Acta Pharmaceutica Sinica B 2025;15(2):669−680

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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ORIGINAL ARTICLE

Safety, pharmacokinetics, and dosimetry of ^{177}Lu-AB-3PRGD2 in patients with advanced integrin $\alpha_v\beta_3$-positive tumors: A first-in-human study

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Received 16 June 2024; received in revised form 18 September 2024; accepted 25 September 2024

KEY WORDS

^{177}Lu-AB-3PRGD2;
Integrin $\alpha_v\beta_3$;
Targeted radionuclide therapy;
First-in-human study;
Pharmacokinetics;
Dosimetry;
Safety;
Advanced tumors

Abstract Integrin $\alpha_v\beta_3$ is overexpressed in various tumor cells and angiogenesis. To date, no drug has been proven to target it for therapy. A first-in-human study was designed to investigate the safety, pharmacokinetics, and dosimetry of ^{177}Lu-AB-3PRGD2, a novel integrin $\alpha_v\beta_3$-targeting radionuclide drug with an albumin-binding motif to optimize the pharmacokinetics. Ten patients (3 men, 7 women; aged 45 ± 16 years) with integrin $\alpha_v\beta_3$-avid tumors were recruited to accept ^{177}Lu-AB-3PRGD2 injection in a dosage of 1.57 ± 0.08 GBq (42.32 ± 2.11 mCi), followed by serial scans to obtain its dynamic distribution in the body. Safety tests were performed before and every 2 weeks after the treatment for 6−8 weeks. No adverse event over grade 3 was observed. ^{177}Lu-AB-3PRGD2 was excreted mainly through the urinary system, with intense radioactivity in the kidneys and bladder. Moderate distribution was found in the liver, spleen, and intestines. The estimated blood half-life was 2.85 ± 2.17 h. The whole-body

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Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.10.012

effective dose was 0.251 ± 0.047 mSv/MBq. The absorbed doses were 0.157 ± 0.032 mGy/MBq in red bone marrow and 0.684 ± 0.132 mGy/MBq in kidneys. This first-in-human study of ^{177}Lu-AB-3PRGD2 treatment indicates its promising potential for targeted radionuclide therapy of integrin $\alpha_v\beta_3$-avid tumors. It merits further studies in more patients with escalating doses and multiple treatment courses.

1. Introduction

Integrin $\alpha_v\beta_3$ is significantly overexpressed on various tumor cells and the neovascular endothelial cells[1,2]. It plays a crucial role in the growth, invasion, and metastasis of malignant tumors, but expresses at deficient levels in mature vascular endothelial cells and most normal organs, making it a hot target for diagnosis and therapy of broad-spectrum tumors[3–5]. The Lasker Prize for Basic Medical Research in 2022 was bestowed upon three scientists for their discovery of integrins[1]. However, up to now, no drug has been approved for the integrin $\alpha_v\beta_3$-targeted diagnosis or therapy.

A variety of arginine-glycine-aspartate (RGD) peptides have been recognized to specifically bind to integrin $\alpha_v\beta_3$[6,7]. In the last few decades, RGD peptide-based radiopharmaceuticals have been widely studied for molecular imaging of integrin $\alpha_v\beta_3$-positive tumors with high integrin $\alpha_v\beta_3$ expression, including lung cancer, breast cancer, and other malignancies[8–10]. Several diagnostic drugs targeting integrin $\alpha_v\beta_3$, including ^{99m}Tc-3PRGD2 and ^{68}Ga-PRGD2, have been preliminarily validated by our group for imaging of lung cancer and other tumors *via* single photon emission computed tomography/computed tomography (SPECT/CT) or positron emission tomography/computed tomography (PET/CT)[11–14], with a promising prospect for its clinical application. However, the integrin $\alpha_v\beta_3$-targeted therapy studies are relatively few and mainly limited to preclinical research.

Targeted radionuclide therapy (TRT) is a promising method that uses radiolabeled targeting vectors to emit charged particles to treat advanced tumors[15]. These vectors can specifically seek the molecular or functional targets overexpressed in tumors and systemically treat the local and metastatic tumors through intravenous administration[16]. ^{177}Lu-DOTATATE and ^{177}Lu-PSMA-617 have achieved tremendous success in the treatment of advanced neuroendocrine tumors and castration-resistant prostate cancer, respectively[17,18]. Radionuclide therapy using labeled RGD peptide analogs to target the integrin $\alpha_v\beta_3$-avid tumors holds promise and may have broader clinical applications in the treatment of a variety of malignancies[6].

