Preclinical evaluation and pilot clinical study of [\(^{18}\text{F}\)]AlF-labeled FAPI-tracer for PET imaging of cancer associated fibroblasts

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**Abstract** In recent years, fibroblast activation protein (FAP) has emerged as an attractive target for the diagnosis and radiotherapy of cancers using FAP-specific radioligands. Herein, we aimed to design a novel [\(^{18}\text{F}\)]-labeled FAP tracer ([\(^{18}\text{F}\)]AlF-P-FAPI) for FAP imaging and evaluated its potential for clinical application. The [\(^{18}\text{F}\)]AlF-P-FAPI novel tracer was prepared in an automated manner within 42 min with a non-decay corrected radiochemical yield of 32 ± 6% (n = 8). Among A549-FAP cells, [\(^{18}\text{F}\)]AlF-P-FAPI demonstrated specific uptake, rapid internalization, and low cellular efflux. Compared to the patent tracer [\(^{18}\text{F}\)]FAPI-42, [\(^{18}\text{F}\)]AlF-P-FAPI exhibited lower levels of cellular efflux in the A549-FAP cells and higher stability in vivo. Micro-PET imaging in the A549-FAP tumor model indicated higher specific tumor uptake of [\(^{18}\text{F}\)]AlF-P-FAPI (7.0 ± 1.0% ID/g) compared to patent tracers [\(^{18}\text{F}\)]FAPI-42 (3.2 ± 0.6% ID/g) and [\(^{68}\text{Ga}\)]Ga-FAPI-04 (2.7 ± 0.5% ID/g). Furthermore, in an initial diagnostic application in a patient with nasopharyngeal cancer, [\(^{18}\text{F}\)]AlF-P-FAPI and [\(^{18}\text{F}\)]FDG PET/CT showed comparable results for both primary tumors and lymph node metastases. These results suggest that [\(^{18}\text{F}\)]AlF-P-FAPI can be conveniently prepared, with promising characteristics in the preclinical evaluation. The feasibility of FAP imaging was demonstrated using PET studies.

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1. Introduction

Fibroblast activation protein (FAP), a type II transmembrane serine protease, exhibits post-proline dipeptidyl peptidase and endopeptidase activity. Under physiological conditions, FAP is expressed at negligible or non-detectable levels in most normal adult tissues, except for multipotent bone marrow stromal cells (BMSCs) and alpha cells of Langerhans islets. However, FAP is highly upregulated in cancer-associated fibroblasts (CAFs) in greater than 90% of epithelial tumors and in the extracellular matrix of the tumor microenvironment. It has been established that FAP levels are related to the survival and prognosis in cancer patients, indicating that they have a vital role in cancer development. Therefore, FAP has become a promising target for cancer diagnosis and treatment. Recently, several positron emission tomography (PET) radiotracers for FAP were successfully developed and imaged in vivo. Among these, the $^{68}$Ga-labeled ligands have been extensively studied and $^{[68\text{Ga}]}$Ga-FAPI-04 has been applied in clinical imaging for various cancers. However, $^{68}$Ga-labeled tracers have a relatively short half-life of $^{68}$Ga ($t_1/2 = 67.7\text{ min}$, 88.9% $\beta^+$), and are limited in the availability of radionuclide from $^{68}$Ge/$^{68}$Ga-generators. In contrast, $^{18}$F ($t_1/2 = 109.8\text{ min}$, 96.7% $\beta^+$) is the most widely-used radionuclide in PET, can be produced in larger doses by one cyclotron production, and can be delivered over longer distances. Hence, there is a strong demand for $^{18}$F-labeled FAP targeting tracers. Thus far, several $^{18}$F-labeled FAP-targeting radiotracers have been reported. Giesel et al. reported using a $^{18}$F-labeled FAP tracer, $^{[18\text{F}]}$FAPI-74, for the imaging of lung cancer patients in a clinical setting; however, the preclinical data is not yet available. Another tracer, $^{[18\text{F}]}$FGlc-FAPI, was reported based on $^{[18\text{F}]}$fluoroglycosylation for FAP imaging by Toms et al. However, the excretion of $^{[18\text{F}]}$FGlc-FAPI was through the kidneys and hepatobiliary pathway in a preclinical study, which might be a problem for use in the clinical study. Therefore, efforts are underway to develop novel $^{18}$F-labeled FAP tracers to achieve higher tumor uptake using a straightforward method.

