First-In-Human Results On The Biodistribution, Pharmacokinetics, And Dosimetry of [^{177}Lu]Lu-DOTA.SA.FAPi and [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂ in Patients with Various End-stage Cancers

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Abstract

Purpose: The present study aimed to evaluate and compare the biodistribution, pharmacokinetics, dosimetry of [^{177}Lu]Lu-DOTA.SA.FAPi, and [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ in patients with various cancers.

Methods: The FAPi agents, [^{177}Lu]Lu-DOTA.SA.FAPi and [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ were administered in two different groups of patients. Three patients (mean age; 50 years) were treated with a median cumulative activity of 2.96 GBq (IQR: 2.2 – 3 GBq) [^{177}Lu]Lu-DOTA.SA.FAPi and seven (mean age; 51 years) were treated with 1.48 GBq (IQR: 0.6 – 1.5) of [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$. Patients in both the groups underwent serial imaging whole-body planar and SPECT/CT scans that were acquired between 1 hour and 168 hours post-injection (p.i.) The residence time and absorbed dose estimate in the source organs and tumor were calculated using OLINDA/EXM 2.2 software. Time versus activity graphs were plotted to determine the effective half-life (T_e) in the whole body and lesions for both the radiotracers.

Results: Physiological uptake of [^{177}Lu]Lu-DOTA.SA.FAPi was observed in the kidneys, colon, pancreas, liver, gall bladder, oral mucosa, lacrimal glands, and urinary bladder contents. Physiological biodistribution of [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ involved liver, gall bladder, colon, pancreas, kidneys, and urinary bladder contents, lacrimal glands, oral mucosa, and salivary glands. The whole body effective dose for [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ was significantly higher than [^{177}Lu]Lu-DOTA.SA.FAPi [$2.26\text{E-}01 \pm 1.24\text{E-}01$; vs. $6.22\text{E-}02 \pm 9.96\text{E-}03$ mSv/MBq, $P=0.058$). In the [^{177}Lu]Lu-DOTA.SA.FAPi group, the highest absorbed dose were noted in the Kidneys (0.618 ± 0.015 Gy/GBq), followed by a colon (right colon: 0.472 Gy/GBq and left colon: 0.43 Gy/GBq). In the [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ group, the colon received the highest absorbed dose (right colon: 1.16 Gy/GBq and left colon: 2.87 Gy/GBq), and demonstrated a significantly higher mean absorbed dose than [^{177}Lu]Lu-DOTA.SA.FAPi ($P < 0.011$). [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ had significantly longer median whole-body T_e compared to that of [^{177}Lu]Lu-DOTA.SA.FAPi [46.2 h (IQR: $38.5 - 70.1$) vs. 23.1 h (IQR: $17.8 - 31.5$); $P=0.0167$]. The median absorbed doses to the lesions were $6.03\text{E-}01$ (IQR: $2.30\text{E-}01 - 1.81\text{E+}00$) Gy/GBq and $6.70\text{E+}00$ (IQR: $3.40\text{E+}00 - 4.9\text{E+}01$) Gy/GBq dose per cycle in the [^{177}Lu]Lu-DOTA.SA.FAPi, and [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ groups, respectively.

Conclusion: The first clinical dosimetry study demonstrated significantly higher tumor absorbed doses with [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ compared to [^{177}Lu]Lu-DOTA.SA.FAPi. [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ is safe and unveiled new frontiers to treat various end-stage cancer patients with a theranostic approach.

Introduction

The tumor microenvironment (TME) plays a crucial role in tumor remodeling and is an important contributor to tumor growth and promoting drug resistance. Within the TME, cancer-associated fibroblasts (CAFs) have a multifaceted function and are major contributors to TME remodeling. The high abundance of CAFs in a wide range of tumors offers important implications to target various cancers.

The fibroblast activation protein (FAP α), a type II transmembrane serine protease, is highly expressed in CAFs. Histopathologic studies reported the prevalence of FAP-positive cancer-associated fibroblasts in ~90% of epithelial tumors [1]. The ubiquitous expression of fibroblast activation protein (FAP) makes it an interesting target for imaging and therapy of a wide spectrum of malignancies [1]. FAP promotes tumor growth, proliferation, and angiogenesis [2]. Hence, targeting this protein with several probes, including antibodies, immunoconjugates, and small molecular FAP inhibitors, may be an interesting approach for tumor detection and suppression.

Although FAP imaging is in the early developmental stage, several FAP inhibitor based small molecules (chelator-linker-FAP inhibitor conjugates) have been developed. Mostly, heterocyclic linker units between chelator and inhibitor were introduced by piperazine series [3, 4] and squaramide based [5, 6, 7] FAP-inhibitor precursors, all based on the highly affine and selective inhibitor lead structure UAMC1110 as described by van der Veken's group [8, 9] have been developed for diagnostic and therapeutic use. (Fig. 1)

Haberkorn's group reported a series of piperazine-based FAP-inhibitors labeled with the positron emitter gallium-68, which were successfully used for imaging various cancers [3, 4], particularly when utilizing the most prominent molecules among their structures such as FAPI-04, FAPI-21, and FAPI-46 [4].

Roesch's group, in collaboration with our group, introduced a modified ligand keeping the pharmacophore intact as new FAPI PET tracers. The critical subunits constituted the squaramide (SA) linker unit coupled with the DOTA/ DATA^{5m} bifunctional chelators and a FAP inhibitor targeting moiety. Both agents were coupled with generator produced gallium-68 and revealed promising imaging and theranostic benefits on in vitro, preclinical, and clinical studies [5, 6]. It is well established now that these FAP inhibitors show expression in various cancers [5–7, 10]. The monomeric DOTA.SA.FAPI labelled with gallium-68 showed the most favorable properties from the imaging point of view which includes high tumor-to-background ratios [TBR], and demonstrates a great applicability for theranostic treatment approach of various cancers.

We applied a theranostic approach of [⁶⁸Ga]Ga guided [¹⁷⁷Lu]Lu-DOTA.SA.FAPI therapy in an advanced stage breast cancer (histology status: ER⁻, PR⁻, HER2/neu⁺) patient who failed multiple lines of treatment, and demonstrated an promising improvement in the quality of life. This radio-ligand therapy concept unveiled a new milestone in precision oncology. However, the findings were preliminary, and the detailed pharmacokinetics and dosimetry data were underway [7]. On visual analysis, we noticed early washout of [¹⁷⁷Lu]Lu-DOTA.SA.FAPI radiotracer. To overcome this problem, in consultation with our group, Moon et al., [11] modified the structure and introduced dimeric systems for prolonged tumor retention. Using the SA.FAPI monomer as the base, they developed two homodimeric structures such as DOTA(SA.FAPI)₂ and DOTAGA (SA.FAPI)₂ (Fig. 1).

The DOTAGA.(SA.FAPI)₂ is based on the monomeric DOTA.SA.FAPI structure, but unlike the monomer, two identical SA.FAPI units are bound to a trifunctional DOTAGA chelator forming a homodimeric system.