A few RGD-based TRT studies have been reported in animal models[19–24]. Both ^{177}Lu and ^{90}Y were used for labeling RGD peptides to treat integrin $\alpha_v\beta_3$-avid tumors, however, ^{177}Lu was preferred for its relatively lower toxicity[19,20]. Multivalent, PEGylated, cyclic RGD peptides were proved to have high tumor-targeting capability and therapeutic effects[20,21]. Although our prior study indicated that ^{177}Lu-3PRGD2 treatment probes could significantly inhibit the growth of U87-MG tumors, there was still significant room for optimization regarding the limitation of rapid drug clearance and low tumor uptake[20]. Serum albumin, a protein that is abundant in serum, can be reversibly bound by negatively charged or hydrophobic small molecules, making it possible to produce long-acting therapeutic drugs to reduce the frequency and dosage of radiopharmaceuticals[22]. Albumin binders, such as Evans blue (EB) and palmitic acid, were introduced to synthesize ^{177}Lu-EB-RGD and ^{177}Lu-Palm-3PRGD2, which showed a significant increase of tumor uptake and retention, leading to enhanced efficacy of targeted therapy compared to ^{177}Lu-RGD and ^{177}Lu-3PRGD2, respectively[23,24].

Here, we present a novel radionuclide therapeutic drug candidate, ^{177}Lu-AB-3PRGD2, with a PEGylated cyclic RGD dimer to target integrin $\alpha_v\beta_3$ and an albumin-binding motif to optimize the pharmacokinetics. This first-in-human study was designed to investigate the safety, pharmacokinetics, and dosimetry of ^{177}Lu-AB-3PRGD2 in patients with integrin $\alpha_v\beta_3$-avid tumors demonstrated by ^{68}Ga-DOTA-3PRGD2 PET/CT.

2. Materials and methods

2.1. Patient recruitment

This prospective study was approved by the Institutional Review Board of the Peking Union Medical College Hospital (ZS-2868), and written informed consent was obtained from each participant. The study was registered at ClinicalTrials.gov (NCT05013086). From February of 2023 to October of 2023, 10 patients (3 men, 7 women) aged 23–70 (45 ± 16) years with body mass index (BMI) of 13.40–29.30 (20.79 ± 5.13) kg/m^2 were recruited, including 8 patients with adenoid cystic carcinoma (ACC), 1 patient with intrahepatic cholangiocarcinoma (ICC), and 1 patient with uterine leiomyosarcoma (uLMS). The main characteristics of participants are listed in Table 1.

The inclusion criteria were: (1) age >18 years old; (2) with precise pathological diagnosis and have failed or progressed after conventional clinical treatments, including surgery, chemotherapy, or radiotherapy; (3) baseline ^{68}Ga-DOTA-3PRGD2 PET/CT revealed at least one avid lesion with uptake higher than normal liver; (4) expected survival time >6 months; (5) liver and kidney functions and blood routine tests were generally normal, or with white blood cells (WBC) ≥2.5 × 10^9/L, platelets (PLT) ≥100 × 10^9/L, hemoglobin (HB) ≥90 g/L; total bilirubin ≤1.5 × the institutional upper limit of normal (ULN), alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≤3.0 × ULN or ≤5.0 × ULN (liver metastasis), serum creatinine (Cr) ≤1.5 × ULN. The exclusion criteria were: (1) received Strontium-89, Samarium-153, Radium-223, or any other radionuclide treatment within 6 months; (2) received chemotherapy, radiotherapy, immunotherapy, or clinical trial drugs within 28 days; (3) with claustrophobia or radiation phobia; (4) in serious

Table 1 Main characteristics of the participants.

Patient number	Gender	Age	BMI (kg/m^2)	Pathology	Lesion location	Prior treatment	Dose: GBq (mCi)
1	F	36	19.1	ACC	Head and neck, lung, bone	Surgery, radiotherapy	1.38 (37.40)
2	M	57	26.3	ACC	Head and neck, lung, pleura, intercostal space	Surgery, chemotherapy, radiotherapy	1.48 (40.10)
3	M	50	29.3	ACC	Lung, pleura, bone, liver	Surgery, radiotherapy	1.56 (42.10)
4	M	48	22.6	ACC	Lung	Surgery, targeted therapy	1.63 (44.00)
5	F	27	13.4	ACC	Head and neck, bone	Surgery, radiotherapy, targeted therapy	1.56 (42.20)
6	F	27	21.3	ACC	Head and neck, liver, bone	Surgery, radiotherapy	1.62 (43.90)
7	F	70	24.2	uLMS	Lymph node, lung	Surgery, radiotherapy, immunotherapy	1.62 (43.70)
8	F	52	20.8	ICC	Lymph node, liver	Surgery, TACE, targeted therapy, immunotherapy	1.58 (42.80)
9	F	23	16.9	ACC	Bone	Surgery, chemotherapy, radiotherapy, targeted therapy	1.59 (42.90)
10	F	57	14.0	ACC	Head and neck, lung, bone	Surgery, chemotherapy, radiotherapy, targeted therapy	1.63 (44.10)

