

Research Article

Fluorine-18 labeling of ML04 – presently the most promising irreversible inhibitor candidate for visualization of EGFR in cancer

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Summary

Overexpression of the EGFR has been linked to cell malignancy, metastasis and poor prognosis thus making it a target for several FDA approved drugs such as Gefitinib and Erlotinib. Unfortunately, these drugs have yielded suboptimal clinical results. In order to evaluate and monitor EGFR-targeted treatment response at the molecular level, several PET biomarkers have been developed. One of the lead irreversible inhibitors (**1**) has been labeled with carbon-11, however the short half-life of this radioisotope limited the time window for *in vivo* studies. Compound **1** was successfully labeled with fluorine-18 via a multi-step radiosynthesis with 14% decay-corrected overall radiochemical yield, 98% radiochemical purity, specific activity of 1800 Ci/mmol ($n = 10$) at end of bombardment, and a total radiosynthesis time of 4 h including purification and formulation. [¹⁸F]-**1** will allow for prolonged *in vivo* studies including Micro-PET analysis of EGFR tumor-bearing animal models. Copyright © 2006 John Wiley & Sons, Ltd.

Received 14 February 2006; Accepted 10 March 2006

Key Words: EGFR; [¹⁸F]; PET; cancer

Introduction

Epidermal growth factor (EGF) and erb-2 (HER-2) kinase receptors are among growth factor receptor kinases which play a major role in cancer initiation, development and progression.^{1–4} Overexpression of the EGF receptor

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Contract/grant sponsor: TK-Signal Ltd
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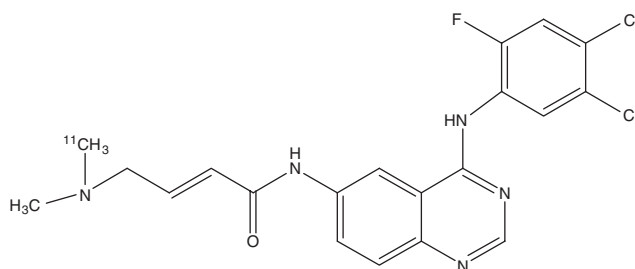


Figure 1. Chemical structure of [^{11}C]-ML04

(EGFR) or of one of its ligands has been linked to cell malignancy, metastasis, and poor prognosis in patients.⁵ Thus, the EGFR has become an attractive target for the design and development of compounds that can specifically bind the receptor, and inhibit its tyrosine kinase activity and its signal transduction pathway in cancer cells.⁶ The EGFR reversible inhibitor, Iressa[®] (ZD 1839, Gefitinib) was recently approved by the FDA for treatment of non-small-cell-lung-carcinoma (NSCLC) and prostate cancer.⁷ Gefitinib binds at the ATP site and inhibits the kinase activity of EGFR and has been in clinical use since 2002. In addition, other anti-EGFR targeted inhibitors, such as Tarceva[®] (OSI-774, Erlotinib) and the anti-EGFR antibody Erbitux[®], are presently undergoing clinical Phase 3 trials. Erlotinib and Gefitinib yield similar results in that they are both effective only in a small percentage of patients in whom EGFR possesses activating mutations in the kinase domain.^{8–10} Since accurate measurements of EGFR phosphorylation in the human tumor are lacking, it is actually not possible to assess whether the poor response to Gefitinib or Erlotinib is indeed due to a lack of the specific activating mutations, the absence of a survival function of EGFR, or to insufficient long-term occupancy of the receptor by reversible inhibitors. Consequently, there has been a growing interest in the use of EGFR-TK inhibitors as radiotracers for molecular imaging of EGFR overexpressing tumors via nuclear medicine modality^{11–15} such as positron emission tomography (PET). During the course of this research, a potent ($\text{IC}_{50} = 0.1 \text{ nM}$) and selective EGFR-TK irreversible inhibitor ML04 (**1**) was labeled with carbon-11¹¹ (Figure 1), and its potential as PET biomarker was investigated both *in vitro* and *in vivo*. Due to the short half-life of carbon-11 ($t_{1/2}$ 20.39 min), the *in vivo* studies in tumor-bearing rats with [^{11}C]-ML04 were limited to a short time window. However, since this inhibitor was presently proved to be the most suitable candidate for visualization of EGFR in cancer, we were motivated to expand the study time window, and to label ML04 with a longer-lived radioisotope, [^{18}F] ($t_{1/2}$ 109.8 min), at the anilino moiety.

