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Radiosynthesis and biological evaluation of ^{18}F-labeled 4-anilinoquinazoline derivative (^{18}F-FEA-Erlotinib) as a potential EGFR PET agent

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ABSTRACT

Epidermal growth factor receptor (EGFR) has gained significant attention as a therapeutic target. Several EGFR targeting drugs (Gefitinib and Erlotinib) have been approved by US Food and Drug Administration (FDA) and have received high approval in clinical treatment. Nevertheless, the curative effect of these medicines varied in many solid tumors because of the different levels of expression and mutations of EGFR. Therefore, several PET radiotracers have been developed for the selective treatment of responsive patients who undergo PET/CT imaging for tyrosine kinase inhibitor (TKI) therapy. In this study, a novel fluorine-18 labeled 4-anilinoquinazoline based PET tracer, 1*N*-(3-(1-(2-^{18}F-fluoroethyl)-1*H*-1,2,3-triazol-4-yl)phenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (^{18}F-FEA-Erlotinib), was synthesized and biological evaluation was performed in vitro and in vivo. ^{18}F-FEA-Erlotinib was achieved within 50 min with over 88% radiochemical yield (decay corrected RCY), an average specific activity over 50 GBq/μmol, and over 99% radiochemical purity. In vitro stability study showed no decomposition of ^{18}F-FEA-Erlotinib after incubated in PBS and FBS for 2 h. Cellular uptake and efflux experiment results indicated the specific binding of ^{18}F-FEA-Erlotinib to HCC827 cell line with EGFR exon 19 deletions. In vivo, Biodistribution studies revealed that ^{18}F-FEA-Erlotinib exhibited rapid blood clearance both through hepatobiliary and renal excretion. The tumor uptake of ^{18}F-FEA-Erlotinib in HepG2, HCC827, and A431 tumor xenografts, with different EGFR expression and mutations, was visualized in PET images. Our results demonstrate the feasibility of using ^{18}F-FEA-Erlotinib as a PET tracer for screening EGFR TKIs sensitive patients.

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is a key mediator of cell division and proliferation upon stimulation of the epidermal growth factor (EGF). Dysregulation of EGFR signaling as a consequence of amplification, overexpression, and mutation of EGFR gene frequently occurs in various tumor types, including head and neck, ovarian, breast, lung, brain and colon cancers.[1–3] Over the past decade, six EGFR TKIs have been approved as anti-cancer drugs for targeting EGFR by the US Food and Drug Administration (FDA). Meanwhile, many TKIs continue to be explored at the preclinical stage and in clinical trials.[4–6] Compared with standard chemotherapy, TKIs demonstrate a greater objective response rate (66.6% versus 30.9%) and better 1-year progression free survival (42.9% versus 9.7%).[7–9] Despite their promising therapeutic effects as cancer treatment drugs, patients overall responsiveness to therapy is undependable due to the heterogeneity of cancer.[10,11] In non-small cell lung cancer (NSCLC), the exon 19 deletion or the L858R mutation increase kinase activity and lead to excellent sensitivity to TKIs such as the first generation TKIs Gefitinib and Erlotinib.[12,13] Meanwhile, most patients develop resistance against these first generation TKIs even if they had great initial response.[14] Therefore, screening for EGFR status was necessary to select TKIs sensitive individuals before treatment.

The most common and conventional screening method is direct sequencing, to detect EGFR status which requires an invasive puncture but the result is highly variable because of the heterogeneity

