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# One-step radiosynthesis of $^{18}$F-IRS: A novel radiotracer targeting mutant EGFR in NSCLC for PET/CT imaging

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ABSTRACT

EGFR (epidermal growth factor receptor) targeted therapy has shown great success in clinical comparing with chemotherapy in EGFR mutation NSCLCs. Such as, gefitinib, first generation EGFR TKI, has obviously prolonged the FPS (progression free survival) of the subgroup of patients, but to those who did not get a certain mutation in EGFR kinase domain, the outcome is poor. In view of this situation, scientists have synthesized many radiotracers for selecting the right people by PET/CT imaging to NSCLC TKI therapy. In this study, we developed a novel PET radiotracer $^{18}$F-IRS in one-step with a radio yield 20% (non-corrected), radiochemistry > 98.5%, specific activity > 105G Bq/μmol, the pharmacokinetics and capacity of the tracer binding to mutant EGFR were evaluated both in vitro and in vivo.



For the last decade, non small cell lung carcinoma (NSCLC) is still the leading causes of cancer death [1]. Conventional treatment method, like chemotherapy, has only lead to marginal effect, but great toxicity. With the development of genomics, the epidermal growth factor receptor (EGFR) has been known widely by its signaling pathway which is concerned with the growth, proliferation, differentiation of advanced non small cell lung cancer [2,3]. It has become a crucial molecular target for NSCLC TKIs targeted therapy [4–6], especially for those patients who have got a certain mutation in EGFR kinase domain. Gefitinib, first generation EGFR-TKI, has significantly prolonged the progression free survival (PFS) of patients who have got a certain mutation (EGFR 19 deletion or L858R) in EGFR kianse domain compared with chemotherapy, but to EGFR wild type, secondary mutation T790M, its outcomes were not good [7–10]. So, despite its promising effect in the treatment of advanced NSCLC, detection of EGFR mutation status is still needed before treatment.

The major method of EGFR status detection gene sequencing analysis needs an invasive puncture, which has many limitations [11,12]. It can not reflect EGFR status of all tumor regions due to the heterogeneity of cancer [13,14], and repeating invasive puncture will make patient suffer much more pain during the process of treatment. Molecular imaging with PET tracer may provide a noninvasive method that could be repeatedly operated and reflect receptor status in real-time [15–19]. In view of gefitinib has shown great response in targeted therapy of NSCLC patients who have got a certain mutation in EGFR kinase domain, to detect EGFR status by molecular imaging is evaluable. So, scientists have developed many PET tracers which could specific bind to EGFR kinase domain, such like $^{18}$F-gefitinib, $^{11}$C-gefitinib, $^{11}$C-erlotinib [20–22], but they have a common insufficient, high lipophilicity results in too much tracer accumulation in liver, which lead to a high background noise, and will affect the quality of imaging pictures. Also, with the short half-life 20 min, radionuclide $^{11}$C was not ideal and suitable for PET imaging, in the other hand, for $^{18}$F labeling, it need multi steps to label the TKIs. So, we suppose that whether chemical modification using polyethyleneglycol will reduce lipophilicity of the tracer, lower its accumulation in liver, and improve the quality of PET imaging pictures. Here we designed and synthesized a *p*-toluene sulfonic acid (TSO) precursor IRST in multi steps, and radiolabeled in one step. The process of IRST (compound 10) synthesizing was shown as below (Scheme 1), and the detailed method was described in Supplemental information.

At the end of synthesis, the chemical purity of IRST was analyzed by HPLC (Agilent 1200) with the elunt (methanol:water = 80:20), UV detector wavelength 254 nm, and the chemical purity was 99.80 ± 0.31% (Fig. 1 and Table 1).

