Identifying non-small-cell lung tumours bearing the T790M EGFR TKI resistance mutation using PET imaging.

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<th>Journal:</th>
<th>Journal of Labelled Compounds and Radiopharmaceuticals</th>
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<tr>
<td>Manuscript ID</td>
<td>JLCR-19-0038.R2</td>
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<tr>
<td>Wiley - Manuscript type:</td>
<td>Research Article</td>
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<tr>
<td>Date Submitted by the Author:</td>
<td>n/a</td>
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<tr>
<td>Complete List of Authors:</td>
<td>Goggi, Julian; Singapore Bioimaging Consortium, IMIL Haslop, Anna; Singapore Bioimaging Consortium, LMC Ramasamy, Boominthan; Singapore Bioimaging Consortium, IMIL Cheng, Peter; Singapore Bioimaging Consortium, IMIL Jiang, Lingfan; Singapore Bioimaging Consortium, LMC Soh, Vanessa; Singapore Bioimaging Consortium, LMC Robins, Edward; Singapore Bioimaging Consortium, LMC; National University Singapore Yong Loo Lin School of Medicine, Clinical Imaging Research Centre</td>
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<tr>
<td>Primary Keywords:</td>
<td>PET diagnostics</td>
</tr>
<tr>
<td>Secondary Keywords:</td>
<td>F-18</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Non small-cell lung cancer (NSCLC), Tyrosine kinase inhibitor (TKI), Resistance, Epidermal growth factor receptor (EGFR) mutation</td>
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Identifying non-small-cell lung tumours bearing the T790M EGFR TKI resistance mutation using PET imaging.

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Running title: Imaging the T790M EGFR TKI resistance mutation in NSCLC
Abstract
Specific mutations have been shown to significantly improve response to epidermal growth factor tyrosine kinase inhibitor (EGFR-TKI) treatment in patients with lung cancer. Identifying patients with these mutations, however, remains a major clinical challenge. Radiolabelled EGFR-TKIs have successfully identified tumours bearing mutations that confer sensitivity to EGFR-TKIs using PET. Unfortunately, these do not detect tumours with the acquired EGFR T790M mutation which conveys resistance to standard TKIs. However, new EGFR TKIs specific for T790M have been developed and provide hope for patients with this acquired mutation, necessitating the development of PET radiopharmaceuticals to identify those patients who may benefit from targeted EGFR-TKI therapy. In the present study we attempt to radiolabel WZ4002 in order to assess the specificity of binding to tumours with the T790M mutation in vivo. [18F]FEWZ was assessed in vitro to determine efficacy relative to the starting compound and in vivo to measure the biodistribution and specificity of binding to EGFR wild type, L858R and T790M bearing tumours. [18F]FEWZ is the first evidence of a radiolabeled 3rd generation anilinopyrimidine derived tyrosine kinase inhibitor targeting T790M mutation bearing tumours in vivo.