Results and discussion

The major disadvantage of labeling **1** with [^{11}C] was the short half-life of the isotope which limited the *in vivo* studies to rather short time periods. Since

[¹⁸F] has a longer half-life which enables for a longer follow-up period of time following injection of the radiotracer, **1** was designated for labeling with [¹⁸F]. With this in mind, two different precursors (**2,3**) were synthesized for radiolabeling with [¹⁸F], requiring three additional steps after the labeling step to yield the desired labeled compound. The radiosynthesis route was based on a well-known nucleophilic substitution of the two leaving groups: trimethylammuniumtriflate and a nitro group, attached at the anilino moiety of compounds **2** and **3**, respectively. The synthesis of precursor **2** is outlined in Figure 2. 1,2-Dichloro-4,5-dinitro-benzene was reacted with hydrazine monohydrate and Raney-Nickel to yield compound **4** with 25% yield. 4-chloro-6-nitro-quinazoline in *i*-PrOH was added dropwise to compound **4** in the presence of triethylamine in *i*-PrOH and heated to 80°C to obtain compound **5** with 42% yield. Compound **5** was reacted with methyltrifluoromethanesulfonate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine in dichloromethane/acetonitrile anhydrous for 6 h at RT to obtain compound **2** with 25% yield and 80–90% purity. Compound **2** was accompanied by the by-product **6**. It is well known that compounds containing the ammonium quarternary salts such as compounds **2** and **6** are not stable and sometimes their purification leads to decomposition.¹⁶ Although compounds **2** and **6** were purified by a short silica column, they could not be purified any further by recrystallization. The synthesis of precursor **3** is outlined in Figure 3. 4,5-Dichloro-2-nitro-aniline was refluxed with 4-chloro-6-nitro-quinazoline and *N,N*-diisopropylethylamine in *i*-PrOH for 12 h to yield compound **3** with a 20% yield and 97% purity. Attempts to radiolabel

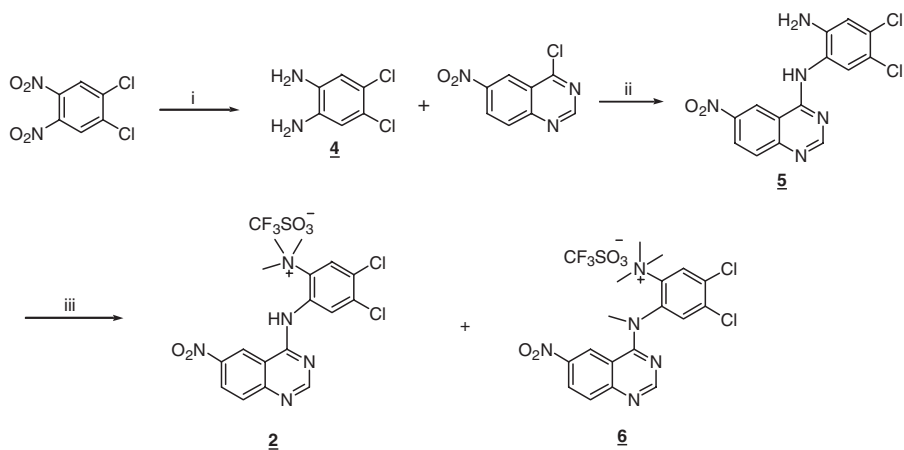


Figure 2. (i) H₂NNH₂·H₂O, Ra-Ni, EtOH:H₂O (9:1), 60°C, 3h; (ii) *i*-PrOH, Et₃N, 80°C, 5h; (iii) CF₃SO₃CH₃, 2,6-*tert*-butyl-4-methylpyridine, CH₂Cl₂/CH₃CN, RT, 6h

