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Original article

# Synthesis and initial tumor affinity testing of iodine-123 labelled EGFR-affine agents as potential imaging probes for hormone-refractory prostate cancer

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ABSTRACT

The epidermal growth factor receptor (EGFR) is over-expressed in a variety of human cancers, including in hormone-refractory prostate carcinomas, in which the EGFR has been associated with advanced disease stage, resistance to standard treatment and poor prognosis. Therefore, the EGFR is considered to be a promising molecular target for molecular imaging and therapy for hormone-refractory prostate cancer. This work describes the synthesis and initial tumor affinity testing of the EGFR antagonist $^{123}$I-mAb425 and the EGF receptor tyrosine kinase (EGFR-TK) inhibitor $^{123}$I-PD153035 as potential imaging probes for studying EGFR-expressing prostate cancer using single photon emission tomography.

*Methods:* $^{123}$I-mAb425 and $^{123}$I-PD153035 were prepared, starting from the IgG2a antibody and EGFR antagonist mAb425, that binds to the external domain of the EGF receptors, and from the EGFR-TK inhibitor PD153035, targeting the intra-endothelial tyrosine kinase domain of the EGFR, respectively. The potential of $^{123}$I-mAb425 and $^{123}$I-PD153035 to target EGFR-positive prostate carcinoma was tested on androgen-insensitive PC-3 and DU-145 prostate carcinoma cell lines, and on the androgen-sensitive LNCaP prostate cancer cell line for comparison. In vivo, the capability of $^{123}$I-mAb425 and $^{123}$I-PD153035 to target hormone-refractory prostate cancer was assessed in RNU rats or nu/nu mice bearing human PC3 prostate cancer xenografts.

*Results:* $^{123}$I-mAb425 was obtained in >90% radiochemical yield using the IODO-GEN® method. $^{123}$I-PD153035 was synthesized by a non-isotopic [$^{123}$I]iodo-debromination of PD153035 in 50–60% radiochemical yield in a total synthesis time including HPLC separation of 70 min. In vitro $^{123}$I-mAb425 and $^{123}$I-PD153035 accumulated highly in human PC-3 and DU-145 prostate cancer cells. Radioactivity incorporation into PC-3 and DU-145 tumor cells following 15-min incubation at 37 °C varied from 25% to 48% of the total loaded activity per $10^6$ tumor cells (560–1230 cpm/1000 cells). In comparison, the uptake of the EGFR-affine probes into LNCaP prostate carcinoma cells was significantly low ($105 \pm 25$ cpm/1000 cells). Inhibition experiments revealed that $^{123}$I-mAb425 is taken up into tumor cells via the same pathway as the naturally occurring epidermal growth factor (EGF), while $^{123}$I-PD153035 accumulation in prostate cancer cells occurs presumably via the same pathway as the selective EGFR-Tyrosine kinase antagonist AG1418. In vivo, the human prostate cancer xenografts in mouse war accurately visualized after i.v. administration of $^{123}$I-mAb425 by a gamma camera.

*Conclusion:* These data suggest that $^{123}$I-mAb425 and $^{123}$I-PD153035 are promising candidates as imaging probes for EGFR-positive prostate cancer and warrant further in vivo validations to ascertain their potential as imaging agents for clinical used.



## 1. Introduction

Prostate cancer is the most commonly diagnosed malignancy and third leading cause of cancer death among men in the western world [1,2]. Despite of its epidemiological significance, accurate staging of the disease at initial diagnosis as well as in case of recurrent disease, has been ascertain with the current imaging techniques. These include computed tomography, magnetic resonance imaging and positron emission tomography with fluorodeoxyglucose [3–6]. Therefore, newer imaging modalities have being developed to accurately identify patients at different stages of disease and to facilitate treatment decision for a better outcome for patients. Among the novel imaging methods recently developed for prostate cancer, positron