Keywords: Non small-cell lung cancer (NSCLC), epidermal growth factor receptor (EGFR), mutation, resistance, tyrosine kinase inhibitor (TKI)
Introduction
Non-small cell lung cancer (NSCLC) is the most common lung cancer accounting for ~85% of all diagnoses [1]. Epidermal growth factor receptor (EGFR) is highly expressed in NSCLC and strongly implicated in tumor development. EGFR targeted tyrosine kinase inhibitors (EGFR-TKIs) have been developed to compete for to prevent phosphorylation of the ATP-binding site on EGFR and activation of downstream signaling controlling cell proliferation and tumor growth. EGFR TKIs have shown considerable success as a as first-line treatment leading to progression-free survival with little toxicity compared to standard cytotoxic chemotherapy, but only in patients with favourable mutational status. Specific mutations affect the ATP binding domain of EGFR and have been shown to significantly improve response to TKI treatment in patients with lung cancer [2]. Identifying patients with these specific tumour mutations, however, remains a major clinical challenge. Obtaining representative tumor tissue samples by biopsy for mutation analysis is limited by practical issues, such as tumour inaccessibility, the paucity of tumour cells and heterogeneity. Recent studies have suggested that biopsy is not feasible in up to 20% of patients [3]. Molecular testing of circulating tumour DNA provides no information on tumour burden or location and is inherently insensitive [4]. Thus, there is a pressing need to develop non-invasive ways to stratify those patients who would be most likely to benefit from TKI therapy. Positron emission tomography (PET) is uniquely placed to assess patient suitability as it is able to assess receptor binding with incredible sensitivity and specificity allowing assessment of drug binding and delivery. Previous studies have attempted to radiolabel various TKIs in order to develop imaging agents to stratify patients in vivo [5-11]. Numerous first- and second generation EGFR TKIs have been radiolabelled in order to identify tumours that bear activating mutations, del19 (EGFR-del19) and L858 (EGFR-L858R), that infer sensitivity to TKI therapy. One of the earliest, [11C]erlotinib, has shown the ability to detect activating mutations both preclinically [5, 12, 13] and clinically [6, 14]. More recently, [18F]MPG, a PEGylated anilinoquinazoline derivative, has been identified as PET racer capable of determining EGFR activating mutation status. When applied pre-clinically [18F]MPG was able to selectively target human EGFR-activating mutations preclinically both in vitro and in vivo. In the subsequent first clinical trial, [18F]MPG PET was able to discriminate between EGFR-mutant-positive and mutant-negative tumours with a sensitivity of 86.5% and a specificity of 81.8%. Higher tumor uptake of the in [18F]MPG radiopharmaceutical correlated well ( >80%) with therapeutic response to first generation EGFR-tyrosine kinase inhibitor therapies (gefitinib)[15]. Unfortunately, acquired resistance frequently develops after treatment with TKIs, usually within a year. The development of resistance is well correlated with the appearance of a second point mutation in the EGFR TK domain. The EGFR T790M, threonine-790 to methionine point mutation (EGFR-
L858R/T790M) is a resistance mutation that results in a mean survival of less than 2 years after its emergence [1]. The first- and second generation EGFR TKIs that have been radiolabelled are unable to detect tumours with this acquired resistance [16].

The development of new EGFR TKIs which specifically target the T790M resistance mutation, such as osimertinib, provide hope for the treatment of NSCLC with this acquired mutation [17], necessitating the development of new non-invasive in vivo methods to help identify those patients who may specifically benefit from targeted T790M mutation therapy. In the present study we attempted to radiolabel one of these new generation of TKIs, WZ4002, in order to assess the specificity of binding to tumours with the acquired T790M mutation conferring resistance in vivo to standard TKIs such as erlotinib and gefitinib.

Materials and methods

General

All chemicals and solvents (including anhydrous solvents) obtained commercially were of analytical grade and used directly without further purification. [$^{18}$F]Fluoride was produced by the Clinical Imaging Research Center (CIRC) via the $^{18}$O(p,n)$^{18}$F reaction and delivered as [$^{18}$F]fluoride in $^{18}$O-water. Radiosynthesis and azeotropic drying was performed via manual manipulation using a heating block and steady stream of nitrogen. Isolation and purification of [$^{18}$F]FEWZ was carried out on a gradient semi-preparative HPLC system comprising of two Knaur Smartline 1050 pumps, manual injection valve (6-port/3-channel), SmartMix 100 solvent mixer, Smartline UV-Detector 2520 ($\lambda$=254 nm) and Flow-Count radio-HPLC NaI detection system. Quality control analysis was performed on a UFLC Shimazdu HPLC system equipped with dual wavelength UV detector and a NaI/PMT-radiodetector (Flow-Ram, LabLogic). FEWZ reference and desmethyl-WZ4002 (WZOH) compounds were purchased from BioDuro, China.