Polyethylene glycol (PEG) has been widely used for extending the in vivo half-life of various drugs. In this study, we introduced a PEG linker between the chelator NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) and the pharmacophore FAP inhibitor to prepare an imaging probe ($^{[18\text{F}]}$AlF-P-FAPI) via $^{18}$F-chelation. Additionally, we directly compared the in vitro and in vivo characteristics of $^{[18\text{F}]}$AlF-P-FAPI with two patent tracers, $^{[18\text{F}]}$FAPI-42 and $^{[68\text{Ga}]}$Ga-FAPI-04. Lastly, we used $^{[18\text{F}]}$AlF-P-FAPI to perform a pilot study in a patient with cancer.

2. Materials and methods

2.1. Chemistry

All the reagents and solvents were purchased commercially, were of analytical grade, and were used without further purification. DOTA-FAPI-04 was obtained from Nanchang TanzhenBio Co., Ltd. (Nanchang, China) with high chemical purity (>95%). High-resolution mass spectrometry (HRMS) data were acquired using a Thermo Fisher Scientific Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The synthesis of non-radioactive compounds, including the reference compounds $^{[19\text{F}]}$AlF-P-FAPI and $^{[19\text{F}]}$FAPI-42, and their precursors, are described in detail in Supporting Information Schemes S1 and S2.

2.2. Radiochemistry and quality control

No-carrier-added $^{[18\text{F}]}$fluoride was synthesized using an 18-MeV proton bombardment of a high-pressure $^{18}$O/H$_2$O target utilizing a General Electric PET trace biomedical cyclotron (PET 800; GE, USA). Sep-Pak Plus QMA and Sep-Pak C18-Light cartridges were used for purification.

Figure 1  The structures of $^{[68\text{Ga}]}$Ga-FAPI-04, $^{[18\text{F}]}$FGlc-FAPI, $^{[18\text{F}]}$FAPI-74, $^{[18\text{F}]}$FAPI-42, and $^{[18\text{F}]}$AlF-P-FAPI.
purchased from Waters Associates. Radioactivity was quantified through the use of a Capintec CARPAC-R dose calibrator (NJ, USA).

\[ ^{18}F \]-labeled FAP tracers labeling via Al\(^{18}F\)-chelation were produced on a modified AllInOne synthesis module (Trasis, Ans, Belgium), as reported previously\(^2\),\(^3\),\(^4\). After production in a cyclotron, \( ^{18}F \) was transferred into the module and trapped on a Sep-Pak Plus QMA, preconditioned with 5 mL of 0.5 mol/L sodium acetate buffer at \( pH = 3.9 \) and 10 mL of water. Subsequently, \( ^{18}F \) (37–74 GBq) was eluted using 0.35 mL of 0.5 mol/L sodium acetate buffer \( pH = 3.9 \) to a mixture of \( AC1 \) (40.0 mmol, 20.0 \( \mu L \), 2.0 mmol/L in 0.2 mol/L sodium acetate buffer \( pH = 4.0 \)) and precursor (NOTA-P-FAPI or NOTA-FAPI-42; 80.0 nmol) in 300 \( \mu L \) dimethyl sulfoxide (DMSO). After being heated for 15 min at 105 °C, the mixture was then cooled and diluted with 5 mL of water. Next, the diluted solution was transferred over an activated C18 cartridge, pre-conditioned with 5 mL of ethanol (EtOH) and 10 mL of water. Afterwards, the cartridge was washed with 20 mL of water and flushed with nitrogen sequentially. The desired radiolabeled compound was eluted from the C18 cartridge with 1 mL of ethanol/water (1/1, \( v/v \)), and the C18 cartridge was flushed with 10 mL of 0.9% NaCl. The eluate was passed through a sterile Millipore filter (0.22 \( \mu m \)) into a sterile vial. The final drug product solution was evaluated through a quality control, described in more detail in the Supporting Information.