ACC: adenoid cystic carcinoma; ICC: intrahepatic cholangiocarcinoma; uLMS: uterine leiomyosarcoma; TACE: transcatheter arterial chemoembolization.

illness with difficulty for further diagnosis and treatment. The trail profile is displayed in Fig. 1.

2.2. *PET/CT examinations*

All patients underwent both ^{68}Ga-DOTA-3PRGD2 PET/CT and ^{18}F-FDG PET/CT examinations within two weeks before ^{177}Lu-AB-3PRGD2 treatment, and the time interval between ^{68}Ga-DOTA-3PRGD2 and ^{18}F-FDG PET/CT was within 48 h. After intravenous injection of ^{68}Ga-DOTA-3PRGD2 in a dosage of 1.48−1.85 MBq (0.04−0.05 mCi) per kg body weight or ^{18}F-FDG in a dosage of 5.55−7.40 (0.15−0.20) mCi/kg, the patients rested quietly for approximately 45 or 60 min, respectively. Whole-body PET/CT was performed using a PoleStar m660 PET/CT scanner (SinoUnion Healthcare Inc., Beijing, China). Whole-body imaging was performed from mid-thigh to the top of the head. A low-

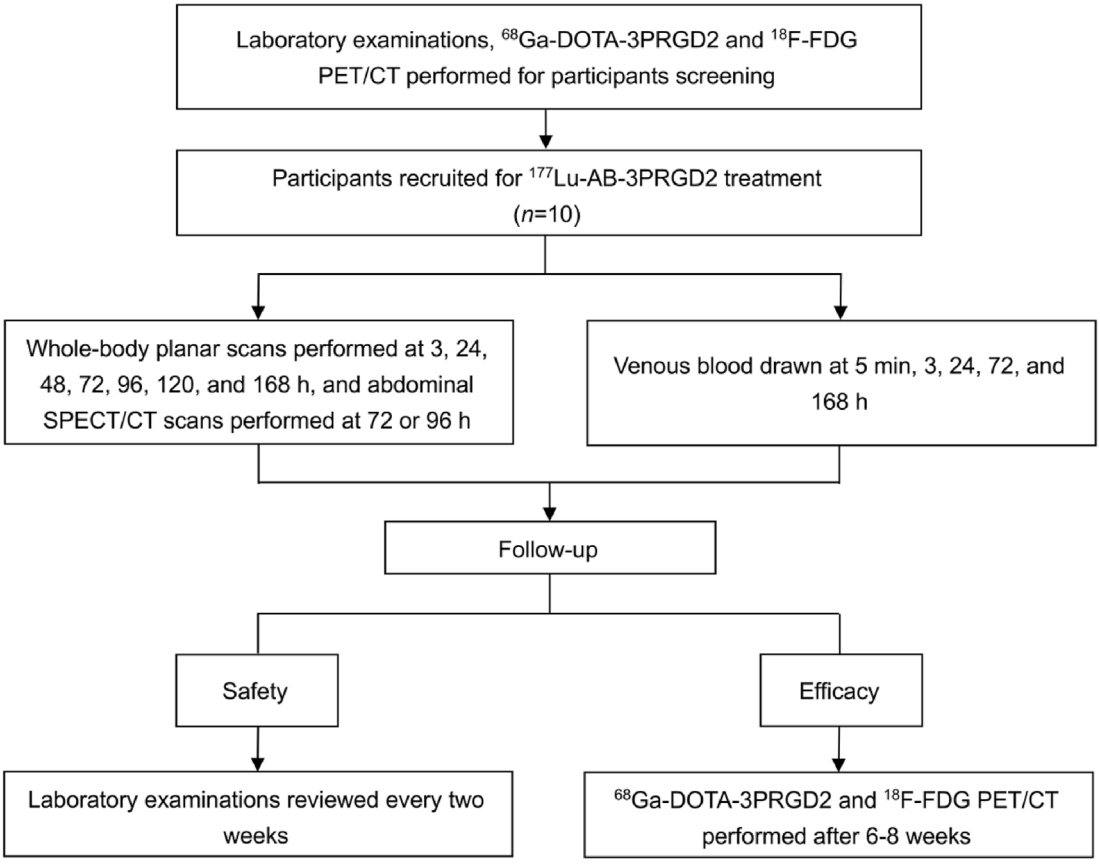

Figure 1 Trail profile.