Additionally, for the possibility of complexing radiometals such as lutetium-177 or actinium-225, at least seven coordination's are required; hence, DOTAGA as chelator was used in the case of the dimer. (Fig. 1)

Various derivatives were investigated in *in vitro* binding assays to FAP, DPPs (proline-specific enzymes dipeptidyl peptidases), and PREP (prolyl oligopeptidase), and revealed high affinity and protease selectivity to FAP and towards DPPs and PREP (Table 1).

Table 1
IC50 values for FAP and the related serine proteases (PREP, DPP4, DPP8, DPP9). (Data from reference 5 and 11)

Compound	IC ₅₀ (μM)			IC ₅₀ (nM)	
	DPP4	DPP8	DPP9	PREP	FAP
DOTAGA.(SA.FAPi) ₂	0.40 ± 0.07	0.42 ± 0.04	0.16 ± 0.02	0.39 ± 0.02	0.92 ± 0.06
[^{nat} Lu]Lu-DOTAGA.(SA.FAPi) ₂	0.63 ± 0.07	0.41 ± 0.03	0.18 ± 0.02	0.56 ± 0.04	1.54 ± 0.15
[^{nat} Ga]Ga-DOTA.SA.FAPi	> 1	N/A	> 1	8.7 ± 0.9	1.4 ± 0.2
[^{nat} Lu]Lu-DOTA.SA.FAPi	> 1	N/A	> 1	2.5 ± 0.4	0.8 ± 0.2
FAP Fibroblast Activation Protein; DPPs Proline-specific enzymes Dipeptidyl Peptidases; PREP Prolyl Oligopeptidase.					

It is of interest to know whether in vitro results of homodimers still hold true from the clinical aspect; therefore, the aim of the present study was to compare the in vivo biodistribution, pharmacokinetics, absorbed dose estimates, and effective half-lives of [¹⁷⁷Lu]Lu-DOTA.SA.FAPi monomer and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ dimer in cancer patients.

Materials And Methods

Patient Recruitment

The study was duly approved by the ethics committee of the All India Institute of Medical Sciences, New Delhi. Patients were included for [¹⁷⁷Lu]Lu-DOTA.SA.FAPi or [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ treatment if they had histologically confirmed carcinoma, documented radiological/molecular or biochemical disease progression on previous lines of treatment, and have exhausted all lines of treatments, ECOG status up to 4, cancers that demonstrated high FAPi expression on [⁶⁸Ga]Ga-DOTA.SA.FAPi PET/CT scan (SUVmax>3), and patients who signed the informed consent form.

Patients who received prior anti-cancer therapy in less than four weeks' time, patients with Hb<9 g/dL, leukocyte counts less than 4.0 × 10⁹/L, platelet counts less than 75,000 per mL, inadequate liver function parameters, serum creatinine >1.2 mg/dL were excluded from the study.

The study was first initiated using [^{177}Lu]Lu-DOTA.SA.FAPi, but after the preliminary qualitative results of serial imaging, we observed low radiotracer retention at about 1 to 2 days p.i. in the target lesions. To improve the radiotracer's retention time, further modifications of the radiopharmaceutical's design led to the development of DOTAGA.(SA.FAPi) $_2$ homo-dimer.

Pertaining to the time difference in chemical modifications in the molecule, the recruiting time-points in both patients groups were different. A total of 3 patients (mean: 50 ± 17.2 (31-63) years, 3 females) were recruited from May 2020 to August 2020 in the [^{177}Lu]Lu-DOTA.SA.FAPi group. Seven patients (mean: 51 ± 12.7 (26 - 63) years, 4 males and 3 females) were recruited between November 2020 to March 2021 in the [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ group. Dosimetry analysis was conducted, compared, and analyzed between patients treated with [^{177}Lu]Lu-DOTA.SA.FAPi and [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$.

[^{68}Ga]Ga-DOTA.SA.FAPi PET/CT imaging

Scans were obtained on a dedicated GE Discovery 710* 128 Slice PET/CT Scanner, with a 40-mm detector at a rotation speed of 0.35 seconds. Whole-body PET/CT scans were acquired 1 hour after the administration of [^{68}Ga]Ga-DOTA.SA.FAPi (mean injected activity: 148 MBq). Patients were positioned in a supine position, and an initial scout was acquired, followed by a diagnostic dose CT with 300–350 mAs, 120 kVp, slice thickness 5 mm, and pitch 1 and PET acquisition with 2 minutes per bed.

The images were subjected to dead-time, random, scatter, and decay correction. The PET image reconstruction was performed using an ordered subset expectation maximization algorithm (OSEM) (21 subsets 3 iterations). All images were processed and analyzed on the GE Xeleris workstation.

[^{177}Lu]Lu-DOTA.SA.FAPi and [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ Radiolabelling

25 nmol of [^{177}Lu]Lu-DOTA.SA.FAPi and DOTAGA.(SA.FAPi) $_2$ were radiolabelled with [^{177}Lu]LuCl $_3$ which was obtained from BRIT, India, in sodium acetate buffer, pH 4, in 0.01 M supra pure HCl. The radiolabelled solution was heated at 95 °C for 30 min. Radiochemical quality control was carried out using the instant thin-layer chromatography method with sodium citrate buffer as the solvent and radiolabelled products with >90% purity were administered.

Post-therapy [^{177}Lu]Lu-DOTA.SA.FAPi and [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ whole body scintigraphy

The planar acquisition of whole-body scans was performed using a dual-headed gamma camera (GE, Discovery NM/CT 670). The camera was equipped with a high-energy general-purpose (HEGP) parallel-hole collimator, and the energy peak was cantered at 113 keV and 208 keV with a 10% window width. Dual-energy Scatter corrections were applied at 90 KeV and 170 keV with a window width of 10%. Serial whole-body emission scans were performed at 1 (pre-void), 6, 24, 48, and 144 hours (h) after treatment for the [^{177}Lu]Lu-DOTA.SA.FAPi group and at 1 (pre-void), 4-6, 24, 48, 96, and 144 to 168 h in the [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ group. Simultaneous anterior and posterior emission scans were acquired at a speed

of 15 cm/min and a matrix size of 256 X 1024. Delayed images were acquired up to 168 h post-injection to prevent the overestimation of doses.

Similarly, SPECT/CT scans of the abdomen and the lesions were acquired in both the radiotracer groups at serial time points but were mainly used to demarcate the overlapping gut and kidney activity and to calculate the volume of the tumor. SPECT/CT acquisition parameters included a total angular range of 360 degrees, an angle view of 6 degrees, acquired at 25 seconds per view, and a matrix size of 512 X 512.

Image Analysis

In the dosimetry analysis, salivary glands, kidneys, pancreas, liver, gall bladder, right colon, left colon, tumor lesions, and whole body were included for dose calculation. The first whole-body image post-injection before voiding was considered to include 100% of injected activity. The region of interests (ROI's) was drawn on the source organs showing uptake of [¹⁷⁷Lu]Lu-DOTA.SA.FAPi and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ on both anterior (A) and posterior images (P). The ROI of the initial scan was cloned to the subsequent serial time-point images of the patient.