2.3. Surface plasmon resonance (SPR) binding assays

The experiments were performed using PlexArray HT (Plexera Bioscience, Seattle, WA, USA). The FAP ligands (10 mmol/L in ddH\(_2\)O) were prepared in 384 wells and spotted on 3D photolithography (LithoLab, Japan). The FAP ligands (10 mmol/L in PBS running buffer while a 10 mmol/L protein sample) were added to the wells, where the surface then amounted to 2.8 J/cm\(^2\). After undergoing UV treatment, the chips were rinsed in PBS, and the surface energy change (1200 RU). Binding data were collected using a commercial SPR imaging analysis software (Plexera SPR Data Analysis Model, Plexera).

2.4. Partition coefficient

The \( n \)-octanol/PBS partition coefficients of \[^{18}F\]AlF-P-FAPI, \[^{18}F\]FAPI-42, and \[^{68}Ga\]Ga-FAPI-04 were determined, as previously described\(^2\),\(^3\).

2.5. Cell lines

The human cell lines A549 (ATCC, USA) were maintained in DMEM (Gibco, USA) supplemented with 1% streptomycin and penicillin (Gibco, USA) and 1% streptomycin and penicillin (Gibco, USA) at 37 °C under 5% CO\(_2\). The A549-FAP cell line, which stably expressed human FAP, was acquired using lentiviral infection, following a two-week screening process with 2 \( \mu g \) of puromycin (Thermo Fisher, USA).

2.6. Cell studies and animal models

The detailed protocols for \textit{in vitro} cell studies and animal experiments, including cell uptake, eflux, internalization, and tumor transplanation, are provided in the Supporting Information. All animal studies were carried out and granted approval according to the guidelines of the Institutional Animal Care and Use Committee of Nanfang Hospital of Southern Medical University.

2.7. \textit{In vitro} and \textit{in vivo} stability

Detailed procedures for \textit{in vitro} and \textit{in vivo} stability of \[^{18}F\]AlF-P-FAPI and \[^{18}F\]FAPI-42 are included in the Supporting Information.

2.8. Micro-PET imaging

For dynamic micro-PET imaging studies, either \(^{18}F\)- or \(^{68}Ga\)-labeled tracer (5.55–11.1 MBq, \( n = 3 \)) were injected through the lateral tail vein into mice bearing A549-FAP tumor xenograft using an Inveon Micro-PET/CT scanner (Siemens; Erlangen, Germany). Image studies were conducted using a three-dimensional ordered-subset expectation maximum (OSEM) algorithm (Siemens, Erlangen, Germany). A549-FAP tumor-bearing mice were co-injected for the blocking study using DOTA-FAPI-04 (100 nmol/mouse, \( n = 3 \)) as a competitor. The images and regions of interest (ROIs) were produced using Inveon Research Workplace 4.1 software (Siemens, Erlangen, Germany).

2.9. Biodistribution studies

Organ distribution studies were carried out using mice with xenografted A549-FAP tumors with and without DOTA-FAPI-04 (100 nmol/mouse, \( n = 4 \)). The mice were then injected through the tail vein using \[^{18}F\]AlF-P-FAPI (1.11–1.85 MBq) and euthanized 1 h after injection. Organs of interest and tumor were quickly dissected and weighed, and radioactivity was quantified using a \( \gamma \)-counter and calculated as the percentage of injected dose per gram of tissue (% ID/g).