dose CT scan was first performed for positioning, followed by a PET scan with 5−6 bed positions depending on the height of the patient, with each bed position scanning for 2 min. The ordered-subset expectation maximization (OSEM) method was used to reconstruct the PET image data (SinoUnion PoleStar: 2 iterations, 10 subsets, Gaussian filter, half-maximum width 4.5 mm, matrix 192 × 192), and attenuation correction was performed with the low-dose CT.

^{68}Ga-DOTA-3PRGD2 and ^{18}F-FDG PET/CT scans were repeated 6−8 weeks after the treatment for response evaluation.

2.3. *Synthesis and quality control of ^{177}Lu-AB-3PRGD2*

No carrier-added $^{177}LuCl_3$ for clinical use was purchased from ITM (Germany), with a specific activity of ≥3000 GBq/mg and a concentration of 36−44 GBq/mL. Typically, 3.7 GBq (100 mCi) $^{177}LuCl_3$ was added to 200 μL of 0.5 mol/L NaOAc (pH 5.6), and the ^{177}Lu dilution was injected into the vial with 100 μg of DOTA-AB-3PRGD2 (Fig. 2A) provided by the Medical Isotopes Research Center, School of Basic Medical Sciences, Peking University. The mixture was heated in an upright position at 100 °C for 30 min, then purified through a C18 cartridge and sterilized through a 0.22-μm aseptic filtration membrane. Radiochemical purity was tested using analytical thin-layer chromatography (Bioscan, USA). CH3OH: NH_4OAc (1:1, *v*/*v*) was used as the developing solution. The radioactive labeling yield was greater than 90%, and after purification, the radiochemical purity of ^{177}Lu-AB-3PRGD2 should exceed 95% before clinical use (Fig. 2B). The sterility test and bacterial endotoxin test performed according to the Chinese Pharmacopoeia 2020 were negative for 3 consecutive batches to qualify the clinical use of subsequent batches.

2.4. *^{177}Lu-AB-3PRGD2 treatment*

^{177}Lu-AB-3PRGD2 was diluted in 30 mL physiological saline to obtain ^{177}Lu-AB-3PRGD2 injection. The ^{177}Lu-AB-3PRGD2 treatment was performed in the ward for radionuclide therapy. The administered radioactivity was 1.56 ± 0.08 GBq (42.32 ± 2.11 mCi) for the patients and the dose for each patient is listed in Table 1.

2.5. *Whole body planar and SPECT/CT*

Whole-body planar scans of the patients after ^{177}Lu-AB-3PRGD2 injection and a SPECT/CT scan of the abdomen were performed using a Philips Precedence scanner (Philips Healthcare, Andover, Massachusetts, USA) with medium-energy general-purpose collimators, peaking at 208 KeV with a 20% energy window. The whole-body planar images were acquired at 3 ± 0.5, 24 ± 1, 48 ± 1, 72 ± 1, 96 ± 1, 120 ± 1, and 168 ± 1 h after the ^{177}Lu-AB-3PRGD2 injection, using an acquisition matrix of 256 × 1024 and a scan speed of 15 cm/min. The dual-head SPECT/CT scans were acquired at 72 or 96 h post-injection, with each head acquiring 32 frames in a 40 s exposure time per frame. Low-dose CT was obtained for positioning and attenuation correction.

2.6. *Blood sample collection and count measurement*

After the injection of ^{177}Lu-AB-3PRGD2, 1−2 mL of venous blood was drawn at 5 ± 1 min, 3 ± 0.5 h, 24 ± 1 h, 72 ± 1 h, and 168 ± 1 h to measure the radioactivity using a Cobra II auto-gamma counter (Perkin-Elmer).

2.7. *Adverse events collection and safety tests*

Adverse events and subjective health complaints were collected after treatment. Laboratory tests, including blood routine tests and hepatic and renal function tests, were performed before and every two weeks until 6−8 weeks later. The adverse events and laboratory tests were categorized according to the Common Toxicity Criteria for Adverse Events 5.0 (CTCAE V5.0).