Radiochemistry

[$^{18}$F]FEWZ

Aqueous [$^{18}$F]fluoride (6-7 GBq in approximately 2 ml) was passed through a resin cartridge (QMA) where the [$^{18}$F]fluoride was trapped. [$^{18}$F]Fluoride was eluted using a mixture of Kryptofix-222 (5 mg, 14.3 $\mu$mol), potassium carbonate (1 mg, 7.2 $\mu$mol), in acetonitrile (0.85 ml) and water (0.15 ml). The solvent was
removed by azeotropic drying at 120°C under a stream of nitrogen (100 ml/min). Afterwards, aliquots of acetonitrile (3 x 0.5 ml) was added, and the distillation was continued (120°C) to dryness. After cooling to room temperature, a solution of ethylene ditosylate (2 mg, 5.4 µmol) in acetonitrile (0.5 ml) was added. The reaction mixture was heated to 90°C for 10 mins prior to purification via semi-preparative HPLC (Phenomenex Luna 5 µm C18 250 x 4.6 mm column, isocratic 70:30 acetonitrile:water mobile phase, flow rate 3 ml/min, t_R = 6.8 – 7.0 minutes). 2-[¹⁸F]Fluoroethyltosylate was then trapped on a C18 light Sep-Pak and eluted with dry DMF (0.4 ml) into a V-vial containing the WZOH precursor (2 mg, 4.2 µmol) in DMF (0.1 ml) with cesium carbonate (5 mg, 15.4 µmol) and dibenzo-21-crown-7 (6.2 mg, 15.4 µmol). The mixture was then heated for 15 minutes at 110°C before the desired product was purified via semi-preparative radio-HPLC (Nucleodur C18 Pyramid 5 µm 250 x 10 mm column, 60% MeCN, 40% ammonium formate (25 mM) in H₂O, 0.1% Et₃N mobile phase, flow rate 3 ml/min, t_R = 12.7 – 14.3 minutes). [¹⁸F]FEWZ was collected in approximately 3 - 5 ml of mobile phase from the semi-preparative radio-HPLC and further diluted through the addition of distilled water (15 ml) before the bulk solution was passed through an activated C-18 Waters Sep-Pak Plus cartridge to retain [¹⁸F]FEWZ. The desired product was eluted from the cartridge with acetone (1 ml) into a sterile 10 ml vial, and then dry N₂ was used to evaporate the acetone solvent to dryness. Ethanol (0.1 ml) and PBS (0.9 ml) was then added and the 1 ml solution passed through a 0.2 µm CA filter to yield the final product. [¹⁸F]FEWZ was identified by comparison with an authentic sample of FEWZ with a retention time (t_R) of 13.6 minutes by analytical radio-HPLC (Phenomenex Luna 5 µm C18(2) column 100Å, column length 250 x 4.6 mm; gradient solvent system 10-90 % acetonitrile with water (0.1% TFA) over 15 minutes, 1 ml/min; λ = 254 nm). [¹⁸F]FEWZ was prepared in a total synthesis time of 120 minutes from [¹⁸F]fluoride, with an activity yield of 2 - 4%, radiochemical purity of ≥97% and molar activity of 37± 21 GBq/µmol.

*Ex vivo* metabolite analysis [¹⁸F]FEWZ

Rat plasma was doped at time 0 with 1 MBq of [¹⁸F]FEWZ and 100 µL samples taken for analysis at 2, 10, 30, 60 and 90 min. An equal amount of acetonitrile was added to the supernatant plasma samples and vortexed to precipitate out remaining proteins. The samples were centrifuged at 2,500 x g for 5 min at 4°C and the supernatant analyzed by analytical radio-HPLC (Phenomenex Luna 5 µm C18(2) column 100Å, column length 250 x 4.6 mm; gradient solvent system 10-90 % acetonitrile with water (0.1% TFA) over 15 minutes, 1 ml/min; λ = 254 nm).
In vivo plasma metabolite analysis of \([^{18}\text{F}]\text{FEWZ}\)

