tissue preparation and processing, differences between primary tumor biopsies versus metastatic biopsies, and intrinsic versus induced PD1/PD-L1 expression levels that may change over time. This should be investigated in future immunoPET studies.