2.8. *Dosimetry analysis*

Hermes Hybrid Viewer Dosimetry module (HERMES Medical Solutions Inc., Greenville, MA, USA) was used to derive the

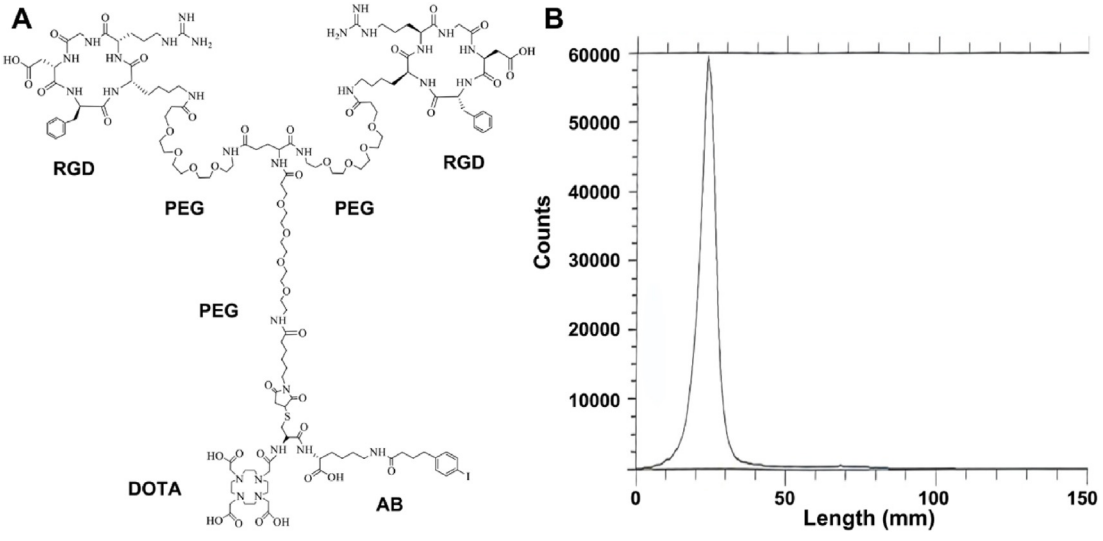

Figure 2 The precursor structure and radiochemical purity of ^{177}Lu-AB-3PRGD2. (A) Structural formula of DOTA-AB-3PRGD2. The molecular formula is $C_{137}H_{215}IN_{30}O_{45}S$, and molecular weight is 3161.36. (B) Radiochemical purity of ^{177}Lu-AB-3PRGD2.