Background counts were obtained from the thigh region. For overlapping organs such as the right kidney had overlapping intestinal uptake, the counts were considered to the left kidney. The corresponding time-point Tx-SPECT/CT scans were also referred to prevent overlap. Background correction of lesion counts was done by subtracting counts in background ROI of the similar area drawn close to the lesions.

Finally, attenuated, background, and scatter corrected percentage injected activity (%IA) in each source organ including salivary glands (parotid and submandibular glands), kidney, liver, gall bladder, pancreas, right and left colon, and the tumor was calculated according to the equation 3.

$$\%IA_{uncorr} = \frac{Ct_{ROI} / pixel}{Ct_{WB} / pixel} \times 100 \quad (1)$$

Where:

%IA_{unCorr}: Uncorrected Percentage of injected activity

Ct_{ROI/pixel}: counts/pixel in a region of interest

Ct_{WB/pixel}: counts in the whole-body image

$$\%IA_{Corr} = \frac{Ct_{ROI} / pixel}{Ct_{WB} / pixel} \times DF \times 100 \quad (2)$$

where:

%IA_{Corr}: Corrected Percentage of injected activity (Corrected with Decay factor)

Ct_{ROI/pixel}: counts/pixel in the region of interest

Ct_{WB/pixel}: counts/pixel in Whole-body image

DF: decay factor (¹⁷⁷Lu- 0.9 for 24 hrs)

Internal Dose Estimation

The percentage injected activities against time were entered in the kinetic input model of the OLINDA/EXM v2.2 software to calculate the area under the curve that represented the number of disintegrations or residence time or cumulative activity in each source organ. The residence times were input to the ICRP-89 female and male models to derive absorbed doses of organs and whole-body effective doses.

Tumor Dosimetry

For the tumor dosimetry, a sphere model implemented within OLINDA/EXM v2.2 was used. For each considered lesion, the volume was evaluated on pre-therapy [⁶⁸Ga]Ga-DOTA.SA.FAPi PET/CT and Tx SPECT-CT of the area of interest using the commercially available workstation (GE Xeleris).

For the estimation of tumor absorbed dose, the dose equation based on the MIRD formalism is expressed below [12, 13] [Equation 3].

$$D = \tilde{A} * S = A_0 * \tau * S \quad (3)$$

Here, τ is the residence time, $\tilde{A}$ is the cumulated activity, A_0 is the patient's administered activity, and S is the mean absorbed dose per unit cumulated activity.

Finally, the residence times of source organs and tumors were entered in the adult female or male ICRP 89 model for normal organs and the sphere model, respectively, that derived the organ absorbed doses, effective dose for each organ as per the ICRP 103 model, and whole-body effective dose, in terms of mSv/MBq. The time-activity graphs and effective half-lives (T_e) of various organs and tumors were generated using GraphPad Prism software (v9.1).

Blood dosimetry

Blood dosimetry was conducted in all patient belonging to the [¹⁷⁷Lu]Lu-DOTA.SA.FAPi and was feasible only in three patients in the [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ group. One millilitre of venous blood sample was taken at 0.5 (prevoid), 3.5, 24, 48, 72, 96, 120, 144 and 168 h after injection from each patient. The marrow dose was derived using the method of Sgouros [14].

Safety

Safety was assessed by dosimetry and adverse events assessment according to the National Cancer Institute's Common Toxicity Criteria (NCI-CTCAE) version 5.0.

Statistical Analysis

The D'Agostino Pearson test was used to check for the normal distribution of data. Based on the distribution, summary statistics were obtained in terms of mean, median, standard deviation (SD), range, and interquartile range (IQR) were calculated for all continuous variables based on the distribution of data. Mann-Whitney test for Independent samples used to compare the organ, tumor absorbed doses, and the T_e between the radiotracers. P-value < 0.05 was considered statistically significant. Statistical analysis was performed with MedCalc statistical software version 12.

Results

Patients

[^{177}Lu]-Lu-DOTA.SA.FAPi and [^{177}Lu]-Lu-DOTAGA.(SA.FAPi) $_2$ were administered as a therapy after pretherapeutic confirmation of adequate FAP expression ($\text{SUV}_{\text{max}} > 3$) of the metastases on [^{68}Ga]-Ga-DOTA.SA.FAPi-PET/CT. The mean SUV_{max} values and tumor-to-background (pancreas) ratios in both groups were 8.1 ± 0.8 (6.7–9), 4 ± 0.5 (3.3–4.8), and 10.2 ± 2.1 (7.2–14.2), 4.347 ± 1.1 (2.7–6.7), respectively. (Supplementary Table 1 & Table 2).

Table 2
Patient demographics

Parameters	[¹⁷⁷ Lu]Lu-DOTA.SA.FAPi	[¹⁷⁷ Lu]Lu-DOTAGA. (SA.FAPi) ₂
Number of patients	3	7
Age (years ± SD; range)	50 ± 17.2 (31–63)	51 ± 12.7 (26–63)
Gender		
Male	0	4
Female	3	3
Type of cancer		
Breast cancer	3	1
Thyroid cancer	0	5
Paraganglioma	0	1
The extent of disease on [⁶⁸ Ga]Ga-DOTA.SA.FAPi PET/CT scan		
Primary	1	0
Lymph nodes	1	3
Skeletal metastases	3	4
Brain metastases	1	0
Liver metastases	1	1
Lung mass	0	1
Injected activity (GBq; IQR)	2.96 GBq (IQR: 2.2–3 GBq).	1.48 GBq (IQR: 0.6–1.5)

The demographics of patients treated with [¹⁷⁷Lu]Lu-DOTA.SA.FAPi and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ are mentioned in Table 2.

A complete concordance was observed in the FAPi expression of lesions between pre-therapy Dx- [⁶⁸Ga]Ga-DOTA.SA.FAPi PET/CT and post-therapeutic [¹⁷⁷Lu]Lu-DOTA.SA.FAPi/[¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ scans (Table 2).

Three breast cancer patients from the [¹⁷⁷Lu]Lu-DOTA.SA.FAPi group (mean age ± SD: 50 ± 17.2 years; range: 31–63 years) were injected with a median cumulative activity of 2.96 GBq (IQR: 2.2–3 GBq). In the [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ group, seven patients (mean age ± SD: 51 ± 12.7 years; range: 26–63 years) were injected with a median dosage of 1.48 GBq (IQR: 0.6–1.5 GBq) at the first cycle of treatment. While

patients in [¹⁷⁷Lu]Lu-DOTA.SA.FAPi group received only one treatment cycle; patients in the [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ dimer group received two cycles of treatment at a median interval of 2 months.

Safety

[¹⁷⁷Lu]Lu-DOTA.SA.FAPi and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ doses were well tolerated. None of the patients experienced any early adverse events after the administration of the agents. One patient with extensive skeletal metastases and pre-existing grade I anaemia experienced grade III and grade I anaemia and thrombocytopenia, respectively. No other grade III/IV toxicities were noted. Table 3 summarises the median pre-treatment and 6 months post-treatment hematological, kidney, and liver function parameters that showed that both radiotracers were well endured.