2.10. PET/CT imaging and analysis

The PET/CT imaging study was performed on a total-body PET/CT scanner (uEXPLORER, United Imaging Healthcare; Shanghai, China) and granted approval by the Ethics Committee of Nanfang
Table 1 Tests items, acceptance criteria and test methods for [18F]AlF-P-FAPI.

| Test item                        | Acceptance criteria                                      | Test method                     |
|---------------------------------|---------------------------------------------------------|--------------------------------|
| Appearance                      | Colorless and particle-free                             | Visual inspection               |
| pH                              | 5.0–8.0                                                 | pH strip                        |
| Radiochemical purity            | ≥90%                                                    | Radio-HPLC                      |
| [18F]AlF-P-FAPI                 |                                                         |                                |
| Sum [18F]F and [18F]AlF         | ≤5%                                                     |                                |
| Chemical purity                 |                                                         |                                |
| Amount of AlF-NOTA-P-FAPI, NOTA-P-FAPI, and metal complexes of NOTA-P-FAPI | <30 μg per injected volume | HPLC with UV detector          |
| Amount of sum of unidentified chemical impurities | ≤10 μg per injected volume |                                |
| Integrity of sterile filter membrane | Bubble point ≥1.5 bar                                   | Bubble point determination |
| Residual solvent                | EtOH: ≤10% v/v                                          |                                |
|                                | DMSO: ≤0.1% v/v                                         |                                |
| Total radioactivity             | 50–5000 MBq/mL                                          | Dose calibrator                 |
| Molar activity                  | ≥3 GBq/μmol                                             | HPLC with UV detector and dose calibrator injector |
| Maximum injection volume        | ≤10 mL                                                  |                                |
| Radionuclide identity—approximate half-life (t1/2) | t1/2 = 110 ± 5 min                                      | Two time point radioactivity measurement in dose calibrator |
| Radionuclide identity—gamma spectrometry | Gamma energy is 0.511 ± 0.02 MeV, or a total peak 1.022 ± 0.02 MeV | Gamma spectrum on NaI (TI) spectrometer |
| Radionuclide purity             | ≥99.8% of the activity of fluorine-18                   | Gamma spectrum on NaI (TI) spectrometer |
| Sterility                       | No growth after 14 days of incubation at 37 °C          | The Chinese Pharmacopoeia       |
| Bacterial endotoxins            | <17.5 EU per mL                                         | LAL testa                       |

*aLimulus amoebocyte lysate (LAL).

Hospital (No. NFEC-2020-205), registered at chictr001@chictr.org.cn (ChiCTR2100045757) and was conducted according to the latest guidelines of the Declaration of Helsinki. These were conducted following the Declaration of Helsinki. The patient signed an informed consent form before participating in the study. A 41-year-old patient with nasopharyngeal cancer was examined using 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG) and [18F]AlF-P-FAPI on two consecutive days. The patient received an intravenous injection of [18F]FDG (3.7 MBq/kg) while fasting for at least 6 h on the first day and [18F]AlF-P-FAPI (3.7 MBq/kg) on the second day without fasting. One hour after the injection of [18F]FDG or [18F]AlF-P-FAPI, the patient underwent imaging and PET images were reconstructed using ordered subsets expectation-maximization algorithm (2 iterations, 10 subsets, 192 × 192 matrix) and corrected for CT-based attenuation, dead time, random events, and scatter.

2.11. Statistical analysis

Data were reported as mean ± standard error of the mean (SEM), and significance of comparison between the two data sets was determined using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Significance was defined at P < 0.05.

3. Results

3.1. Chemistry and radiochemistry

The labeling precursors (NOTA-P-FAPI and NOTA-FAPI-42) and reference compounds ([18F]AlF-P-FAPI and [18F]FAPI-42) were successfully prepared with high chemical purity (>95%) and identified through the use of mass spectrometry (Supporting Information).

[18F]-labeled FAP tracers ([18F]AlF-P-FAPI and [18F]FAPI-42) were successfully prepared in an automated manner via Al18F-chelation on the AllInOne module. The total synthesis time was 40 ± 2 min, starting from the end of [18F]F- transfer to the synthesis module to obtain the final product. The non-decay corrected labeling yield for [18F]AlF-P-FAPI was 32 ± 6% (n = 8) and the measured specific activity was 46–182 GBq/μmol (n = 8). The non-decay corrected radioactive yield for [18F]FAPI-42 was 28 ± 8% (n = 10), and the specific activity was found to be 52–200 GBq/μmol (n = 10). The acceptance criteria for [18F]AlF-P-FAPI is summarized in Table 1 based on the monograph of “2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG) injection” in the Chinese Pharmacopoeia.