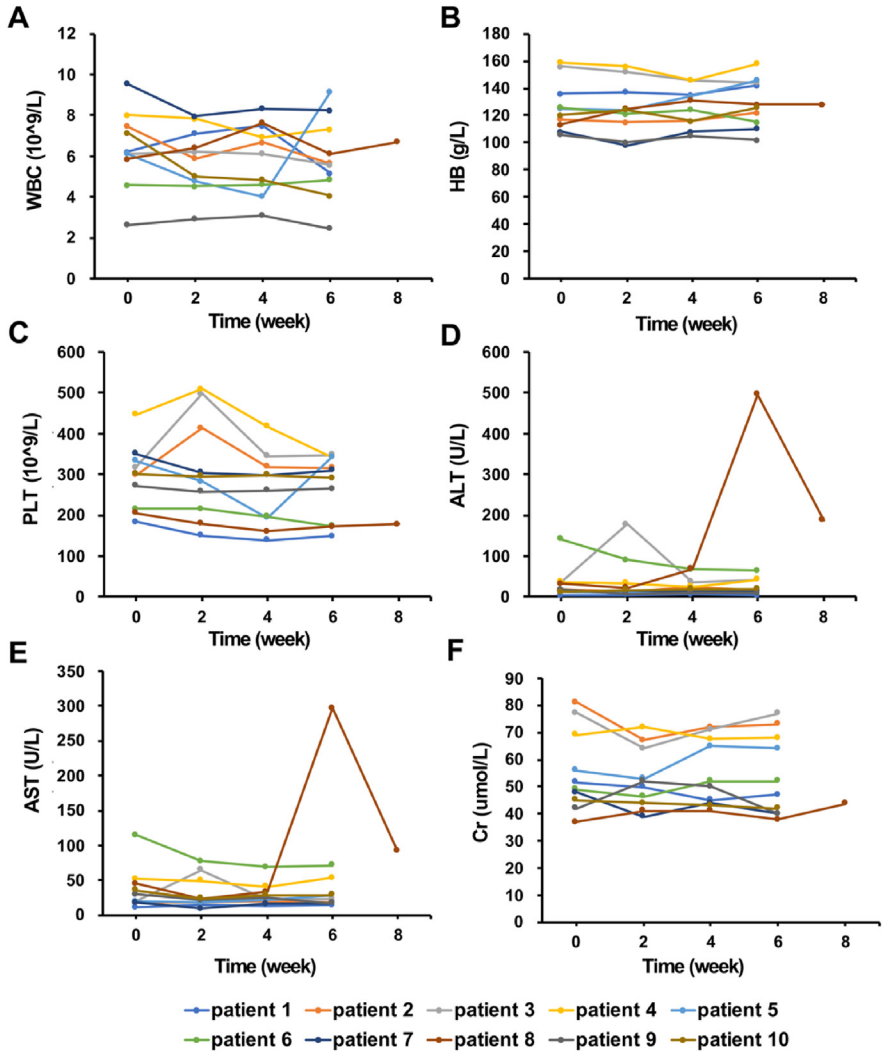

Figure 3 Serial changes of main indicators for hematological toxicity (A−C), hepatotoxicity (D−E), and nephrotoxicity (F) at baseline and 2, 4, 6, and 8 weeks after the ^{177}Lu-AB-3PRGD2 treatment. WBC: blood white blood cell counts; HB: blood hemoglobin level; PLT: blood platelet counts; ALT: serum alanine aminotransferase level; AST: serum aspartate aminotransferase level; and Cr: serum creatinine level.

absorbed doses of the organs and whole-body effective doses. The volumes of individual organs, including the brain, heart, kidneys, liver, lungs, pancreas, red bone marrow, spleen, and urinary bladder, were delineated on serial SPECT/CT slice-by-slice and projected onto the whole-body images to obtain their residence time and other values. The residence times of different patients were determined by fitting the time−activity curves obtained from multiple time-point imaging data and calculating the integral under the curves.

The dosimetry module in conjunction with Olinda/EXM uses the well-established MIRD technique to determine organ and whole-body doses from the radionuclide within the body. The equations for absorbed dose in an organ are shown in Eqs. (1)−(2):

$$D_{r_\kappa} = \sum_h \tilde{A}_h S(r_\kappa \leftarrow \mathrm{r_h}) \tag{1}$$

where r_k represents a target region, r_h represents a source region, $\tilde{A}_h$ is the cumulated activity (integrated area under time activity curve), and S is a factor to convert the integrated activity into absorbed dose.

$$S(\mathrm{r}_\kappa \leftarrow \mathrm{r_h}) = \frac{\kappa \sum_i n_i E_i \Phi_i (r_\kappa \leftarrow \mathrm{r_h})}{m_{r_\kappa}} \tag{2}$$

where n_i is the number of radiations with energy E emitted per nuclear transition, E_i is the energy per radiation (MeV), ϕ_i is the fraction of energy emitted that is absorbed in the target, m is the mass of the target region and k is the proportionality constant.

2.9. *Statistical analysis*

Calculations were performed using the SPSS software (IBM SPSS Statistics for Windows, version 25.0; Armonk, NY, USA). Continuous variables are summarized as mean ± standard deviations (SD). Categorical variables were described as numbers and percentages. Detailed correlations between the pre-therapy standardized uptake value (SUV) of ^{68}Ga-DOTA-3PRGD2 and the effective absorbed dose of the main normal organs were assessed using Spearman's rank correlation coefficient. The mean counts obtained by SPECT/CT were defined as counts_mean. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Table 2 Adverse events reported in each patient during the follow-ups assessed according to CTCAE 5.0.

Patient number	Baseline status	Grade of adverse event/week			
		2	4	6	8
1	Normal	0	0	0	–
2	Normal	0	0	0	–
3	ALT: 35.1 U/L	2	0	0	–
	AST: 19.9 U/L	1	0	0	–
4	Normal	0	0	0	–
5	Normal	0	0	0	–
6	ALT: 141.5 U/L	0	0	0	–
	AST: 115.0 U/L				
7	HB: 108 g/L	2	1	1	–
8	ALT: 32.5 U/L	0	1	3	3
	AST: 45.0 U/L	0	0	3	1
9	WBC: 2.62 × 10^9/L	0	0	0	–
10	Normal	0	0	0	–

ALT: alanine aminotransferase; AST: aspartate aminotransferase; HB: Hemoglobin; WBC: white blood cells.