Table 3

Comparison of hematological, renal and liver function parameters pre and at median of 6 months post-treatment.

[¹⁷⁷ Lu]Lu-DOTA.SA.FAPi group				[¹⁷⁷ Lu]Lu-DOTAGA.(SA.FAPi) ₂ group		
Parameters	Baseline (Mean, 95% CI of mean)	Post-treatment (Mean, 95% CI of mean)	P value	Baseline (Mean, 95% CI of mean)	Post- treatment (Mean, 95% CI of mean)	P value
Haemoglobin (g/dL)	10.9 (8.9–11.7)	11 (8.7–11.6)	-	10.8 (9.1– 11.9)	10.07 (6.9–11)	0.112
Platelets (lakhs/μL)	199 (178–201)	199 (188–201)	-	225 (156– 295)	198 (81–239)	0.255
Leukocytes 109/L	6500 (5600–7800)	7700 (6780–7877)	-	6781.6 (4216– 9348)	6947.4 (5239– 8655)	0.857
Creatinine (mg/dL)	0.8 (0.67–0.9)	0.77 (0.7–0.9)	-	0.70 (0.17– 1.2)	0.50 (0.36–0.64)	0.385
ALP	212 (168–225)	188 (160–234)	-	98.3 (73.4– 123)	90.5 (70.6–110)	0.304
(-): P value cannot be estimated due to low sample size						

Biodistribution and Pharmacokinetics of Normal Organs

[¹⁷⁷Lu]Lu-DOTA.SA.FAPi

On qualitative analysis, maximum normal physiological uptake was observed in the kidneys, followed by the colon/large intestines (ascending, transverse, and descending colon). Other organs included the liver, pancreas, gall bladder, oral mucosa, lacrimal gland, and urinary bladder contents. Time-activity curves were derived by either mono or biexponential curve fitting. [¹⁷⁷Lu]Lu-DOTA.SA.FAPi is excreted via both renal excretion and hepatobiliary clearance. A combined wash-in and washout trend of the radiotracer in the kidneys were observed in all patients. The wash-in of radiotracer in the kidneys was initiated as early as 6 h p.i. and continued up to 144 h after treatment. Pure washout of the trend was observed for lacrimal glands, oral mucosa, liver, and pancreas. (Fig. 2)

Transit of radiotracer in the gut (ascending and descending colon) was first observed at 24 h post-injection and reached its peak uptake at 48 h. At 144 h p.i., a complete washout of the radiotracer was observed from the gut. Figure 2 shows the [¹⁷⁷Lu]Lu-DOTA.SA.FAPi serial whole-body scintigraphy images obtained up to 144 h of breast cancer patients with extensive skeletal metastases. The image demonstrates the biodistribution of FAPi in various organs. The graphical representation details the pattern of clearance of [¹⁷⁷Lu]Lu-DOTA.SA.FAPi from the organs and whole-body, which was predominantly bi-phasic. (Fig. 2)

[¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂

Physiological biodistribution of [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ involved liver, gall bladder, large intestines (transverse, ascending, and descending colon), pancreas, kidneys, urinary bladder contents, and to a lesser extent in the lacrimal glands, oral mucosa, salivary glands (Fig. 3). Visual analysis revealed the colon as the organ with the highest FAP uptake. Route of excretion was predominantly via biliary followed by renal excretion. A pure washout trend was observed by the kidneys, and a combined wash-in and washout trend of radiotracer was observed by the biliary route and fitted with bi-exponential curves. Kidney excretion was seen as early as 1 h p.i. continued up to 168 h (Fig. 3).

Excretion of radiotracer from the hepatobiliary system/liver was initiated 4 h p.i., and rapidly reduced by 50% at 24 h p.i. (5.2% – 1 hp.i. to 2.4% – 24 h p.i.); further reached negligible concentration at 168 h p.i. (1% IA). The radiotracer concentration in the colon was observed at 24 h p.i. (mean %IA: 14%) and showed the maximum uptake at 48 h p.i. (%IA: 17.8%) and washed out to as low as 3% at 168 h post-infusion. Clearance of gut activity by approximately 7.4 fold was observed between 96 h and 168 h. However, The radiotracer concentration in the gut widely varied among the patients depending on the tumor burden, intestinal motility, and excretion. Patients with a high tumor burden received lower absorbed doses to the colon and kidneys due to the "tumor-sink" effect.

Dosimetry estimate and T_e of Normal organs

The mean absorbed doses and the whole body effective dose of [¹⁷⁷Lu]Lu-DOTA.SA.FAPi and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ are mentioned in Table 4. The whole body effective dose for [¹⁷⁷Lu]Lu-DOTAGA.

(SA.FAPi)₂ was significantly higher than [¹⁷⁷Lu]Lu-DOTA.SA.FAPi [2.26E-01 ± 1.24E-01; vs. 6.22E-02 ± 9.96E-03 mSv/MBq, P=0.058).

Table 4

Absorbed dose and effective dose estimate of [^{177}Lu]Lu-DOTA.SA.FAPI and [^{177}Lu]Lu-DOTAGA.(SA.FAPI) $_2$

[^{177}Lu]Lu-DOTA.SA.FAPI			[^{177}Lu]Lu-DOTAGA.(SA.FAPI) $_2$		
Organ	Mean absorbed doses (mSv/MBq)	ED ICRP-103 (mSv/MBq)	Organ	Mean absorbed doses (mSv/MBq)	ED ICRP-103 (mSv/MBq)
Adrenals	7.79E-03 ± 3.69E-04	7.19E-05 ± 3.42E-06	Adrenals	1.27E-02 ± 4.63E-03	1.19E-04 ± 4.05E-05
Brain	2.06E-05 ± 2.09E-05	2.06E-07 ± 2.09E-07	Brain	1.17E-04 ± 3.93E-05	1.17E-06 ± 3.93E-07
Breasts	4.39E-04 ± 2.64E-04	5.27E-05 ± 3.16E-05	Breasts	6.39E-04 ± 5.30E-05	7.67E-05 ± 6.37E-06
Esophagus	1.80E-03 ± 9.12E-04	7.19E-05 ± 3.65E-05	Esophagus	3.07E-03 ± 6.35E-04	1.23E-04 ± 2.55E-05
Eyes	2.13E-05 ± 2.04E-05	0	Eyes	9.96E-05 ± 4.11E-05	0
Gallbladder Wall	6.06E-03 ± 1.91E-04	5.59E-05 ± 1.80E-06	Gallbladder Wall	7.95E-01 ± 2.58E-01	7.34E-03 ± 2.39E-03
Left colon	4.30E-01 ± 9.40E-02	2.08E-02 ± 4.58E-03	Left colon	2.87E + 00 ± 1.74E + 00	1.39E-01 ± 8.43E-02
Small Intestine	2.71E-03 ± 1.74E-04	2.50E-05 ± 1.55E-06	Small Intestine	9.24E-03 ± 4.92E-03	8.53E-05 ± 4.53E-05
Stomach Wall	2.03E-03 ± 3.74E-04	2.43E-04 ± 4.48E-05	Stomach Wall	6.62E-03 ± 1.84E-03	7.96E-04 ± 2.23E-04
Right Colon	4.72E-01 ± 3.93E-02	2.29E-02 ± 1.91E-03	Right Colon	1.16E + 00 ± 8.58E-01	5.64E-02 ± 4.18E-02
Rectum	5.08E-04 ± 5.31E-05	1.17E-05 ± 1.25E-06	Rectum	2.02E-03 ± 1.04E-03	4.69E-05 ± 2.46E-05
Heart Wall	1.40E-03 ± 1.09E-03	1.29E-05 ± 1.01E-05	Heart Wall	2.57E-03 ± 1.39E-03	2.37E-05 ± 1.28E-05
Kidneys	6.18E-01 ± 1.54E-02	5.70E-03 ± 1.42E-04	Kidneys	3.74E-01 ± 2.57E-01	3.45E-03 ± 2.37E-03
Liver	1.15E-01 ± 9.02E-03	4.61E-03 ± 3.56E-04	Liver	2.09E-01 ± 2.38E-02	8.36E-03 ± 9.57E-04
Lungs	6.10E-02 ± 1.04E-01	7.31E-03 ± 1.2E-02	Lungs	2.21E-03 ± 5.66E-04	2.67E-04 ± 7.03E-05