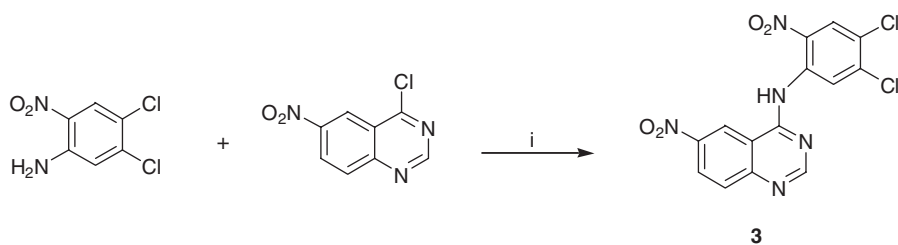


Figure 3. (i) *i*-PrOH, 90°C, 4 h

Table 1. Attempts to radiolabel compounds **2**, **3** with [¹⁸F] under different conditions

Temperature (°C)	Time (min)	Solvent
100, 120, 140	20	DMSO
100, 120, 140	20	CH ₃ CN
100, 120, 140	20	DMF
120	5, 10, 15	DMSO
120	5, 10, 15	CH ₃ CN
120	5, 10, 15	DMF

compounds **2**, **3** with [¹⁸F] were performed in a commercial module under different conditions such as time, solvent, and temperature (Table 1). However, none of these experiments have been successful. In addition, reaction of compound **6**, which contains a tertiary amine, yielded similar results and eliminated the possibility that the hydrogen of the secondary amine in compounds **2** and **3** inactivated the fluoride by protonation, and inhibited the transformation. Therefore, the most logical explanation for the unsuccessful results is that the electron donating secondary amine group in compounds **2** and **3** increased the electron density at the aromatic ortho position attached to the two leaving groups of compounds **2**, **3** and made the substitution unfeasible. Consequently, another radiosynthesis route similar to the one that was developed for ML01¹⁷ and which was recently used to label Gefitinib¹⁶ and other EGFR inhibitors,¹⁸ was performed. However, using this process, we encountered several disadvantages, including prolonged radiosynthesis time, radiation exposure and low yield due to the half-life of [¹⁸F] (109.8 min) and the number of the radiochemical steps that needed to be taken. To mitigate these problems, we designed and successfully used a simple semi-automated radiosynthesis system that decreases synthesis time and increases yield. The first two steps were performed in a commercial module, and the last four with the homemade semi-automated system. With this method, the exposure to radiation was decreased, and the radiosynthesis time was cut down to a total of 4 h, including purification

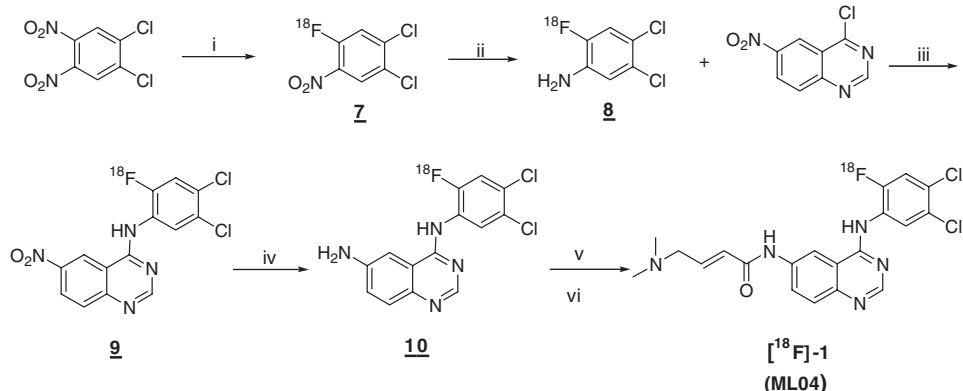


Figure 4. (i) K^{18}F , kryptofix, DMF, 115°C , 20 min; (ii) $\text{H}_2\text{NNH}_2\cdot\text{H}_2\text{O}$, Ra-Ni, EtOH: H_2O (9:1), 58°C , 7 min; (iii) i-PrOH, $100\text{--}105^\circ\text{C}$, 15 min; (iv) $\text{H}_2\text{NNH}_2\cdot\text{H}_2\text{O}$, Ra-Ni, EtOH: H_2O (9:1), 65°C , 10 min; (v) bromo/chlorocrotonylchloride, *N,N*-DIPEA, THF, 0°C , 15 min; (vi) dimethylamine in THF (2 M), 0°C , 15 min