3.2. In vitro evaluation

The partition coefficients (logD) demonstrated high hydrophilic properties for [18F]AlF-P-FAPI and [18F]FAPI-42 with values of −2.72 ± 0.07 and −2.43 ± 0.02, respectively, which is similar to the clinically used radiotracer 68GaGa-FAPI-04 (−2.63 ± 0.04).

The stability of [18F]AlF-P-FAPI and [18F]FAPI-42 in PBS, mouse serum, and mouse blood is shown in Fig. 2. Determining the in vitro stability in PBS and mouse serum at 37 °C for 2 h indicates high stability across both tracers. We did not observe any decomposition (radiochemical purity >95%) across both tracers using radio-performance liquid chromatography (radio-HPLC). The in vivo stability of [18F]AlF-P-FAPI and [18F]FAPI-42 was also tested from mice blood at different times intervals post-injection (p.i.). The parent of [18F]AlF-P-FAPI was greater than 95% within the tested time. Nevertheless, the parent of [18F]FAPI-42 at 15, 30, and 60 min constituted 20%, 15%, and 12%, respectively.
The binding affinity of NOTA-P-FAPI, NOTA-FAPI-42, and DOTA-FAPI-04 for FAP was determined using SPR imaging, the results of which are shown in Fig. 3A. The $K_d$ values for NOTA-P-FAPI and NOTA-FAPI-42 were $0.73 \times 10^{-10}$ mol/L and $0.11 \times 10^{-10}$ mol/L, respectively, and comparable to that of DOTA-FAPI-04 ($0.63 \times 10^{-10}$ mol/L). These results indicate that the three radiotracers have a high affinity towards FAP.

The FAP-positive A549-FAP cells and FAP-negative A549 cells for cell studies were identified in the Supporting Information. The binding properties of $[^{18}F]$AlF-P-FAPI and $[^{18}F]$FAPI-42 to FAP were evaluated across different cell lines and cell lines transfected with human FAP (Fig. 3B–E). $[^{18}F]$AlF-P-FAPI and $[^{18}F]$FAPI-42 indicated high uptake in FAP-positive A549-FAP and 293T-FAP cell lines and were significantly blocked by adding the competitor DOTA-FAPI-04. Additionally, FAP-negative A549 and 293T cells did not show any significant uptake of both tracers, suggesting high specificity of both tracers. Time-dependent uptake of $[^{18}F]$AlF-P-FAPI demonstrated a fast cellular uptake (>14% ID/1 million cells after 5 min; Fig. 3C) with a linearly increasing uptake which reached a maximum of 66.4 ± 2.3% ID/1 million cells at 120 min, and can be significantly blocked by the adding DOTA-FAPI-04 to less than 0.8% ID/1 million cells. Internalization assays in A549-FAP cells...
demonstrated a rapid and high uptake of $[^{18}\text{F}]$AlF-P-FAPI and $[^{18}\text{F}]$FAPI-42 after 10 min of incubation with 76% and 65% internalized activity, respectively. Efflux experiments demonstrated that $[^{18}\text{F}]$AlF-P-FAPI exhibits a lack of significant cellular efflux in A549-FAP cells, which shows more than 95% of retention of the originally accumulated radioactivity during the 2-h incubation time. The efflux of $[^{18}\text{F}]$FAPI-42 from A549-FAP cells was almost linear during the 2-h incubation time. After the 2-h incubation, approximately 75% of the radioactivity was released from tumor cells.

3.3. Micro-PET/CT and biodistribution studies

The dynamic micro-PET studies on mice bearing xenografts from human FAP-positive tumor cells were conducted using $[^{18}\text{F}]$AlF-P-FAPI. These results are represented through coronal micro-PET images taken at different time interval p.i. (Fig. 4A). The PET images and time–activity curves demonstrate a rapid and high initial A549-FAP tumor uptake, with a constant increase over the total scan time of 120 min (Fig. 4B).