All values are mentioned as mean ± SD

$[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPI}$			$[^{177}\text{Lu}]\text{Lu-DOTAGA.}(\text{SA.FAPI})_2$		
Ovaries	$9.08\text{E-}04 \pm 7.3\text{E-}05$	$3.63\text{E-}05 \pm 2.94\text{E-}06$	Ovaries	$2.23\text{E-}03 \pm 2.50\text{E-}04$	$8.55\text{E-}05 \pm 1.4\text{E-}05$
Pancreas	$3.69\text{E-}03 \pm 2.33\text{E-}04$	$3.41\text{E-}05 \pm 2.16\text{E-}06$	Pancreas	$6.51\text{E-}01 \pm 1.37\text{E-}01$	$6.01\text{E-}03 \pm 1.26\text{E-}03$
Prostate	-	-	Prostate	$2.57\text{E-}03 \pm 1.35\text{E-}03$	$1.19\text{E-}05 \pm 6.24\text{E-}06$
Salivary glands	$6.56\text{E-}05 \pm 6.79\text{E-}05$	$6.56\text{E-}07 \pm 6.79\text{E-}07$	Salivary glands	$1.17\text{E-}01 \pm 9.53\text{E-}03$	$1.17\text{E-}03 \pm 9.53\text{E-}05$
Red Marrow	$9.84\text{E-}04 \pm 2.58\text{E-}04$	$1.18\text{E-}04 \pm 3.07\text{E-}05$	Red Marrow	$1.73\text{E-}02 \pm 1.82\text{E-}02$	$2.08\text{E-}03 \pm 2.18\text{E-}03$
Osteogenic Cells	$1.18\text{E-}03 \pm 2.89\text{E-}04$	$1.18\text{E-}05 \pm 2.89\text{E-}03$	Osteogenic Cells	$8.57\text{E-}03 \pm 6.95\text{E-}03$	$3.33\text{E-}04 \pm 6.48\text{E-}04$
Spleen	$3.99\text{E-}03 \pm 2.18\text{E-}04$	$3.68\text{E-}05 \pm 2.01\text{E-}06$	Spleen	$6.36\text{E-}03 \pm 1.52\text{E-}03$	$5.87\text{E-}05 \pm 1.40\text{E-}05$
Testes	-	-	Testes	$1.71\text{E-}04 \pm 9.97\text{E-}05$	$6.87\text{E-}06 \pm 3.99\text{E-}06$
Thymus	$1.22\text{E-}03 \pm 1.49\text{E-}03$	$1.13\text{E-}05 \pm 1.37\text{E-}05$	Thymus	$1.00\text{E-}03 \pm 3.09\text{E-}04$	$9.24\text{E-}06 \pm 2.85\text{E-}06$
Thyroid	$4.53\text{E-}04 \pm 5.25\text{E-}04$	$1.81\text{E-}05 \pm 2.10\text{E-}05$	Thyroid	$3.98\text{E-}04 \pm 8.53\text{E-}05$	$1.59\text{E-}05 \pm 3.40\text{E-}06$
Urinary Bladder Wall	$4.05\text{E-}04 \pm 4.12\text{E-}05$	$1.62\text{E-}05 \pm 1.65\text{E-}06$	Urinary Bladder Wall	$1.28\text{E-}03 \pm 4.29\text{E-}04$	$5.01\text{E-}05 \pm 1.73\text{E-}05$
Uterus	$7.84\text{E-}04 \pm 7.47\text{E-}04$	$3.62\text{E-}06 \pm 3.44\text{E-}07$	Uterus	$2.18\text{E-}03 \pm 2.70\text{E-}04$	$9.77\text{E-}05 \pm 1.58\text{E-}05$
Total Body	$1.10\text{E-}02 \pm 1.72\text{E-}03$	$0.00\text{E} + 00$	Total Body	$2.33\text{E-}02 \pm 6.15\text{E-}03$	0
Effective Dose Equivalent			Effective Dose Equivalent		
Effective Dose		$6.22\text{E-}02 \pm 9.96\text{E-}03$	Effective Dose		$2.26\text{E-}01 \pm 1.24\text{E-}01$
All values are mentioned as mean $\pm$ SD					

In the $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPI}$ group, the non-target organs with the highest absorbed dose, were noted in Kidneys (0.618 ± 0.015 Gy/GBq), followed by a colon (right colon: 0.472 Gy/GBq and left colon: 0.43 Gy/GBq). On the other hand, with $[^{177}\text{Lu}]\text{Lu-DOTAGA.}(\text{SA.FAPI})_2$ colon received the highest absorbed dose right colon: 1.16 Gy/GBq and left colon: 2.87 Gy/GBq and demonstrated a significantly higher mean absorbed dose than $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPI}$ ($P < 0.011$). The marrow dose was $9.84\text{E-}04 \pm 2.58\text{E-}04$ and

1.73E-02 ± 1.82E-02 Gy/GBq for the [¹⁷⁷Lu]Lu-DOTA.SA.FAPi, and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ groups, respectively.

Contrary to [¹⁷⁷Lu]Lu-DOTA.SA.FAPi, which showed mono-exponential whole-body clearance, [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ radiotracer followed a bi-exponential clearance. [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ had significantly longer median whole-body T_e compared to that of [¹⁷⁷Lu]Lu-DOTA.SA.FAPi [46.2 h (IQR: 38.5–70.1) vs. 23.1 h (IQR: 17.8–31.5); P=0.0167].