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emission tomography (PET) with $^{11}$C-choline, $^{11}$C-acetate and $^{18}$F-labelled choline derivatives have shown promising results in a limited number of patients [7–10]. However, the short half-life of $^{11}$C (20 min) means that $^{11}$C-labelled tracers can be used only at facilities that have an on-site cyclotron. For this reason $^{11}$C-choline and $^{11}$C-acetate have been impractical for use as a routine PET tracer in the majority of imaging centers. $^{18}$F-fluoroethylcholine and $^{18}$F-acetate are currently being investigated as imaging agent for prostate cancer. So far, no significant improvement in sensitivity and specificity has emerged for prostate cancer. Compared with $^{11}$C- and $^{18}$F-labelled tracers, imaging probes labelled by radioisotopes such like iodine-123 offer practical benefits due to a long half-life of 13 h for iodine-123 and would be more appropriate for a widespread clinical application using a gamma camera. In a previous work, we reported on the iodinated non-naturally occurring cyclic amino acid 8-[$^{123}$I]iodo-l-1,2,3,4-tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid (ITIC(OH)), that demonstrated high affinity for prostate cancer in preclinical studies [11,12]. Unfortunately, a preliminary investigation in humans suggested that ITIC(OH) is not suitable as imaging probe for clinical use in prostate cancer (unpublished data). The lack of adequate radiopharmaceuticals for routine diagnosis of prostate cancer prompted us to explore further specific pathways involved in the development or progression of prostate cancer. In this regard, recent studies have demonstrated that the epidermal growth factor receptor (EGFR) is up-regulated in a variety of human cancers, including in prostate carcinoma, and that metastatic tissue expressed higher levels of EGFR than primary cancers [13]. Moreover, the over-expression of EGFR correlates with rapid growth, metastasis, resistance to conventional chemotherapy or radiotherapy, and poor prognosis [14–17]. Therefore, the EGFR has been considered as a very promising molecular target for molecular imaging and therapy. This work describes the synthesis and tumor affinity testing of the iodine-123 labelled EGFR antagonist $^{123}$I-mAb425, and the EGF receptor tyrosine kinase inhibitor $^{123}$I-PD153035 as potential radiopharmaceuticals for imaging EGFR-expressing prostate cancer non-invasively using single photon emission tomography.

## 2. Materials and methods

### 2.1. Reagents

Sodium [$^{123}$I]iodide for radiolabelling was obtained from Forschungszentrum Karlsruhe (Karlsruhe, Germany). The monoclonal antibody mAb425 was a gift of Dr. Class (Hahnemann University Hospital, Philadelphia, USA). PD1503035, Iressa®, IMC-C225, AG1478 und EGF were purchased from Biotrend (Köln, Germany) or provided from the Oncologic Laboratory of the Saarland Medical Centre (Homburg/Saar, Germany). IODO-GEN® pre-coated tubes for iodination were from Pierce (Rockford, IL, USA, distributed by KMF Laborchemie Handels GmbH, Sankt Augustin, Germany). The amount of 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (IODO-GEN®) in the tubes, as given by the supplier was 50–100 μg 2-Aminobicyclo[2.2.1]heptane-2-carboxcylic acid (BCH) was purchased from Sigma–Aldrich (Deisenhofen, Germany). Buffered saline solution PBS (pH 7.2, without $MgCl_2$, $CaCl_2$), human insulin (40 IE/μl), physiologic saline solution, ethanol, Heparin and pentobarbital (Narcoren®) were obtained from the Saarland Medical Centre main Pharmacy (Homburg/Saar, Germany). Unless otherwise stated, all other solvents were of analytical or clinical grade and were obtained either from Merck (Darmstadt, Germany) or purchased via the local university hospital pharmacy. Radioactivity in cells was measured on a Berthold LB 951G scintillation counter (Berthold, Wildbad, Germany) after reference samples (triplicates) of the injected dose were prepared as standards.