After intravenous injection of the radiopharmaceutical, blood samples (\(\sim 50 \mu\text{L}\)) from the retro-orbital plexus were manually collected into 0.5 ml heparinized polypropylene centrifuge tubes, at 2, 10, 30, 60 and 90 min p.i. Blood samples were centrifuged at 2,500 \(\times\) g for 10 minutes. An equal amount of acetonitrile was added to the supernatant plasma samples and vortexed to precipitate out remaining proteins. The samples were centrifuged at 2,500 \(\times\) g for 5 min at 4\(^\circ\)C and the supernatant analyzed by radio-HPLC (Phenomenex Luna 5 \(\mu\text{m}\) C18(2) column 100Å, column length 250 \(\times\) 4.6 mm; gradient solvent system 10-90 \% acetonitrile with water (0.1\% TFA) over 15 minutes, 1 ml/min; \(\lambda = 254\) nm).

Animal model development

Outbred male nude NCr mice were purchased from in vivos (Singapore). Animal experiments were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines under ethics number IACUC 151089.

Human A549, HCC827 and H1975 NSCLC cells were purchased from the ATCC. Cells were cultured in Dulbecco’s modified Eagle medium (DMEM, Invitrogen), supplemented with 10\% fetal bovine serum, 100 U/ml penicillin and 100 \(\mu\text{g/ml}\) streptomycin, at 37\(^\circ\)C in a humidified atmosphere, with 5\% CO\(_2\). Initial growth kinetics were assessed to ensure optimal tumour growth for imaging, the cells were inoculated subcutaneously at 3-5 million per cell line (depending on growth rate) into alternate shoulders and the mid back of the mice to avoid areas of high background signal due to the hepatobiliary and urinary routes of excretion. At 3 weeks post-inoculation the subcutaneous A549, HCC827 and H1975 NSCLC tumours reached a volume of 200–250 mm\(^3\) and the in vivo assessment was initiated.

In vivo imaging studies

The triple tumor-bearing mice (n=7) were imaged dynamically over 90 minutes post injection with \([^{18}\text{F}]\text{FEWZ}\). The mice were injected with a solution of \([^{18}\text{F}]\text{FEWZ}\) (\(\sim 10\) MBq in 0.2 ml) via the lateral tail vein, and the animals imaged under isoflurane anaesthesia (2\% alveolar concentration) while biological monitoring for respiration and temperature was performed using a BioVet system (m2m imaging, Cleveland, OH). Small-animal PET imaging was performed dynamically from 0 to 90 min post injection (p.i.), on an Inveon PET/CT system (Siemens Inc., Washington DC). Images were generated from sinogram data, rebinned to 2-dimensional format by the Fourier rebinning algorithm, followed by 3-dimensional
ordered subsets expectation maximization reconstruction. Low dose CT images (40 kV, 500 µA; 4×4 binning, 200 µm resolution) were acquired for anatomical information. Images were reconstructed using the image reconstruction, visualization, and analysis program supplied by the manufacturer. PET and CT data were analyzed by using Amide software (Sourceforge 10.3, http://amide.sourceforge.net). The PET and CT images were co-registered, to confirm anatomical location of the tumors. Uptake of radioactivity in the tumor was determined by placement of a Region of Interest (ROI) around the tumor border delineated using the CT images. The tissue concentrations were measured using ROI analysis and are presented as percent injected dose/gram (%ID/g) for tumors, brain, liver, intestine, muscle, lung, kidneys and bone.
Results