Tumor Pharmacokinetics, Effective half-lives, and Absorbed dose estimate

The values for the corresponding absorbed doses for various tumor lesions for [¹⁷⁷Lu]Lu-DOTA.SA.FAPi and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ are presented in Tables 5&6. The median masses of the lesions in the [¹⁷⁷Lu]Lu-DOTA.SA.FAPi and [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPi)₂ groups were similar; 32 grams (IQR:16.8–43.3) and 31.7 gm (9.630–118.000), P=0.8658, respectively.

Table 5

Effective half-life (T_e) and Dosimetry estimate of tumor lesions with [^{177}Lu]Lu-DOTA.SA.FAPi

Patient S.No	Cancer type	Site of lesion	T_e in tumor (hours)	No of disintegrations or Residence time	Mass of lesion (grams)	Absorbed dose mGy/MBq
1.	Right breast cancer	Right breast primary tumor	17	2.44E + 00	800	2.52E-01
		Right shoulder skeletal lesion	13.7	3.09E-01	46.1	5.40E-01
2.	B/L breast cancer	Right shoulder skeletal lesion	14	4.30E-01	22	1.57E + 00
		Left shoulder skeletal lesion	16	3.54E-01	15	1.89E + 00
		Left knee skeletal lesion	14	0.41E-01	15	2.13E + 00
3.	Right breast cancer	Ileum	12.6	2.80E -01	32	6.03E-01
		Pubis	12	1.02E-01	35	2.34E-01
Total number of lesions		7				
Median (IQR)			14 (12.8–15.5)	3.00E-01 (1.46E-01 – 4.11E-01)	32 (16.8–43.3)	6.03E-01 (2.30E-01 – 1.81E + 00)

Table 6

Effective half-life (T_e) and Dosimetry estimate of tumor lesions with [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂

Patient S.No	Cancer type	Site of lesion	T_e in tumor (hours)	Number of disintegrations or Residence time	Mass of lesion (grams)	Absorbed dose mGy/MBq
1	Radioiodine refractory follicular thyroid cancer	Right ileum skeletal lesion	99	3.37E + 00	65.4	4.16E + 00
		Femur bone lesion	231	9.80E + 00	158	7.99E + 00
2	Triple negative breast cancer	Right lung mass	40.7	3.47E + 00	50.7	5.51E + 00
3	Radioiodine refractory papillary thyroid cancer	Right lung nodule	86.6	6.02E + 00	1.5	3.17E + 02
4	Radioiodine refractory papillary thyroid cancer	Left shoulder bone lesion	86.62	6.41E + 00	189	2.64E + 00
		Sternum	89.5	4.47E + 00	3.96	8.97E + 01
		Right head of femur lesion	48.6	3.97E + 00	23.2	1.37E + 01
5	Paraganglioma	Skull	27.7	1.82E-01	6.04	2.40E + 00
		Anterior rib lesion	27.9	6.48E-01	5.57	9.33E + 00
		Posterior rib lesion	23.9	8.51E-01	30	2.28E + 00
6	Anaplastic thyroid cancer	Right Neck mass	90.2	1.54E + 01	250	5.02E + 00
7	Medullary thyroid cancer	Liver lesion	115.5	3.51E + 01	33.4	8.44E + 01
Total number of lesions		N = 12				

Patient S.No	Cancer type	Site of lesion	Te in tumor (hours)	Number of disintegrations or Residence time	Mass of lesion (grams)	Absorbed dose mGy/MBq
Median (IQR)			86.6 (34.3– 94.6)	4.22E + 00 (2.11E + 0– 8.10 E + 00)	31.7 (5.8– 111.7)	6.70E + 00 (3.40E + 00 to 4.9E + 01)

All lesions that showed expression on $[^{68}\text{Ga}]\text{Ga-DOTA.SA.FAPi}$ PET/CT scans were visualized on both $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPi}$ and $[^{177}\text{Lu}]\text{Lu-DOTAGA}(\text{SA.FAPi})_2$ post-therapy scans. Uptake in the tumor and metastases was detectable as early as 1 h p.i. for both radiotracers. Interestingly, on qualitative analysis, despite the early and avid uptake of $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPi}$ in the tumor lesions, a rapid washout was observed with only minimal uptake in the lesion at 24 h and no uptake at 48 h post-treatment. In contrast, there were combined wash-in and washout trends observed in the lesions of $[^{177}\text{Lu}]\text{Lu-DOTAGA}(\text{SA.FAPi})_2$ group where lesions demonstrated avid uptake even up to 168 h post-treatment. Similar to the clearance pattern from the whole body, a rapid mono-exponential clearance was observed with $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPi}$ radiotracer compared to the significantly slow and bi-phasic clearance of $[^{177}\text{Lu}]\text{Lu-DOTAGA}(\text{SA.FAPi})_2$ radiotracer.

In total, all lesions received a median absorbed dose of $6.03\text{E-}01$ (IQR: $2.30\text{E-}01$ – $1.81\text{E} + 00$) Gy/GBq in the $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPi}$ group and patients belonging to the $[^{177}\text{Lu}]\text{Lu-DOTAGA}(\text{SA.FAPi})_2$ group received a significantly higher absorbed dose of $6.70\text{E} + 00$ (IQR: $3.40\text{E} + 00$ – $4.9\text{E} + 01$) Gy/GBq dose per cycle. (Tables 5 & 6)

The Te of tumors in both groups reflected the uptake pattern. Unlike $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPi}$, a remarkably higher tumor Te was observed in the patient group treated with $[^{177}\text{Lu}]\text{Lu-DOTAGA}(\text{SA.FAPi})_2$. (Table 7)

Table 7
Comparison of T_e between [^{177}Lu]Lu-DOTA.SA.FAPi and [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ post-therapy scans

T_e	[^{177}Lu]-DOTA.SA.FAPi	[^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$	P-value
Whole body T_e	N = 3 patients	N = 7 patients	
Median (IQR)	23.1 (17.8–31.5)	46.2 (38.5–70.1)	0.0167
Tumor T_e	N = 7 lesions	N = 12 lesions	
Median (IQR)	14 (12.8–15.5)	86.6 (34.3–94.6)	0.0004

The absorbed dose to the bone lesions of [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ group was about 5.6-fold higher than that in the [^{177}Lu]Lu-DOTA.SA.FAPi group, 6.0750 (2.5200–11.5150) Gy/GBq with 8 lesions vs. 1.0865 (0.5400–1.8900) Gy/GBq with 6 lesions, P=0.0019). Due to the difference in the type of cancer and the low sample size, the comparison was not possible for other categories of lesions such as primary tumor, lymph nodes, and visceral metastases.

Response Assessment

Patients in the [^{177}Lu]Lu-DOTA.SA.FAPi group were administered only a single cycle of treatment, hence one hematological and clinical response was assessed. Though patients showed initial response which remained for upto 6 weeks post treatment, they demonstrated relapse in the clinical symptoms. Two patient in this group died.

On the contrary, All patients in the [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ have demonstrated clinical response, have completed a median of 3 cycles of treatment and are alive.