and formulation. The radiosynthesis of [^{18}F]-1 is shown in Figure 4. 1,2-Dichloro-4,5-dinitro-benzene was reacted with a solution of [^{18}F] KF and kryptofix in DMF at 115°C for 20 min to yield compound [^{18}F]-7 with 80% radiochemical yield. Reaction of the latter with hydrazine monohydrate and Raney-Ni at 58°C for 7 min yielded compound [^{18}F]-8 with 70% radiochemical yield. The coupling of 4-chloro-6-nitro-quinazoline and [^{18}F]-8 was performed in i-PrOH at 100°C to yield compound [^{18}F]-9 with 80% radiochemical yield. [^{18}F]-9 was reacted with hydrazine monohydrate and Raney-Ni at 65°C to obtain the reduced compound [^{18}F]-10 with 70% radiochemical yield. Reaction of the latter with bromo/chlorocrotonylchloride and diisopropylethylamine at 0°C followed by reaction with dimethylamine provided [^{18}F]-1 ([^{18}F]-ML04) with 14% overall decay-corrected radiochemical yield, 98% radiochemical purity, specific activity of 1800 Ci/mmol ($n = 10$) and a total radiosynthesis time of 4 h, including purification and formulation. For comparison, ^{18}F -Iressa was obtained after a three-step radiosynthesis (2 h), with 60–70% overall decay-corrected radiochemical yield.¹⁶ However, it should be noted that unlike compound 1 which is an irreversible EGFR inhibitor with a complex chemical structure,⁶ Iressa is a reversible inhibitor which does not contain a complex reactive functional group at the six position of the quinazoline ring that enables the *in vivo* covalent binding with the receptor as does compound 1. Therefore, the fluorine-18 labeling of Iressa is more straightforward. On the other hand, since Iressa is a reversible inhibitor, it should compete with high concentrations of cellular ATP on receptor

binding and might yield a narrow time window for imaging immediately after injection of the labeled biomarker.¹⁷

Experimental section

General

All chemicals were purchased from Sigma-Aldrich, Fisher Scientific, Merck or J. T. Baker. Chemicals were used as supplied, excluding THF, which was refluxed over sodium and benzophenone and freshly distilled prior to use. Mass spectroscopy was performed in EI mode on a Thermo Quest-Finnigan Trace MS-mass spectrometer at the Hadassah-Hebrew University Mass Spectroscopy Facility. ¹H-NMR spectra were obtained on a Bruker AMX 300 MHz apparatus using the hydrogenated residue of the deuterated solvents (DMSO-d₆, δ = 2.5 ppm, CDCl₃, δ = 7.25 ppm) and TMS as internal standard for ¹H-NMR. Elemental analysis was performed at the Hebrew University Microanalysis Laboratory. Thin-layer chromatography (TLC) was run on plates of silica gel 60F₂₅₄ (Merck). The compounds were localized at 254 nm using a UV lamp. Reversed phase HPLC: analytical and semi-preparative column C-18 μBondapak® Waters, with mobile-phase system composed of 45:55 (v/v) acetonitrile:acetate buffer 0.1 M pH 3.8 and 53:47 (v/v) ammonium formate 0.1 M:acetonitrile, respectively. A Varian 9012Q pump, a Varian 9050 variable wavelength detector operating at 254 nm, and a Bioscan Flow-Count radioactivity detector with a NaI crystal. Specific radioactivities were determined by HPLC, using cold mass calibration lines. Radiosyntheses using [¹⁸F] were carried out on a [¹⁸F] module (Nuclear-Interface, Munster, Germany). [¹⁸F] was produced on a cyclotron IBA 18/9 by irradiation of a 2 ml water target using a 18 MeV proton beam on 97%-enriched [¹⁸O] water by the [¹⁸O(p,n)¹⁸F] nuclear reaction and was transferred to the appropriate hot cell.