Design of [18F]FEWZ

The limited library of 3rd generation TKIs was evaluated for affinity, efficacy and suitability for radiolabeling. WZ4002 was chosen as a potential starting structure for synthesizing a fluorine-18 derivative. WZ4002 is a third generation pyrimidine-based irreversible EGFR TKI. In order to adapt the structure of WZ4002 to develop a PET radiopharmaceutical, it was decided that key features of the molecule were to be left intact and that only minimal functional group substitution should be employed as the inclusion of an additional 18F-moiety could potentially be damaging to the molecule’s affinity for the target. Analysis of the WZ4002-EGFR T790M crystal structure [17] and the molecular conformation within the binding pocket of EGFR tyrosine kinase identified a single site for chemical modification. As such, the acrylamide Michael-acceptor functional group, the chloroanilinopyrimidine core of WZ4002 were left unmodified. It was determined that addition of an 18F-moiety at the methoxy- position shown in Figure 1 should cause the least interference to binding and therefore have the smallest impact on the binding affinity to EGFR T790M. Substitution of the methoxy- functional group for a fluoroethoxy- also provides a straight-forward route for the preparation of the fluorine-18 derivative by O-alkylation of the desmethyl-WZ4002 using 2-[18F]fluoroethyl tosylate.

Synthesis of [18F]FEWZ

Synthesis of [18F]FEWZ was accomplished in a two-step protocol. Starting from 1,2-ethylene ditosylate, 2-[18F]fluoroethyl tosylate ([18F]FETs) was prepared by reaction of the ditosylate with [K(K222)][18F]F- in acetonitrile at 90°C for 10 minutes. Following semi-preparative radio-HPLC purification, the product [18F]FETs was trapped on a C18 Sep-Pak cartridge and eluted in dry DMF. [18F]Fluoroalkylation of WZOH, the desmethyl derivative of WZ4002, was accomplished in the presence of cesium carbonate base and phase-transfer catalyst dibenzo-21-crown-7. During the course of synthetic method development for [18F]fluoroalkylation of WZOH, a range of different bases were investigated including potassium carbonate, cesium carbonate, sodium hydride, sodium hydroxide and tertbutylammonium hydroxide. The use of either sodium hydride and sodium hydroxide failed to provide the desired [18F]FEWZ product by analytical radio-HPLC, however, the use of cesium carbonate in the presence of dibenzo-21-crown-7 provided a modest conjugation (15 – 20% as observed by analytical radio-HPLC) of the desired [18F]FEWZ. From the crude reaction mixture, the [18F]FEWZ product was isolated by semi-preparative radio-HPLC and
reformulated for injection in phosphate-buffered saline containing ≤10% ethanol in a total synthesis time of 120 minutes in a modest activity yield of 2 - 4%.

**In vitro analysis**

The specificity of binding of [18F]FEWZ was assessed in human NSCLC cell lines in vitro. [18F]FEWZ displayed little overall binding to A549 cells expressing the wild type EGFR (86,544 ± 7,138 counts/well) compared to HCC827 cells carrying the L585R TKI sensitizing mutation (231,650 ± 18,318 counts/well) and H1975 cells bearing the T790M TKI resistance mutation (209,435 ± 13,585 counts/well). Furthermore pre-administration of 10 µM WZ4002 did not inhibit binding to A549 cells (104.0 ± 6.2%), but significantly decreased binding to both HCC827 (37.0 ± 0.8%) and H1975 cells (32.8 ± 8.0%; Figure 3A). The cytotoxic efficacy of WZ4002 and [19F]FEWZ were also compared in human A549, HCC827 and H1975 NSCLC cells using a CCK-8 assay as previously described [18]. Briefly, tumour cells (2000 per well) were seeded onto 96 well plates and allowed to adhere overnight. Media containing various concentrations of WZ4002 or [18F]FEWZ were added to the appropriate wells and incubated for 72 hours at 37°C. Subsequently CCK-8 reagent was added to the wells and incubated for 4 hours at 37°C and read on a Thermoscan plate reader at wavelength 405 nm. Experiments were performed in triplicate with controls. WZ4002 was efficacious in the EGFR-L858R cell line (HCC827) and the EGFR-L858R cell line (H1975) but not the EGFR-WT cell line (A549) in line with published results (Figure 3B). [19F]FEWZ displayed a slightly reduced efficacy in the HCC827 and H1975 cell lines and no efficacy on the wild type A549 (Figure 3C).