The process of radiosynthesis was performed in GE Health tracerlab FX-Fn synthesizer, shown in Scheme 2, and $^{18}$F-IRS was

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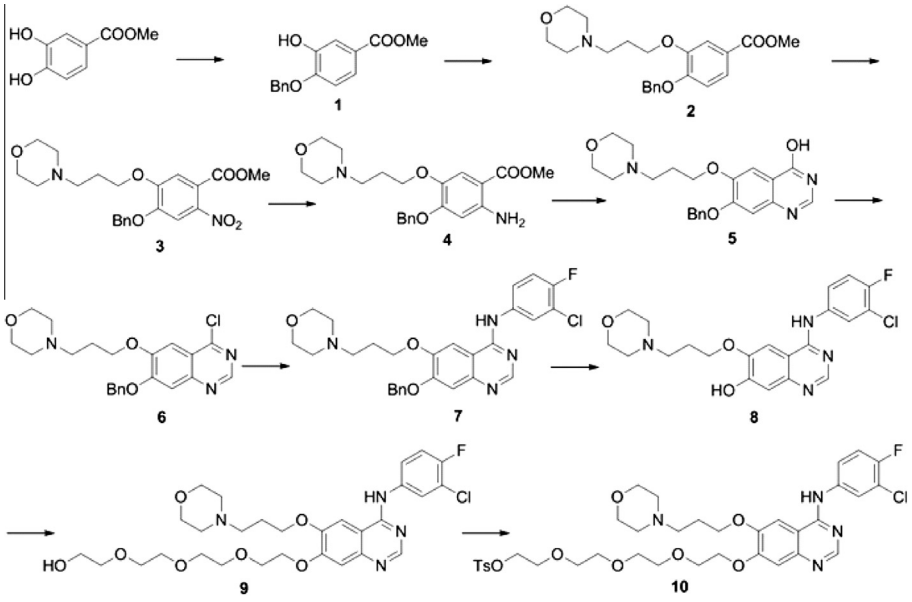


**Scheme 1.** The synthetic process of precursor IRST.

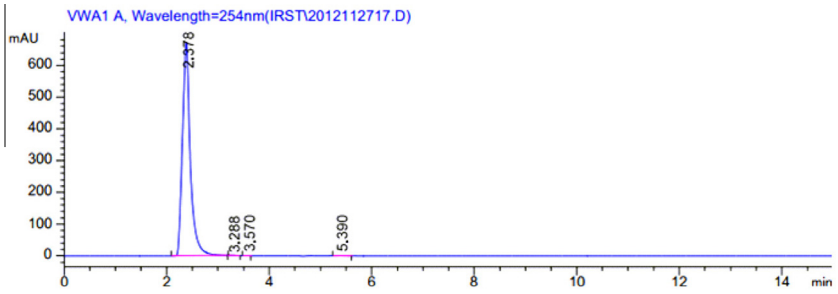


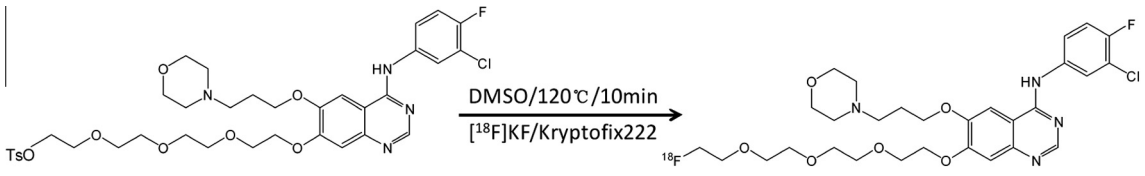

**Fig. 1.** HPLC analysis of precursor IRST.

**Table 1**
Chemical property of IRST.

| Parameter | Method | Specification | Result |
|---|---|---|---|
| Bacterial endotoxins | LAL | <2.0 I.U./25 mg | <0.5 I.U./25 mg |
| Appearance | Organoleptic | Off-white to white solid | White solid |
| Purity | HPLC | >95.0% | 99.80% |
| ESI | LC-MS | $[M+H]^+$, 763.25 | 763.30 |
| Identity | $^1$H NMR | Conformed | Conformed |