Chemistry

*4,5-Dichloro-benzene-1,2-diamine (4)*¹⁹. 1,2-Dichloro-4,5-dinitro-benzene (0.2 g, 0.84 mmol) dissolved in ethanol/water 9:1 (40 ml) was added dropwise to a solution of ethanol/water 9:1 (15 ml), hydrazine monohydrate (4.2 mmol, 204.45 μl) and Raney Nickel solution (1 ml) at 60°C. The reaction mixture was stirred for an additional 1 h. The solution was cooled and filtered over Celite®, and the filtrate was evaporated under reduced pressure. The residue was purified on silica gel column, eluted with dichloromethane to yield 4,5-dichloro-benzene-1,2-diamine **4** (0.05 g, 25%). TLC conditions: 1% MeOH in CH₂Cl₂, *R_f* = 0.38. HPLC conditions: C-18 column, 60% acetate buffer, pH = 3.8; 40% acetonitrile, flow = 1 ml/min, *R_t* = 7.2 min. ¹H-NMR (DMSO): δ 6.59 (s, 2 H), 4.85 (s, 4 H). MS (*m/z*): 177.27 (MH)⁺. M.p. = 159°C. (Lit., M.p. = 161°C).¹⁹

4,5-Dichloro-N-(6-nitro-quinazolin-4-yl)-benzene-1,2-diamine (5). 4-Chloro-6-nitro-quinazoline¹¹ (0.079 g, 0.37 mmol) dissolved in i-PrOH (60 ml) was added dropwise to a solution of 4,5-dichloro-benzene-1,2-diamine (0.1 g, 0.56 mmol) and triethylamine (210 μ l, 1.5 mmol) in i-PrOH (10 ml). The mixture was heated to 80°C for 5 h. The solution was cooled and evaporated, and the product was purified on silica gel column, eluted with 2% MeOH in dichloromethane to yield compound **5** (0.055 g, 42%). TLC conditions: 5% MeOH in CH₂Cl₂, R_f = 0.33. HPLC conditions: C-18 column, 60% acetate buffer, pH = 3.8; 40% acetonitrile, flow = 1 ml/min, R_t = 20 min. ¹H-NMR(DMSO): δ 8.9 (m, 1H), 8.84 (m, 1H), 8.63 (dm, 1H, J = 9.3 Hz), 8.08 (dm, 1H, J = 9 Hz), 7.65 (br s, 1H), 7.04 (br s, 1H). MS (m/z): 350.53 (MH)⁺. M.p. = 192–194°C. Analytically calculated (C₁₄H₉Cl₂N₅O₂·H₂O): C, 45.65; H, 2.98; N, 19.0; Cl, 19.56. Found: C, 45.92; H, 2.80; N, 18.61; Cl, 20.9.

[4,5-Dichloro-2-(6-nitro-quinazolin-4-yl-amino)-phenyl]-trimethyl-ammonium-triflate (2). 4,5-Dichloro-*N*-(6-nitro-quinazolin-4-yl)-benzene-1,2-diamine **5** (0.091 g, 0.26 mmol) was dissolved in CH₂Cl₂:CH₃CN 5:1 (12 ml). 2,6-Di-*tert*-butyl-4-methylpyridine (0.053 g, 0.26 mmol) was added. After 5 min, methyltrifluoromethanesulfonate (29.4 μ l, 0.26 mmol) was added to the basic solution. After 5 min, a dark-red color was produced. The reaction mixture was stirred for 6 h and compound **2** was obtained with 65% conversion accompanied by by-product **6**. The crude product was purified on short silica gel chromatography column, eluted with 2% MeOH in CH₂Cl₂ to yield compound **2** (0.026 g, 25%). Compound **2** could not be purified further by recrystallization, and since the purity of the compound was only 85%, it was not analyzed by elemental analysis. ¹H-NMR(DMSO): δ 8.93 (m, 1H), 8.62 (m, 1H), 8.35 (m, 1H), 8.04 (m, 1H), 7.82 (dm, 1H), 7.68 (m, 1H), 7.59 (m, 1H), 3.68 (s, 9H). MS(m/z) = 392.53 (MH)⁺.