3. Results

3.1. Safety and toxicity

No remarkable acute adverse event was reported during the ^{177}Lu-AB-3PRGD2 injection. During the follow-ups, there was no instance of life-threatening grade 4 or 5 toxicity observed in any of the patients. No significant change was found in blood white blood cell counts ($P = 0.495$), hemoglobin level ($P = 0.837$), platelet counts ($P = 0.459$), and serum alanine aminotransferase (ALT) level ($P = 0.630$), aspartate aminotransferase (AST) level ($P = 0.925$), creatinine level ($P = 0.645$) before and after treatment (Fig. 3A–F).

Adverse events were found in 3 patients (3/10, 30%) (Fig. 3, Table 2). A maximum of grade 3 elevations of ALT and AST was observed in patient No. 8 in the 6th week after treatment, which decreased significantly 2 weeks later without special treatment. Patient No. 3 also showed a maximum of grade 2 ALT elevation and grade 1 AST elevation that recovered two weeks later. Both patients had confounding factors, including multiple intrahepatic cholangiocarcinoma in patient No. 8 and liver metastasis of ACC in Patient No. 3. In addition, Patient No. 7 with borderline hemoglobin reported a grade 2 reduction 2 weeks after therapy, which was partly recovered 4 weeks after the therapy.

3.2. Pharmacokinetics

^{177}Lu-AB-3PRGD2 was excreted mainly through the urinary system, with intense radioactivity in the bladder and kidneys. Radioactivity could also be observed in the intestine, which was changed over time. Moderate physiological uptake was found in the liver and spleen. There was very low uptake in the brain. Representative images of the patients are shown in Fig. 4A–D.

The uptakes in the percentage of injection activity for normal organs and tumor lesions are shown in Fig. 5A and B. The urinary bladder had the highest residence time (8.45 h), followed by the liver (3.96 h), heart (2.06 h), and red bone marrow (2.03 h) (Fig. 5C, Table 3). The decay curve of counts per minute (CPM)