Discussion

The expression of cancer-associated FAP in a broad spectrum of cancers offers an optimal target for various molecular-based FAP inhibitor imaging and therapies [6, 7, 15]

Based on the synthesis of a potent FAP inhibitor UAMC1110 [8, 9], Moon et al., [5] introduced a squaramide linker containing bifunctional DATA 5m and DOTA chelators and a FAP targeting moiety abbreviated as DATA 5m .SA.FAPi and DOTA.SA.FAPi were labeled with gallium-68. Both show sufficient in vitro affinity in nanomolar IC $_{50}$ values for FAP and low affinity in μM IC $_{50}$ ranges for DPPs and PREP. Selectivity to FAP and accordingly towards DPPs and PREP is an important aspect for efficacy targeting FAP. Research on [^{68}Ga]Ga-DOTA.SA.FAPi in an HT-29 human colorectal cancer xenograft mouse model revealed excellent in vivo and ex vivo results [5].

Subsequently, we conducted clinical studies comparing [^{68}Ga]Ga-DOTA.SA.FAPI with [^{18}F]F-FDG in various cancers and demonstrated comparable results and complimentary benefits to [^{18}F]F-FDG PET/CT reporting and demonstrated a scope for [^{68}Ga]Ga-DOTA.SA.FAPI guided theranostic approach for the treatment of various cancers [6].

Ballal et al. further carried out a theranostic approach of [^{68}Ga]Ga-DOTA.SA.FAPI PET/CT guided [^{177}Lu]Lu-DOTA.SA.SA.FAPI radionuclide therapy in an ER⁻, PR⁻, Her2neu⁺ end-stage breast cancer patient [7]. Though, visual analysis on PTx-[^{177}Lu]Lu-DOTA.SA.SA.FAPI whole-body scan demonstrated a high tumor affinity, early washout of the radiotracer, which was completely eliminated by 48 h p.i. which was the major drawback of the molecule. Despite the short-tumor retention time, the patient experienced an improvement in the clinical status.

Among the various FAP targeted molecules, owing to its superior tumor retention and fast clearance from the kidneys, Haberkorn's group [3] evaluated FAPI-04 as a theranostic tool. Preclinical studies in cells expressing human and murine FAP and CD26 resulted in an increased half-life of 3.0 h for [^{177}Lu]Lu-FAPI-04, versus 1.7 h [^{177}Lu]Lu-FAPI-02 in the tumor. Further, they attempted a theranostic approach of [^{68}Ga]Ga-FAPI-04 guided [^{90}Y]Y-FAPI-04 therapy in an end-stage breast cancer patient. Though the tumor absorbed dose for [^{90}Y]Y-FAPI-04 was not conclusive; however, the patient experienced a reduction in pain with no significant toxicities [3].

From the reports of previous studies [3, 7], it is evident that the main challenge for the potential therapeutic application of the FAP tracers was to optimize its tumor retention time. To design an ideal radiotracer for theranostic use and deliver a maximum radiation dose to the desired target lesions, the biological half-life of the FAPI agent should match the physical half-life of the radiometal.

Small-molecule inhibitors with a shorter-biological half-life could be labelled with shorter physical half-life therapeutic radionuclides such as $^{90}\text{Y}/^{188}\text{Re}/^{213}\text{Bi}$, and similar molecules with longer half-life could be tagged to long-lived therapeutic radionuclides like $^{177}\text{Lu}/^{225}\text{Ac}$, etc.

This problem received substantial interest and an approach to improve tumor affinity as well as tumor retention led to the evolution from monomers to dimeric systems such as DOTA based homodimeric structures [DOTA.(SA.FAPI)₂ and DOTAGA.(SA.FAPI)₂] by Moon et al. [11]. Unlike monomeric precursors, the bifunctional chelator at the centre is linked to two squaramide linker/target vector units. The coupling of squaramide linker-target vector (FAP inhibitor) to dimers significantly increases tumor uptake, tumor retention, and low background. DOTA.(SA.FAPI)₂ and DOTAGA.(SA.FAPI)₂ were synthesized, tested for stability *in vitro*, and complexed with gallium-68 and lutetium-177. As evident from the reports of Moon et al. [11] the homodimers display very high *in vitro* affinity for FAP similar to the monomeric structures (similar low nM IC₅₀ values). The use of the DOTAGA chelator for the dimeric version allows to have the same coordination sites as the DOTA monomer structure attributing to the better chelation with heavy radiometals such as lutetium-177, yttrium-90, rhenium-188, actinium-225, bismuth-213, etc. It increases

the tumor retention time by several folds. Interestingly, the homodimeric structure had significantly increased tumor uptake and retention with a low background at 24 h p.i. compared to the monomer.

To introduce the homodimers from bench to bedside, the systematic clinical trials focusing on head-to-head comparisons of the homo-dimers addressing the pharmacokinetics in normal organ and tumor lesions are warranted.

Both radiotracers were well tolerated in all patients with minimal toxicities. While the dose-limiting organ with [^{177}Lu]Lu-DOTA.SA.FAPi was the kidney, followed by a colon, the highest estimated absorbed radiation dose by [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂ dimer was observed in the colon, followed by gall bladder, pancreas, and kidneys.

To achieve a safe limit of 28 Gy [16] to the kidneys, a calculated maximum cumulative activity of 45 GBq [^{177}Lu]Lu-DOTA.SA.FAPi and ~ 10 GBq [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂ can be safely administered. A study by Bodei et al. [17] has followed patients post-PRRT in NET patients and revealed that long-term kidney toxicities are minimal. The safe, tolerable limit of [^{177}Lu]Lu-DOTATATE or [^{90}Y]Y-DOTATOC could reach up to 40 Gy in patients who have no prior risks factor, co-morbidities or previous history of impaired kidney function.

Based on the maximum tolerable dose limit of 38 Gy in the colon based on the stereotactic body radiation therapy (SBRT) data [18], patients can be injected with as much as 84 GBq of [^{177}Lu]Lu-DOTA.SA.FAPi and approximately 20 GBq of [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂. Among our patient series in the [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂ group, one patient with paraganglioma suffered from constipation with the persistence of activity in the gut even up to 168 h p.i. and hence received a relatively higher absorbed to the colon (Fig. 4). The physiological uptake in the gut/intestines varied widely across the patients and was majorly dependent on intestinal motility. Efforts to reduce the risk to the colon and reduce the absorption might be facilitated by suggesting high fatty food to accelerate the washout from the gall bladder and administering laxatives to accelerate the washout of the radiotracer from the gut may be beneficial. However, the effect of the above methods cannot be deduced from the current study results, and it mandates a proper execution and investigation.

The whole body effective dose was significantly higher for [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂ group compared to [^{177}Lu]Lu-DOTA.SA.FAPi. A similar pattern of the whole-body effective dose was observed with [^{177}Lu]Lu-EB-PSMA-617 (0.1 mSv/MBq) [19] and [^{177}Lu]Lu-PSMA-ALB-56 (0.2 Gy/GBq) [20] radiotracers that were introduced to improve the pharmacokinetic profile of the PSMA ligands in treating mCRPC patients. The findings go hand-in-hand with the significantly higher tumor effective half-lives in patients treated with [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂. Our median absorbed doses to the tumor lesions was 6.7 Gy/GBq in patients injected with [^{177}Lu]Lu-DOTAGA.(SA.FAPi)₂, which was 5.16 folds higher than that deposited by [^{177}Lu]Lu-DOTA.SA.FAPi (0.67Gy/GBq). Comparable tumor absorbed doses of 6.64 Gy/GBq were obtained by Kramer et al. [20]. The high absorbed doses from with [^{177}Lu]Lu-DOTAGA.