{4,5-Dichloro-2-[methyl-(6-nitro-quinazolin-4-yl)-amino]-phenyl}-trimethyl-ammonium-triflate (6). Compound **6** was simultaneously purified with **2**, and eluted with 5% MeOH in CH₂Cl₂ to yield **6** (0.005 g, 10%). ¹H-NMR (DMSO): δ 8.6 (m, 1H), 8.34 (dm, 1H), 8.25 (m, 1H), 8.04 (m, 1H), 7.82 (m, 1H), 7.59 (m, 1H), 3.74 (s, 3H), 3.68 (s, 9H). MS(m/z) = 406.4 (MH)⁺.

(4,5-Dichloro-2-nitro-phenyl)-(6-nitro-quinazolin-4-yl)-amine (3). 4-Chloro-6-nitro-quinazoline (0.1 g, 0.47 mmol) was added to a solution of 4,5-dichloro-2-nitro-aniline (0.197 g, 0.95 mmol) and *N,N*-diisopropylethylamine (331 μ l, 1.9 mmol) in i-PrOH (6 ml) and stirred at 90°C for 12 h, yielding a bright-brown precipitate. The solution was cooled, filtered, rinsed with i-PrOH (10 ml), and dried in a vacuum oven to obtain **3** (0.036 g, 20%). TLC conditions: 2% MeOH in CH₂Cl₂, R_f = 0.54. HPLC conditions: C-18 column, 60% acetate buffer, pH = 3.8; 40% acetonitrile, flow = 1 ml/min, R_t = 17.32 min.

$^1\text{H-NMR(DMSO)}$: δ 9.66 (s, 1H), 9.28 (m, 1H), 8.89 (m, 1H), 8.79 (m, 1H), 8.7 (dm, 1H, $J = 9$), 8.45 (d, 1H, $J = 9.6$ Hz), 8.06 (d, 1H, $J = 8.7$ Hz). $\text{MS}(m/z)$: 381.2 (MH)⁺. M.p. = 203–205°C. Analytically calculated (C₁₄H₇Cl₂N₅O₄·H₂O): C, 42.20; H, 2.20; N, 17.58. Found: C, 42.33; H, 1.95; N, 18.2.

Radiochemistry

[^{18}F]fluoride ion was produced using IBA cyclotron by irradiation of [^{18}O]H₂O via the $^{18}\text{O}(p,n)^{18}\text{F}$ nuclear reaction. Syntheses of [^{18}F]-**7** and [^{18}F]-**8** were performed in two commercial automated modules (Nuclear Interface, Münster), and the last four steps with the homemade semi-automated system. The labeled compounds [^{18}F]-**7-11**, and [^{18}F]-**1** were analyzed using HPLC and compared to cold standards.⁶

1,2-Dichloro-4-fluoro-5-nitro-benzene ([^{18}F]-**7**). [^{18}O]H₂O/ $^{18}\text{F}^-$ (1930 mCi) was trapped and then transferred through an ion exchange column (pre-activated with 0.8 ml EtOH and 3 ml HPLC water) and by elution with 0.5 ml potassium carbonate (2.5 mg/0.5 ml) to the reactor. A solution of 1 ml Krytox[®] 222 (18 mg/1 ml CH₃CN) was added. The water was removed by azeotropic distillation with anhydrous acetonitrile at 95°C under reduced pressure for 3 min. 1,2-Dichloro-4,5-dinitro-benzene (10 mg) dissolved in DMF (600 μl) was added. The reactor temperature was increased to 115°C, and the reaction mixture was stirred for 20 min. The mixture was cooled to 30°C, 13 ml of water was added to the reactor, and the mixture loaded on a C-18 cartridge (Waters Sep-Pak, preactivated with 5 ml EtOH and 10 ml of sterile water). Product [^{18}F]-**7** was eluted with 2 ml EtOH to the collection vial, after a total radiosynthesis time of 40 min and was obtained with 80% radiochemical yield. The product was analyzed by a reverse-phase C-18 analytical column and eluted with 55% acetate buffer 0.1 M/45% CH₃CN, flow = 1 ml/min; $R_t = 14.3$ min.