**Metabolism**

*Ex vivo* stability of [18F]FEWZ in doped rat plasma (1 MBq /ml) was examined by reversed-phase HPLC and was found to be 100% stable in plasma over 90 minutes (data not shown).

*In vivo* stability of [18F]FEWZ was determined by metabolite analysis in NCr nude mice at 2, 10, 30, 60 and 90 minutes post injection (30 MBq in 0.2 ml IV). Unfortunately plasma metabolite analysis was not possible as no [18F]FEWZ or metabolites were detectable in plasma at any of the time points studied.

**Biodistribution**

As shown in Figure 4A tumour uptake was rapid with stable retention observed after 5 minutes; EGFR WT (A549; 0.49 ± 0.22%ID/g), EGFR L858R (HCC827: 0.52 ± 0.25%ID/g) and EGFR L858R/T790M (H1975 0.69
± 0.23%ID/g). The uptake at 90 mins post injection is similar in the EGFR-WT tumour (A549; 0.58 ± 0.18%ID/g) and EGFR-L858R (HCC827: 0.52 ± 0.16%ID/g) despite WZ4002 displaying a 200 fold preference for EGFR-L858R over EGFR-WT. Uptake in the EGFR-L858R/T790M tumour however, was significantly greater than the EGFR-WT or EGFR-L858R alone (H1975: 0.90 ± 0.26%ID/g, p<0.05). Tracer delivery to well perfused tissues was initially high, however, [18F]FEWZ displayed rapid excretion through the hepatobiliary route. [18F]FEWZ clearance from other organs was rapid with blood plasma levels of [18F]FEWZ undetectable within 10 minutes. [18F]FEWZ showed no evidence of defluorination or metabolism in vivo, with little uptake in bone or brain observable after 10 minutes, however, assessment was obfuscated by rapid excretion from the blood (Figure 4B).
Discussion

Various radiopharmaceuticals have been assessed for their ability to detect early response to TKI therapy with varying levels of success, both $[^{18}F]$FDG and $[^{18}F]$FLT are able to determine successful response to TKI treatment [19-22], however, they do not help stratify patients to aid in therapy management. Numerous radiolabelled EGFR TKIs have been developed in order to identify tumours with mutations that confer sensitivity to EGFR-TKI therapy (EGFR-del19 or EGFR-L858R) to stratify patients, however, these radiopharmaceuticals do not detect tumours with acquired T790M resistance [16]. In the present study we have attempted to develop a radiopharmaceutical that could selectively identify EGFR-L858R/T790M by $^{18}$F-fluorination of the TKI WZ4002. WZ4002 is an anilinopyrimidine which covalently binds to the Cys797 residue inside the ATP binding cleft and has a chlorine on the pyrimidine ring which interacts with the mutant gatekeeper residue Met790 [23]. This interaction at the mutant gatekeeper residue leads to a 200 fold greater potency against EGFR (L858R/T790M) compared to wild type. Careful evaluation of the radiolabelling position was required in order to minimize potential interference with the key binding points in the ATP binding cleft. The crystal structure of WZ4002 in complex with the EGFR T790M mutant kinase has been described by Zhou et al [23]. We determined that the optimal position for addition of the fluoride was on the methoxy- substituent of the aniline ring which extends towards Leu792 in the ATP binding cleft but hypothesized that this may reduce selectivity. Our in vitro data show that $[^{19}F]$FEWZ retains the ability to target EGFR-L858R/T790M tumour cells (H1975) and EGFR-L858R tumour cells (HCC827) but, as expected, does not specifically bind to EGFR-WT tumour cells (A549; Figure 3A). However, the introduction of fluoride did result in slight reduction in therapeutic efficacy most likely due to the ethyl fluoride hindering interactions with Leu792 (Fig 3B and C). The cytotoxic activity of WZ4002 and $[^{18}F]$FEWZ were compared against the EGFR WT and mutant cancer cells (A549, HCC827 and H1975) using the CCK-8 assay. Addition of the fluoride reduces the cytotoxicity of $[^{19}F]$FEWZ by ~10 fold for both EGFR-L585R and EGFR-L585R/T790M. Despite the apparent reduction in cytotoxicity after fluorination, $[^{18}F]$FEWZ shows potential for the identification of EGFR-L585R/T790M tumours in vivo. The retention of $[^{18}F]$FEWZ in EGFR-L585R/T790M tumours (H1975) is relatively low (0.90 ± 0.26%ID/g at 90 min), however, this is not surprising as the EGFR ATP binding site is intracellular and $[^{18}F]$FEWZ must compete with high intracellular ATP concentrations in order to bind [24]. $[^{18}F]$FEWZ, however, shows much lower retention in EGFR-L585R tumours (HCC827, 0.52 ± 0.16%ID/g at 90 min) despite similar in vitro binding specificity and efficacy, this may be due to the high expression of P-glycoprotein transporters on HCC827 cells leading to rapid clearance [24], as expected $[^{18}F]$FEWZ retention in the EGFR-WT A459 tumours is low.
Conclusion