(SA.FAPI)₂ were in concordance with the high survival rate of advanced stage disease in our patient cohort.

[¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPI)₂ demonstrates rapid internalization, high tumor uptake, prolonged tumor effective half-life, delivers high radiation dose to the tumors even with lower dosages of lutetium. However, along with providing promisingly high tumor doses, [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPI)₂ also attributes higher absorbed dose to the whole body, including other organs at risk as to the gall bladder, pancreas, kidneys, and liver.

It should be underlined that the benefit-to-risk ratio should be weighed for each patient taking into account factors such as tumor burden, the bowel emptying time of each patient, history of hepatobiliary obstruction. Additionally, dose fractionation protocols by inducing small doses of [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPI)₂ per treatment cycle at longer treatment intervals may cover a descent treatment period and at the same time induce tumor regression and provide a window for recovery of vital and clinical toxicities.

Limitations

The current administered activities to patients were arbitrary as no data on the dosimetry estimates were available for any therapeutic FAPI tracer in the literature.

Pertaining to the heterogeneity in the type of cancers and difference in the tumor burdens of the patients between the two radiotracer groups, it is not ideal for conducting a head-to-head comparison. The serial time-point of the acquisition was not uniform due to the differences in the pharmacokinetics between the radiotracers. An inherent drawback of planar dosimetry is the overestimation of dose due to overlap of abdominal organs, but efforts were made to reduce the error by applying appropriate subtraction techniques. The effect of laxatives to promote the early washout of radiotracers and the reduction of radiation burden to the large intestine was out of the scope of this paper.

Future Prospects

Based on the current results, we have initiated a dose-escalation study to evaluate the maximum tolerated absorbed dose to critical organs for [¹⁷⁷Lu]Lu-DOTAGA.(SA.FAPI)₂, thereby, we expect to achieve the best objective response and minimal toxicities at an optimal dosage of lutetium-177.

From the molecular perspective, we intend to reconstruct/improvise the molecule further, improve its pharmacokinetics to promote minimum uptake in the non-target organs by reducing the percentage of injected activity to the dose-limiting organs (hepatobiliary and large bowels), and enhancing tumor internalization and greater "tumor-sink" effect.

Conclusion

Compared to [^{177}Lu]Lu-DOTA.SA.FAPi monomer, [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ homodimer demonstrated longer tumor retention significantly; however, later uptakes in colon and kidneys are higher than the former but are well tolerated. The desired qualities like rapid internalization, higher affinity, longer tumor retention, faster clearance from the non-target organs with [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ unveiled new frontiers for the treatment of various end-stage cancer patients with a theranostic approach.

Declarations

Compliance with Ethical Standards:

Ethical Clearance: Ref. No IEC PG-22/2020 for the clinical use of [^{68}Ga]Ga-DOTA.SA.FAPi, and Ref. No. IEC/1054/5/2020 for the clinical use of [^{177}Lu]Lu-labelled FAPi radiotracers

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Figures

Figure 1

Generations of chelator-linker FAP inhibitor conjugates, a step towards development of FAP-targeted theranostics.

Figure 2

a. $[^{68}\text{Ga}]\text{Ga-DOTA.SA.FAPi}$ PET/CT images of a 49-year-old woman with breast cancer shows biodistribution in the oral mucosa, pancreas, and kidneys and intense expression of DOTA.SA.FAPi in extensive skeletal metastases. b. Serial $[^{177}\text{Lu}]\text{Lu-DOTA.SA.FAPi}$ whole body scintigraphic images for dosimetry, after intravenous injection of 50 mCi of radiotracer, demonstrates normal and minimal biodistribution in the oral mucosa, salivary glands, liver, kidneys and intestines. b. Accumulation in the metastatic sites were observed at 1 and 6 h p.i. and decreased significantly by 24 h p.i., and nearly complete washout by 48 h p.i. c. Time–activity curves for whole body, organs that were easily discernible and metastatic sites generated from region of interest placed on whole-body scintigraphic images.

Figure 3

a. [^{18}F]F-Fluorodeoxyglucose (FDG) PET/CT images of a 50-year-old woman with follicular variant of papillary carcinoma, post radioiodine therapy (cumulative dose of 22.2 GBq) showing soft tissue density mass in left shoulder (arrows) and multiple skeletal lesions. b. Whole body scintigraphy done after additional 7.4 GBq of radioiodine therapy, showing multiple foci of tracer accumulation suggestive of disease progression and was started on Sorafenib (400 mg OD). c. [^{68}Ga]Ga-DOTA.SA.FAPi PET/CT images (done after 6 months of Sorafenib therapy as part of ongoing clinical study when the patient had clinically progressive disease with thyroglobulin 3,00,000 ng/mL) show normal biodistribution in the oral mucosa, salivary glands, liver, pancreas, gall bladder, colon, and kidneys. Intense accumulation of radiotracer in the soft tissue mass (arrows) and multiple skeletal sites (right femur-arrow head). d. Serial [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ whole body scintigraphic images for dosimetry, after intravenous injection of 40 mCi of radiotracer, showing radiotracer retention in the metastatic sites till 168-hours delayed images. e. Time-activity curves for whole body, organs that were easily discernible and metastatic sites generated from region of interest placed on whole-body scintigraphic images. Accumulation in the normal organs peaked during the 24-48 hours and decreased significantly by 96 hours post injection. Left shoulder, sternum and right femur shows persistent retention till 168-hour delayed images. Patient received two cycles of [^{177}Lu]Lu-DOTAGA.(SA.FAPi) $_2$ therapy and showed significant clinical improvement with a decrease of thyroglobulin levels to 27,000 ng/mL. The patient also showed significant decrease in the VASmax score from 10 to 5 in a follow-up of 4.5 months.

Figure 4

a. A 27-year old male diagnosed with paraganglioma was treated with $[^{177}\text{Lu}]\text{Lu-DOTAGA}(\text{SA.FAPi})_2$ treatment and the 1 h scan post-treatment showed (a) normal and minimal biodistribution of radiotracer in the oral mucosa, salivary glands, liver, pancreas, and kidneys. At 1 h p.i. intense accumulation of radiotracer was observed in the skull, and rib lesions. The patient has a history of constipation and hence demonstrated persistent and intense uptake of $[^{177}\text{Lu}]\text{Lu-DOTAGA}(\text{SA.FAPi})_2$ radiotracer in the gut due to reduced intestinal motility even at 168 h (b) post-treatment reflecting higher radiation absorbed dose to the colon compared to the other patients such as the patient treated in Figure 3.

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