4,5-Dichloro-2-fluoro-aniline ([^{18}F]-**8**). Hydrazine monohydrate (200 μl) was added to a reactor containing 200 μl of EtOH:H₂O 9:1 and Ra-Ni (400 μl), and [^{18}F]-**7** in EtOH/H₂O was added drop-wise to the reactor. The synthesis was proceeded at 60°C for 7 min to yield [^{18}F]-**8** with 70% radiochemical yield. The product was diluted with 10 ml water, passed through a C-18 cartridge, and washed with water (5 ml); the column was dried under argon for 5 min. The product was eluted with *i*-PrOH (2.5 ml), and analyzed by a reverse-phase C-18 analytical column, and eluted with 55% acetate buffer 0.1 M/45% CH₃CN, flow = 1 ml/min; $R_t = 11.66$ min.

(4,5-Dichloro-2-fluoro-phenyl)-(6-nitro-quinazolin-4-yl)-amine ([¹⁸F]-9). 4-Chloro-6-nitro-quinazoline (20 mg) dissolved in i-PrOH (0.5 ml) was added to a solution of [¹⁸F]-8 in i-PrOH (2.5 ml). The reaction mixture was refluxed at 100–105 °C for 5 min and a second batch (20 mg) was added. The reaction mixture was refluxed for an additional 15 min to obtain [¹⁸F]-9 with 80% radiochemical yield. The product was analyzed by a reverse-phase analytical column, and eluted with 55% acetate buffer 0.1 M/45% acetonitrile, flow = 1 ml/min; R_t = 20.92 min.

*N*⁴-(4,5-Dichloro-2-fluoro-phenyl)-quinazoline-4,6-diamine ([¹⁸F]-10). The vial containing [¹⁸F]-9 was transformed into another heating bath (65 °C), EtOH:H₂O 9:1 (250 μl), hydrazine monohydrate (450 μl) and Ra-Ni (400 μl) were added. The reaction mixture was stirred and heated to 65 °C for 10 min to obtain [¹⁸F]-10 with 70% radiochemical yield. The solution was cooled, diluted with water (10 ml) and loaded onto a C-18 Sep-pak; the column was dried under argon for 10 min for the next step. The product was analyzed by a reverse-phase analytical column, and eluted with 55% acetate buffer 0.1 M/45% acetonitrile, flow = 1 ml/min; R_t = 9.73 min.

N-{4-[(4,5-dichloro-2-fluorophenyl)amino]quinazolin-6-yl}-dimethylamine-butynamide ([¹⁸F]-1). [¹⁸F]-10 was eluted with dry THF (2.5 ml) to a conical vial at 0 °C. Two hundred and fifty microliters of *N,N*-diisopropylethylamine in THF (20 μl/ml), and 0.8 ml of Br/Cl-crotonylchloride²⁰ in THF (100 mg/1 ml) were added. The reaction mixture was stirred for 20 min at 0 °C, and used for the next step without any further treatment. The product was analyzed by a C-18 analytical column under the same conditions, R_t = 21.5 min (first peak), R_t = 23.8 min (second peak). Dimethylamine (2.0 M) in THF (1 ml) was added to the solution at 0 °C and the reaction proceeded for 15 min. The solution was evaporated under argon to a volume of 700 μl, then a mixture of CH₃CN:H₂O (1:1) was added. [¹⁸F]-1 was analyzed by a reverse-phase C-18 column, R_t = 11.3 min, and purified using an HPLC reversed-phase C-18 preparative column to yield [¹⁸F]-ML04 with an average of 14% radiochemical yield, specific activity of 1800 Ci/mmol, and 98% radiochemical purity (n = 10). HPLC conditions: C-18 preparative column, 53% ammonium formate 0.1 M/47% acetonitrile, flow = 5 ml/min; R_t = 35 min.