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and branched evolution of intratumor.[15] EGFR-targeted molecular imaging has the potential to provide a non-invasive method to reflect receptor status in a real-time manner for identifying responsive patients who may benefit from EGFR targeting drugs.[16] Many PET tracers which specifically bind to EGFR kinase domain have been developed. Examples are the ML series, (^{18}F-ML01,[17] ^{11}C-ML03,[18] ^{11}C or ^{18}F labeled ML04,[19,20] ^{124}I labeled ML06, ML07 and ML08[21]), ^{11}C-PD153035,[22] ^{11}C or ^{18}F labeled gefitinib,[23,24] ^{11}C-erlotinib[25] and ^{18}F-afatinib.[26] Except for ^{11}C-PD153035[27,28] and ^{11}C-erlotinib[29–31] which have been used in clinical research, most of these studies were carried out at the preclinical stage. ^{11}C-erlotinib accumulated in brain metastases in an NSCLC patient with an exon 19 deletion in the EGFR gene.[29] Meanwhile, lymph-node metastases of NSCLC, unidentified by ^{18}F-FDG PET imaging, but instead, identified by ^{11}C-erlotinib PET/CT.[30] ^{11}C-erlotinib scored high as a suitable candidate for PET imaging in NSCLCs tumors with EGFR exon 19 deletions.[31] However, the short half-life (20 min) of the carbon-11 limits its widespread use as a tool for community-based diagnostic screening and therapeutic evaluation, except in instances when the institution has an on-site cyclotron. We envisaged that this shortcoming could be overcome by the development of a fluorine-18 (half-life = 109 min) labeled tracer with kinetics similar to ^{11}C-erlotinib.

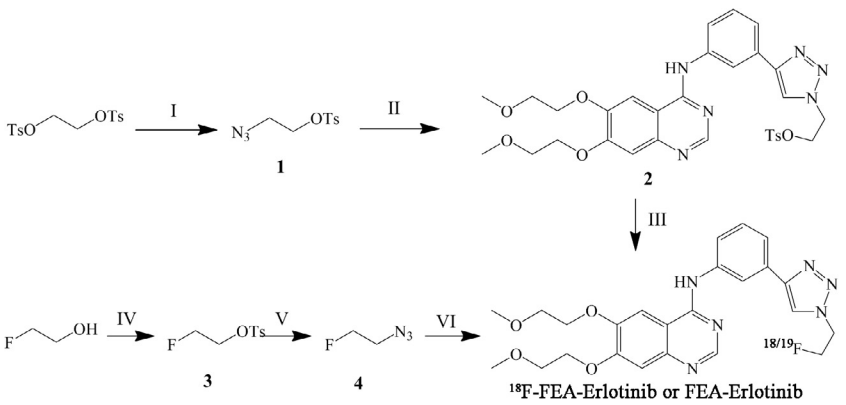
In this study, we designed and synthesized a fluorine-18 labeled erlotinib derivative (^{18}F-FEA-Erlotinib) with a 4-anilinoquinazoline pharmacophore as an EGFR-TKI PET tracer. We used the "click reaction" to label erlotinib from the alkynyl group with ^{18}F. The 1,2,3-triazole scaffold was featured in a vast number of bioactive molecules which have exhibited considerable biological and pharmaceutical activities.[32–34] The syntheses of ^{18}F-FEA-Erlotinib and the reference compound FEA-Erlotinib are shown as Scheme 1; the products were confirmed by NMR and ESI-MS. 2-fluoroethyl azide (FEA) was obtained by two-step reactions from 2-fluoroethanol with substitution reactions, as previously described.[35] The reference compound FEA-Erlotinib (IC_{50} value of 11.3 μM for EGFR) was synthesized by the Cu-catalyzed Huisgen reaction with 60–70% yield ($n \geq 3$).

The radiosynthesis was performed in GE Health tracer lab FX-Fn synthesizer. In the beginning, we made an effort to synthesize ^{18}F-FEA-Erlotinib through ^{18}F-labeled precursor 2-^{18}F-fluoroethyl azide (^{18}F-FEA) "click react" with erlotinib.[36] This approach required two radiochemical reactions and a vacuum distillation for the purification of ^{18}F-FEA. Furthermore, the production rate is unstable and low. So we attempted to radiolabel ^{18}F-FEA-Erlotinib by nucleophilic substitution with fluoride-18 from the *p*-toluene sulfonic acid ester precursor **2**. The reaction was stirred at 130 °C in DMSO for 10 min. The radiolabeled product ^{18}F-FEA-Erlotinib was purified by semi-preparative HPLC, with 88% decay corrected yield (RCY) and over 50 GBq/μmol of the average specific activity. The identification of target product ^{18}F-FEA-Erlotinib was determined by matching the reference compound on the analytical HPLC (Fig. 1). The ^{18}F-FEA-Erlotinib retention time was 8.55 min in the γ-radioactivity detection system, which was matched with the reference compound under UV absorbance peak at 254 nm (8.38 min) under the same conditions.