A comparison of micro-PET imaging of $[^{18}\text{F}]$AlF-P-FAPI, $[^{18}\text{F}]$FAPI-42, and $[^{68}\text{Ga}]$Ga-FAPI-04 was conducted using FAP-positive A549-FAP xenografts (Fig. 4C). Maximum intensity projections (MIP) indicated clear visualization of A549-FAP tumors with the three radiotracers. $[^{18}\text{F}]$AlF-P-FAPI and $[^{18}\text{F}]$FAPI-42 were observed as the predominant liver excretions, as indicated by nonspecific uptake in the gallbladder and intestines. In comparison, $[^{68}\text{Ga}]$Ga-FAPI-04 was found to be mainly excreted via the renal route. Dynamic measurements for 60 min p.i. revealed that the tumor uptake of $[^{18}\text{F}]$AlF-P-FAPI was significantly higher compared to $[^{18}\text{F}]$FAPI-42 and $[^{68}\text{Ga}]$Ga-FAPI-04 ($P < 0.05$). Therefore, $[^{18}\text{F}]$AlF-P-FAPI had a significantly higher tumor-to-background tissue contrast compared to the other two probes (Fig. 4E). In addition to the uptake in the tumor, both $[^{18}\text{F}]$AlF-P-

Figure 4  Representative micro-PET images and time–activity curves. (A) A series of coronal and transverse dynamic PET images of A549-FAP tumor-bearing mice at 5, 30, 60, 90, and 120 min p.i. of $[^{18}\text{F}]$AlF-P-FAPI. The A549-FAP tumors are delineated in white arrows. (B) Time–activity curves of organs and A549-FAP tumor for 2 h p.i. of $[^{18}\text{F}]$AlF-P-FAPI. (C) MIP at 60 min post-intravenous injection of $[^{18}\text{F}]$AlF-P-FAPI, $[^{18}\text{F}]$FAPI-42, and $[^{68}\text{Ga}]$Ga-FAPI-04 with and without DOTA-FAPI-04 as a competitor. Shoulder joint (SJ); tumor (T); gall bladder (GB); liver (L); bladder (BL). (D) Time–activity curves of A549-FAP tumor uptake for 1 h p.i. of $[^{18}\text{F}]$AlF-P-FAPI, $[^{18}\text{F}]$FAPI-42, and $[^{68}\text{Ga}]$Ga-FAPI-04 with and without DOTA-FAPI-04 as a competitor. (E) The ratios of tumor-to-muscle for 1 h p.i. of $[^{18}\text{F}]$AlF-P-FAPI, $[^{18}\text{F}]$FAPI-42, and $[^{68}\text{Ga}]$Ga-FAPI-04, respectively.
FAPI and [18F]FAPI-42 exhibited high uptake in joints (knees and shoulders), with uptake values (in % ID/g) of 5.49 ± 0.25, and 4.07 ± 0.04, respectively, at 1-h p.i. However, [68Ga]Ga-FAPI-04 accumulated within the joints to a lower extent (0.90 ± 0.07% ID/g). The blocking study for the tested three tracers in mice bearing A549-FAP tumors indicated a remarkable decrease in uptake by tumors and joints (Fig. 4C and D), demonstrating the specificity of radiotracers for its target protein.

We conducted biodistribution studies of [18F]AlF-P-FAPI with and without DOTA-FAPI-04 as a competitor 1 h after injection to further confirm micro-PET imaging quantification (Fig. 5). High and specific uptake of tracers was observed in the A549-FAP tumors. The uptake in FAP-positive organs, including tumor and bone, was significantly reduced via co-injection of an excess of DOTA-FAPI-04, indicating FAP-specific targeting.