Conclusion

ML04 was successfully labeled with [¹⁸F] at the anilino moiety, by six radiochemical steps using two commercial modules and a homemade semi-automated system. [¹⁸F]-ML04 was obtained after 4 h synthesis time, including purification and formulation with a 14% decay-corrected overall radiochemical yield, specific activity of 1800 Ci/mmol (n = 10) at EOB, and

radiochemical purity of 98%. [^{18}F]-ML04, will allow for prolonged *in vivo* studies including Micro-PET analysis of EGFR tumor-bearing animal models.

Acknowledgements

Support was provided by TK-Signal Ltd, Jerusalem, Israel, and by Rotem Industries Ltd, Beer-Sheva, Israel.

References

1. Voldborg BR, Damstrup L, Spang-Thomsen M, Poulsen HS. *Ann Oncol* 1997; **8**: 1197–1206.
2. Ritter CA, Artega CL. *Sem Oncol* 2003; **30**: 993–1011.
3. Raymond E, Faivre S, Armand JP. *Drugs* 2000; **60**: 15–23 (discussion 41–2).
4. Salomon DS, Brandt R, Ciardiello F, Normanno N. *Crit Rev Oncol Hematol* 1995; **19**: 183–232.
5. Rae JM, Lippman ME. *Breast Cancer Res Treat* 2004; **83**: 99–107.
6. Mishani E, Abourbeh G, Jacobson O, Dissoki S, Ben-Daniel R, Levitzki A. *J Med Chem* 2005; **48**(16): 5337–5348.
7. Cohen MH, Williams GA, Sridhara R, Chen G, Pazdur R. *Oncologist* 2003; **8**: 303–306.
8. Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Shibata T, Sakiyama T, Yoshida T, Tamura T. *J Clin Oncol* 2005; **23**(28): 6813–6816.
9. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. *Science* 2004; **304**: 1497–1500.
10. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. *N Engl J Med* 2004; **350**: 2129–2139.
11. Mishani E, Abourbeh G, Rozen Y, Jacobson O, Laky D, Ben-David I, Levitzki A, Shaul M. *J Nucl Med Biol* 2004; **31**: 469–476.
12. VanBrocklin HF, Dorff PN, Vasdev N, O'Neil JP, Nandan E, Gibbs AR. *J Label Compd Radiopharm* 2003; **46**: S117.
13. Ackermann U, Tochon-Dabguy H, Nice E, Nerrie M, Young K, Sachinidis J, Scott A. *J Label Compd Radiopharm* 2003; **46**: S118.
14. Shaul M, Abourbeh G, Jacobson O, Rozen Y, Laky D, Levitzki A, Mishani E. *Biol Med Chem* 2004; **12**: 3421–3429.
15. Ortu G, Ben-David I, Rozen Y, Freedman NMT, Chisin R, Levitzki A, Mishani E. *Int J Cancer* 2002; **101**(4): 360–370.
16. Seimille Y, Phelps ME, Czernin J, Silverman DHS. *J Label Compd Radiopharm* 2005; **48**: 829–843.
17. Bonasera TA, Ortu G, Rozen Y, Kraiss R, Freedman NM, Chisin R, Levitzki A, Mishani E. *Nucl Med Biol* 2001; **28**: 359–374.
18. Vasdev N, Dorff PN, Gibbs AR, Nandan E, Reid LM, O'Neil JP, VanBrocklin HF. *J Label Compd Radiopharm* 2005; **48**: 109–115.

19. Acheson RM, Taylor GA, Tomlinson ML. *J Chem Soc* 1958; 3750–3752.
20. Tsou H-R, Mamuya N, Johnson BD, Reich MF, Gruber BC, Ye F, Nilakantan R, Shen R, Discifani C, DeBlance R, Davis R, Koehn FE, Greenberger LM, Wang Y-F, Wissner A. *J Med Chem* 2001; **44**: 2719–2734.