purified by HPLC (Fig. 2). The whole process was only about 30 min, the little time cost was due to the contribution of the *p*-toluene sulfonic acid (TSO), which is active group that could be substituted by halogen $^{18}$F in only one step, and also with the high chemical yield >20%. At the end of synthesis, the radiochemistry was determined by Thin-layer chromatography, and the radiochemistry was >98.5%. $^{18}$F-IRS is stable in both PBS and serum, the radiochemistry of $^{18}$F-IRS incubation in PBS was 98.56 ± 0.60% and 97.87 ± 0.51% at 1 h, 2 h time point, respectively (n = 3). The radiochemistry of $^{18}$F-IRS incubation in serum was 97.69 ± 0.93% and 92.43 ± 1.26% at 1 h, 2 h time point, respectively (n = 3). The LogP value of $^{18}$F-IRS was −0.647 ± 0.096 (n = 3), and the low lipophilicity will lead to less accumulation of $^{18}$F-IRS in liver which may be concerned with the chemical modification using polyethyleneglycol.

To evaluate the pharmacokinetics of $^{18}$F-IRS, ex vivo biodistribution was performed. The uptake of $^{18}$F-IRS in organs were expressed as %ID/g (n = 3), the result was shown in Fig. 3. As the data suggested, the highest level of accumulation of $^{18}$F-IRS in liver was only 2.12 ± 0.60%ID/g at 30 min post injection, which is lower than reported before. The evidence provided by LogP value and biodistribution assay has proved that the chemical modification using polyethyleneglycol do decreased the lipophilicity of the




**Scheme 2.** One-step radiolabeled of $^{18}$F-IRS.

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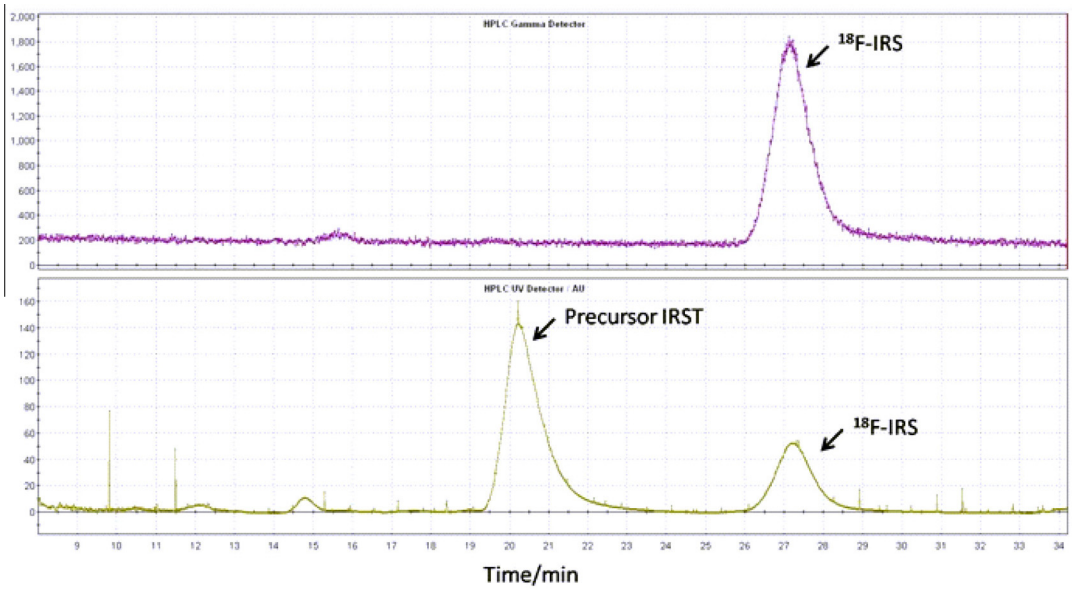