### 2.2. Synthesis of $^{123}$I-mAb425 and $^{123}$I-PD1503035

Radioiodination of mAb425 to $^{123}$I-mAb425 was performed using 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (IODO-GEN®) as oxidant. In a previous experiment, reaction parameters were optimized in terms of IODO-GEN® and mAb425 concentration, temperature and reaction time. For this purpose, the synthesis was performed with different amounts of IODO-GEN® (50–500 μg) and mAb425. We also varied the reaction temperature (room temperature, 30 °C and 50 °C). The radiochemical yield of the preparation was determined at 2, 5, 10, 15 and 30 min, respectively.

The optimized preparation of $^{123}$I-mAb425 was performed as follows: 100 μl of a solution of mAb425 (1.0 mg/ml PBS, pH 7) and sodium [$^{123}$I]iodine (typically 200–500 MBq diluted with in 30–50 μl PBS) was given into an IODO-GEN pre-coated tube containing 50–100 μg of IODO-GEN®. After 5 min at room temperature while shaking from time to time, the reaction mixture was purified on a Sephadex® G-25M PD-10 column (Pharmacia Biotech, Uppsala, Sweden), using PBS as mobile phase. The fraction containing $^{123}$I-mAb425 was collected into a sterile tube, diluted with PBS and sterile-filtered through a 0.22 μm-filter (Millipore, Eschborn, Germany) into an evacuated sterile vial prior to studies. Quality control was performed by radio TLC, using ITLC-SG plates (Gelman Sciences), eluted with PBS (pH 7.0).

$^{123}$I-PD153035 was prepared by [$^{123}$I]iododebromination of PD153035, which contains a bromine atom at the para-position of its phenyl ring (Fig. 1). In detail, a solution of sodium [$^{123}$I]iodide (370–500 MBq) in 0.05 N NaOH and 5 μl aqueous $Na_2S_2O_5$ (4.0 mg $Na_2S_2O_5$/ml) was evaporated to dryness by passing a stream of nitrogen through a reaction vessel at 90 °C, followed by addition of 50 μl of a PD153035 solution (2.0 mg/ml HOAc), 5 μl L-ascorbic acid (10 mg/ml of water) and 10 μl Cu(I) chloride (0.10 mg/ml HOAc). The reaction vessel was heated for 30 min at 170 °C, cooled and the mixture diluted with water/ethanol. $^{123}$I-PD153035 was separated from radioactive impurities and non-radioactive by-products by HPLC, using acetate buffer/acetonitrile (35:65) as mobile phase with simultaneous UV (254 nm) and radioactivity detection. The isolated fraction containing $^{123}$I-PD153035 was evaporated to dryness by passing a stream of nitrogen through a reaction vessel at 90 °C, followed by addition of ethanol and physiological saline (Braun, Melsungen, Germany) and sterile filtered through a 0.22-μm sterile membrane (Millex GS, Millipore, Molsheim, France).

### 2.3. Investigation of the stability of $^{123}$I-mAb425 and $^{123}$I-PD153035 in injection solutions and in rat serum

In order to assess the stability of $^{123}$I-mAb425 in the injection solution, the freshly formulated injection solution containing $^{123}$I-mAb425 was examined by radio TLC at 1, 4, 6, 8 and 12 h after preparation, using ITLC-SG plates (Gelman Sciences) and PBS as mobile phase. The stability of $^{123}$I-PD153035 was determined at 1, 4, 6, 8 and 12 h after preparation, using analytical HPLC and acetate

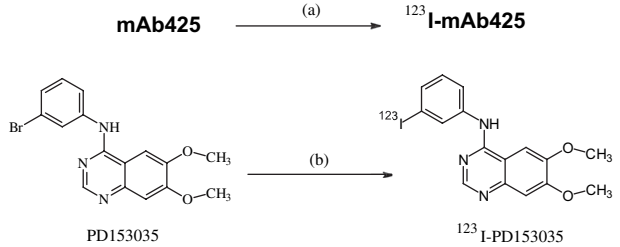


**Fig. 1.** Radiosynthesis of $^{123}$I-mAb425 and $^{123}$I-PD153035. (a): Na [$^{123}$I], Iodo-gen®, for 5 min at room temperature. (b): Na [$^{123}$I], Cu(I)/ascorbic acid/170 °C/30 min.