3.4. PET/CT imaging and analysis

The patient with nasopharyngeal cancer tolerated the examination and did not report any subjective effects after injection of [18F]AlF-P-FAPI. There were no drug-related adverse events or physiologic responses. In the patient, [18F]AlF-P-FAPI PET/CT showed intense uptake of radioactivity within the primary tumor (SUV\textsubscript{max} of 14.1) and lymph node metastases (SUV\textsubscript{max} of 13.7 and 8.6, respectively), similar to [18F]FDG PET/CT (primary tumor: SUV\textsubscript{max} of 17.6; lymph node metastases: SUV\textsubscript{max} of 11.9 and 7.1, respectively, Fig. 6). The uptake of [18F]AlF-P-FAPI within the kidneys and bladder indicated that the main excretion organ was the kidneys. In addition, thyroid glands, the submandibular glands, parotid glands, and pancreas were clearly visible at 1 h p.i. The primary tumor lesion and lymph node metastases were clearly defined on the [18F]AlF-P-FAPI PET/CT due to the low physiologic uptake of [18F]AlF-P-FAPI within the brain. These results suggest that this method has the potential to be translated into a clinical setting.

4. Discussion

The main goal of the present study was to identify a novel 18F-labeled FAP tracer for cancer imaging. Here, we report the synthesis and preclinical characterization of [18F]AlF-P-FAPI, as well as the direct comparison with patent tracers [18F]FAPI-42 and [68Ga]Ga-FAPI-04. In addition, a first-in-man study was conducted using [18F]AlF-P-FAPI.

McBride et al.\textsuperscript{27} developed direct radiolabeling of peptides and proteins through chelation with Al\textsuperscript{18F}. Our previous work also demonstrated that radiolabeling of peptides through complexation of Al\textsuperscript{18F} by radiometal chelator NOTA could be good labeling yield and automated production\textsuperscript{28}. We designed a novel precursor NOTA-P-FAPI using a PEG2 linker between the quinolone-based pharmacophore and the NOTA chelator to take advantage of the Al\textsuperscript{18F}-labeling method. The PEG linker was used to improve the in vivo half-life and metabolic stability\textsuperscript{19,20}. NOTA-FAPI-42 was obtained by substituting the DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic...
acid) motif of DOTA-FAPI-04 by the NOTA motif. Interestingly, the
binding affinity of NOTA-FAPI-42 was approximately six-fold that of
the known tracer DOTA-FAPI-04, while NOTA-P-FAPI displayed a similar
binding affinity to DOTA-FAPI-04.

The preparation of \(^{18}\text{F}\)AlF-P-FAPI and \(^{18}\text{F}\)FAPI-42 was efficient and convenient, using an automated synthesis module with high molar activities. Due to the effect of the linker, the lipophilicity of \(^{18}\text{F}\)AlF-P-FAPI was lower than that of \(^{18}\text{F}\)FAPI-42. Meanwhile, stability studies did not find any degradation of \(^{18}\text{F}\)AlF-P-FAPI in vitro or in vivo, whereas \(^{18}\text{F}\)FAPI-42 was degraded in vivo. These results are consistent with the hypothesis that using PEG can increase hydrophilicity and improve half-life in vivo\(^{19,20}\).

Cellular uptake studies using FAP-positive and FAP-negative cells indicated that both tracers were rapidly internalized with specific binding to FAP. In addition, the amount of internalized activity of \(^{18}\text{F}\)AlF-P-FAPI was higher than that of \(^{18}\text{F}\)FAPI-42. Efflux experiments demonstrated that \(^{18}\text{F}\)AlF-P-FAPI was significantly slower compared to \(^{18}\text{F}\)FAPI-42, which might be caused by the higher stability of \(^{18}\text{F}\)AlF-P-FAPI as compared to \(^{18}\text{F}\)FAPI-42.