**Fig. 2.** Chromatogram from HPLC separation of $^{18}$F-IRS.

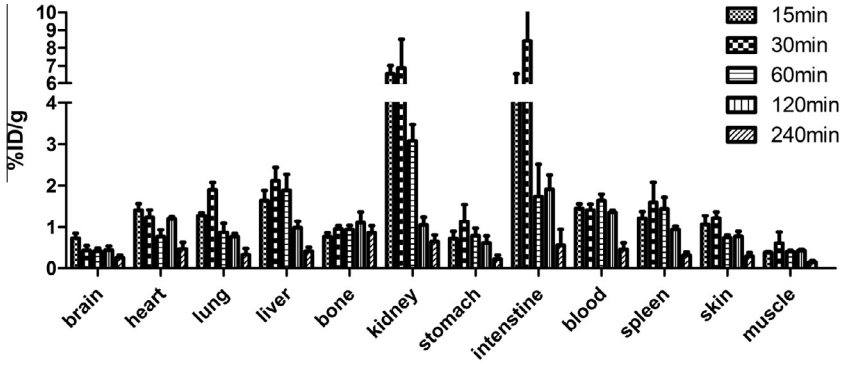


**Fig. 3.** Biodistribution of $^{18}$F-IRS in KM mice.

tracer, which will lead to more tracer circulation in the blood system, and the tumor region may has more tracer accumulation. The accumulation of $^{18}$F-IRS in kidney was as high as 6.86 ± 2.81%ID/g, which means $^{18}$F-IRS was mainly excreted from urinary. The uptake of $^{18}$F-IRS in lung has reached the highest level, only 1.90 ± 0.31%ID/g at 30 min time point, which will be good for PET/CT imaging of NSCLC due to the low background. The activity in blood at 240 min time point was 0.46 ± 0.28%ID/g, which suggested the clearance of $^{18}$F-IRS was rapid.

To test the capacity of $^{18}$F-IRS in binding mutant EGFR and retention, cell uptake and efflux were performed in four NSCLC cell lines, HCC827(19 exon deletion), H1975(L858R/T790M), H358 (EGFR wild type), H520(low EGFR expression). The result was shown in Fig. 4. As the data suggested, the accumulation of $^{18}$F-IRS in HCC827 was much higher than that in H1975, H358, H520 at all time points examined. At 2 h time point, the uptake of $^{18}$F-IRS in HCC827 cells reached the highest level 19.07 ± 0.70% of total input radioactivity, which is about 17.66, 26.12 and 19.46 times higher than that in H1975, H358 and H520 cell lines. In the cell efflux assay, $^{18}$F-IRS has shown good retention in HCC827 cells, even at 2 h time point, the accumulation of $^{18}$F-IRS in HCC827 cells still remained 4.10 ± 1.06% of total input radioactivity, which is much higher than H1975, H358 and H520 cells. The cell uptake and efflux assay have proved $^{18}$F-IRS could specifically bind to HCC827 cells, which may be concerned with EGFR 19 exon deletion in HCC827 cells, so $^{18}$F-IRS has the potential to be used in

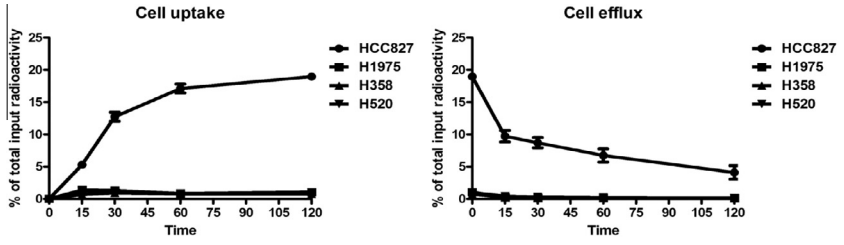


**Fig. 4.** Cell uptake and efflux of $^{18}$F-IRS in NSCLC cell lines.

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EGFR TKIs sensitive patients selection; also due to the good retention in HCC827 cells, it could be used in observing the pharmacokinetics of TKIs for longer time.

In summary, PET/CT imaging with molecular tracer was an ideal method to detect the EGFR status and monitor the response of TKIs therapy. In this study, we have successfully developed a new PET tracer $^{18}$F-IRS in one-step radio-labeling. With the chemical modification by polyethylene glycol, the lipophilicity of $^{18}$F-IRS was decreased by detecting the LogP value, the tracer was also stable in both PBS and serum. The pharmacokinetics of $^{18}$F-IRS was evaluated by biodistribution assay, the data has shown that $^{18}$F-IRS has a low accumulation in liver and lung, which will lead to lower background. Due to the specificity of $^{18}$F-IRS to HCC827 cells, it could be used to select the appropriate patient for TKIs therapy. Considering that the potency of $^{18}$F-IRS, PET/CT imaging study was required.

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## A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.10.084.

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