We have developed a technique to synthesize $^{18}$F-FEYZ for the detection of the EGFR-L585R/T790M in vivo. While $^{18}$F-FEYZ shows some specificity, the overall binding is low, due in part to rapid blood clearance and inaccessibility of the binding site. Further study will be required to successfully develop $^{18}$F-FEYZ as a TKI-PET tracer for the detection of EGFR-L585R/T790M bearing tumours.
**Figure Legends:**

**Figure 1.**


**Figure 2.**

Reaction scheme for the 18F-fluoroalkylation labelling of WZOH to synthesise [18F]FEWZ.

**Figure 3.**

3A. Binding specificity of [18F]FEWZ in vitro expressed as a percentage of total binding for each cell line. 3B. Therapeutic efficacy of WZ4002 in vitro assessed using the CCK-8 assay. 3C. Therapeutic efficacy of [19F]FEWZ in vitro assessed using the CCK-8 assay.

**Figure 4.**

4A. Graph showing retention of [18F]FEWZ measured by PET imaging (~20 MBq dynamic imaging from 0-90 mins post injection under isoflurane anaesthesia). Retention was significantly higher in the EGFR-L585R/T790M H1975 tumours from 60 minutes (n=6, *P<0.05, data shown as %ID/g ± SD). 4B. Graph showing a basic biodistribution of [18F]FEWZ showing routes of excretion (liver, large intestine and kidneys) and defluorination (uptake in bone, blood and brain) measured by PET imaging (n=6, data shown as %ID/g ± SD).

**Acknowledgements**

This work was supported by funding from the Singapore Bioimaging Consortium, A*STAR.

The authors declare no conflict of interest and no disclosures.
REFERENCES

Figure 1.
1D. Chemical structure of [18F]WZ4002.

207x169mm (96 x 96 DPI)
Figure 2.
Reaction scheme for the $[^{18}\text{F}]$fluoroalkylation labelling of WZOH to synthesise $[^{18}\text{F}]$FEWZ.
Figure 3.

3A. Binding specificity of $^{[18F]}$FEWZ in vitro expressed as a percentage of total binding for each cell line. 3B. Therapeutic efficacy of WZ4002 in vitro assessed using the CCK-8 assay. 3C. Therapeutic efficacy of $^{[19F]}$FEWZ in vitro assessed using the CCK-8 assay.
Graph showing retention of $[^{18}\text{F}]\text{FEWZ}$ measured by PET imaging (~20 MBq dynamic imaging from 0-90 mins post injection under isoflurane anaesthesia). Retention was significantly higher in the EGFR-L585R/T790M H1975 tumours from 60 minutes ($n=6$, *P<0.05, data shown as %ID/g ± SD).