The micro-PET imaging results demonstrated that \(^{18}\text{F}\)AlF-P-FAPI had significantly higher tumor uptake compared to the other two patent tracers, while \(^{18}\text{F}\)FAPI-42 had comparable tumor uptake in comparison to \(^{68}\text{Ga}\)Ga-FAP-04. Moreover, the uptake of \(^{18}\text{F}\)AlF-P-FAPI in the tumor was visualized at 30 min p.i., with low uptake in all the non-target organs, except for liver and intestine decline over time, leading to a constant improvement in the tumor-to-background ratios. In addition, both \(^{18}\text{F}\)AlF-P-FAPI and \(^{18}\text{F}\)FAPI-42 exhibited higher uptake in joints (knees and shoulders), compared to \(^{68}\text{Ga}\)Ga-FAP-04, consistent with the literature reported for \(^{18}\text{F}\)FGlc-FAPI\(^7\). It has been reported that FAP is expressed in BMSCs of mice, which are capable of directed migration towards various tumor types, involving tissue remodeling\(^2,29,30\). The ex vivo distribution studies of \(^{18}\text{F}\)AlF-P-FAPI indicated the highest uptake within the gall bladder, indicating excretion mainly through the hepatobiliary pathway. The compound DOTA-FAPI-04 has been proven to be a FAP competitor in the previous literature\(^16\). The in vivo blocking experiment demonstrated that co-injection of DOTA-FAPI-04 significantly diminished the tumor and joints uptake of \(^{18}\text{F}\)AlF-P-FAPI, suggesting its high specificity for FAP within these regions.

Based on prior preclinical studies, we conducted the pilot clinical study of \(^{18}\text{F}\)AlF-P-FAPI in one nasopharyngeal cancer patient to examine the potential clinical application of FAP imaging. There were no subjective effects reported by the patient p.i. with a tracer. The excretion pathway of \(^{18}\text{F}\)AlF-P-FAPI in human predominantly renal elimination is similar to that of reported tracers \(^{18}\text{F}\)FAPI-74 and \(^{68}\text{Ga}\)Ga-FAP-04\(^{4,31}\). Due to different species, the excretion of \(^{18}\text{F}\)AlF-P-FAPI in humans is different from that of mice. Our preliminary experience in one patient showed that \(^{18}\text{F}\)AlF-P-FAPI had a high level of physical uptake in thyroid glands, submandibular glands, parotid glands, and pancreas, which has also been observed in clinical studies for \(^{18}\text{F}\)FAPI-42\(^3,33\). Compared to \(^{18}\text{F}\)FDG, the pilot study of \(^{18}\text{F}\)AlF-P-FAPI demonstrated comparable tumor detectability in nasopharyngeal cancer. \(^{18}\text{F}\)AlF-P-FAPI, as a FAP tracer for PET imaging, has a higher specificity compared to \(^{18}\text{F}\)FDG and is independent of blood-sugar and physical activity. Although limited by the number of patients available, our pilot study of \(^{18}\text{F}\)AlF-P-FAPI demonstrated promising imaging properties for cancer associated fibroblasts imaging. Furthermore, a study directly comparing \(^{18}\text{F}\)-labeled FAP tracer versus \(^{68}\text{Ga}\)Ga-FAP-04 in tumor patients is underway.

5. Conclusions

We developed a fluorine-18 labeled FAP-targeting tracer through automated preparation for imaging of cancer associated fibroblasts. The preclinical evaluation of \(^{18}\text{F}\)AlF-P-FAPI exhibited suitable characteristics for tumor imaging of FAP expression in mice. Compared to patent tracers \(^{18}\text{F}\)FAPI-42 and \(^{68}\text{Ga}\)Ga-FAP-04, \(^{18}\text{F}\)AlF-P-FAPI exhibited a higher tumor uptake and retention. The primary clinical studies have demonstrated the safety and feasibility of \(^{18}\text{F}\)AlF-P-FAPI for further clinical translation.

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Author contributions

Study conception and design, Kongzhen Hu, Ganghua Tang, and Jin Su; acquiring data, Kongzhen Hu, Junqi Li, Yong Huang, Li Li, Shinmin Ye, and Yanjiang Han; analysis and interpretation of data, Kongzhen Hu, Lijuan Wang, Yong Huang, Junqi Li, and Jin Su; diagnostic imaging, Lijuan Wang and Huaing Wu; data management, Shun Huang; drafting the manuscript, Kongzhen Hu; revising the manuscript, Ganghua Tang and Jin Su. All authors read and approved the final manuscript.

Conflicts of interest

All authors declare no potential conflicts of interest.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2021.09.032.

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