^{18}F-FEA-Erlotinib is stable in both phosphate buffered saline (PBS) and fetal bovine serum (FBS) in vitro at 37 °C for 2 h. The radiochemistry purity of the tracer was over 97% after 2 h (n = 4). The LogP value of ^{18}F-FEA-Erlotinib was 2.36 ± 0.01 (n = 3) indicated the radiotracer was a lipophilic compound, similar to erlotinib. The lipophilic characteristic of a radiolabeled pharmaceutical is important in predicting its excretion pathway.

To test the capacity of ^{18}F-FEA-Erlotinib in binding EGFR, the cellular uptake and efflux were evaluated, using three cell lines, HCC827 (EGFR positive expression and with 19 exon deletion), HepG2 (low EGFR expression), A431 (high EGFR expression).[37,38] The results are shown in Fig. 2. The accumulation of tracer in HCC827 cells was significantly greater than that in HepG2 and A431 cells. The uptake of ^{18}F-FEA-Erlotinib in HCC827 cells exhibited a fast increase in binding in the first 15 min and almost reached saturation at 30 min (5.54 ± 0.55% of total added dose). Between 30 min and 60 min, there was only a slight increase (5.82 ± 0.62% of total added dose) (Fig. 2a). In the cell efflux assay, ^{18}F-FEA-Erlotinib labeling showed dissociation and efflux from the cells with time. In HCC827 cells, there was about 38% of tracer dissociation after 15 min of incubation. Tracer release reached a plateau at 30 min while approximately 54% of ^{18}F-FEA-Erlotinib (3.1 ± 0.21% of total added dose) remained bound to the cells. It showed good retention after 60 min incubated (2.9 ± 0.43% of total added dose) in serum-free RMPI-1640 medium. In HepG2 and A431 cells, the uptake and efflux of ^{18}F-FEA-Erlotinib were much lower than HCC827 cells, which might be due to a nonspecific uptake (Fig. 2b). After treating HCC827 cells with erlotinib (100 μmol/L) for 60 min,[39] the cellular uptake of ^{18}F-FEA-Erlotinib dramatically decreased from 5.82 ± 0.62% to 0.86 ± 0.25% at 60 min (Fig. 2c). It showed that the uptake of ^{18}F-FEA-Erlotinib in HCC827 cells was strongly inhibited by erlotinib. Our cellular uptake and efflux results proved that ^{18}F-FEA-Erlotinib could specifically bind to HCC827 cells, which might be associated with the exon 19 deletion of EGFR in HCC827 cells. Altogether, our results suggest that the tracer has the potential to be used as a PET probe to select EGFR TKIs sensitive patients.

Biodistribution studies of ^{18}F-FEA-Erlotinib were carried out in normal BALB/c mice at 5, 15, 30 and 60 min to evaluate the



Scheme 1. The synthesis of reference and radioactive compound. (I) DMSO, NaN_3, rt, 12 h; (II) erlotinib, $CuSO_4{\cdot}5H_2O$/sodium L-ascorbate, TBTA, DMF, rt, overnight; (III) K18F/K_{222}, DMSO, 130 °C, 10 min; (IV) TsCl, DMAP, TEA, rt, 48 h; (V) DMF, NaN_3, rt, 12 h; (VI) erlotinib, $CuSO_4{\cdot}5H_2O$/sodium L-ascorbate, TBTA, DMF, rt, overnight.