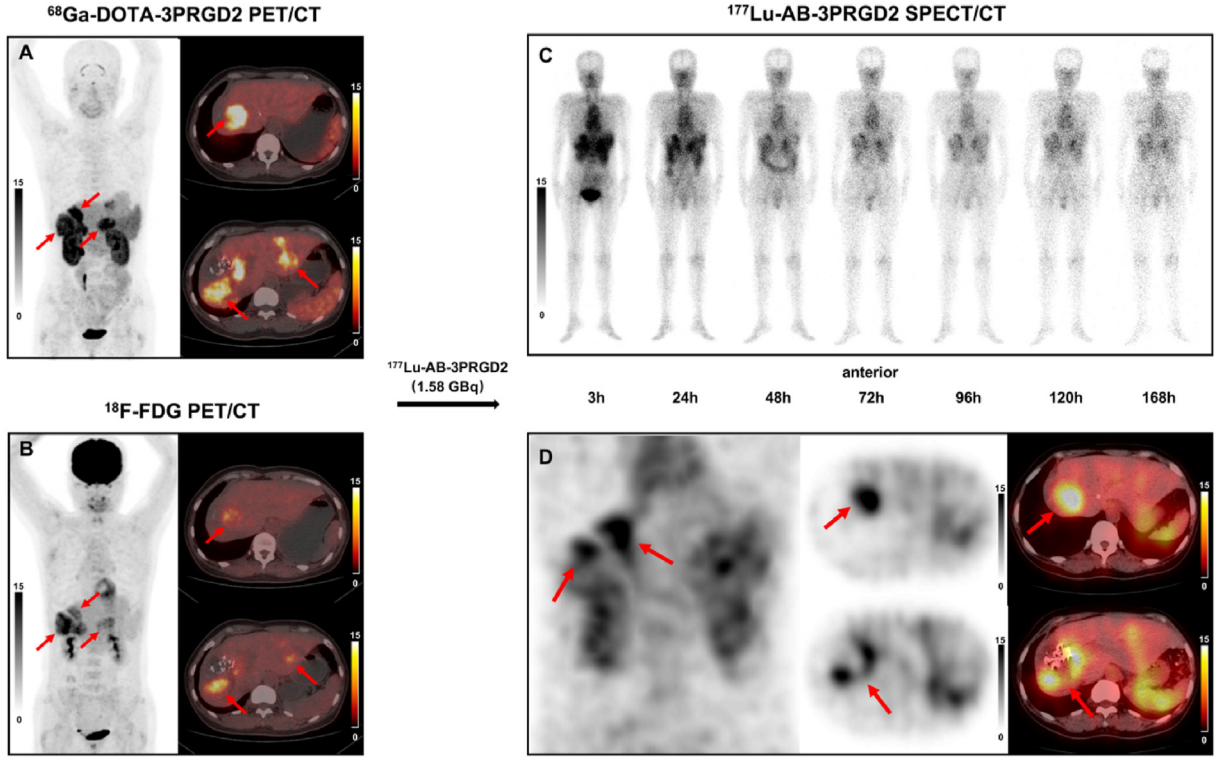

Figure 4 Representative ^{68}Ga-DOTA-3PRGD2 PET/CT and ^{177}Lu-AB-3PRGD2 whole body planar scan and SPECT/CT images in a 52-year-old women. ^{177}Lu-AB-3PRGD2 accumulated in the intrahepatic cholangiocarcinoma and liver metastases. (A) ^{68}Ga-DOTA-3PRGD2 PET/CT, (B) ^{18}F-FDG PET/CT, (C) ^{177}Lu-AB-3PRGD2 whole-body anterior planar images at different time points, (D) ^{177}Lu-AB-3PRGD2 SPECT/CT at 72 h.

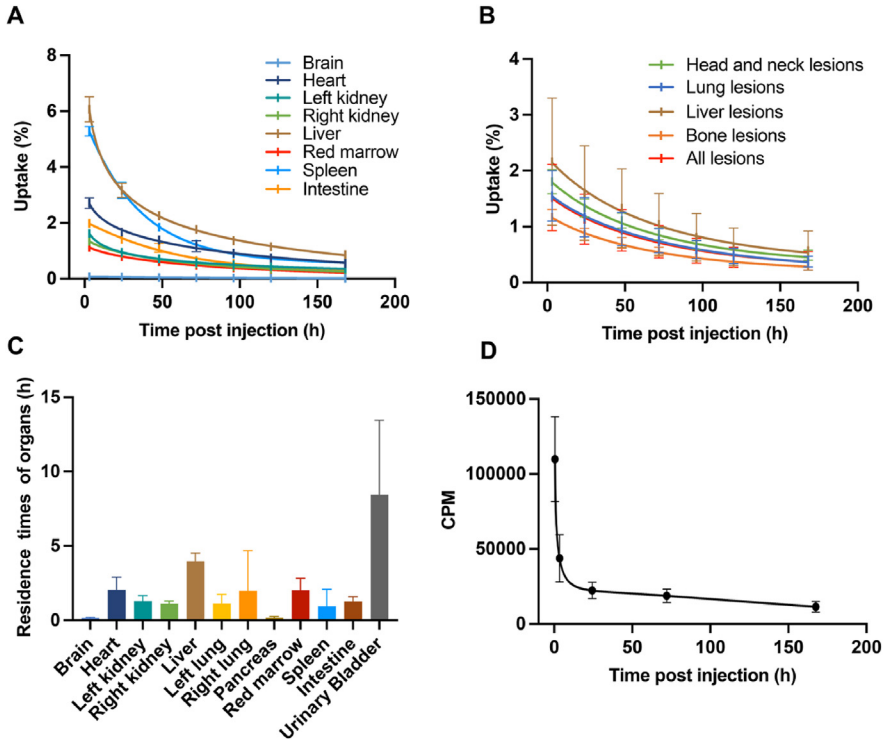

Figure 5 Pharmacokinetics of ^{177}Lu-AB-3PRGD2 in the patients. (A) Changes of normal organ uptake in percentage of injection activity over time; (B) Changes of lesion uptake in percentage of injection activity over time; (C) Residence times of ^{177}Lu-AB-3PRGD2 in normal organs; (D) Radioactive curve of ^{177}Lu-AB-3PRGD2 in the blood of the participants ($n = 10$). CPM: decay-corrected counts per minute.

for ^{177}Lu-AB-3PRGD2 over time was plotted based on blood samples extracted at 5 min and 3, 24, 72, and 168 h (Fig. 5D) and the fitted exponential decay curve. Based on the fitted model, the estimated half-life of ^{177}Lu-AB-3PRGD2 in blood was approximately 2.85 ± 2.17 h.

3.3. *Dosimetry of normal organs*

The whole-body effective dose of ^{177}Lu-AB-3PRGD2 was 0.251 ± 0.047 mSv/MBq. The absorbed doses of normal organs are shown in Table 3. The average red bone marrow dose was 0.157 ± 0.032 mGy/MBq. The absorbed dose of the kidneys was 0.684 ± 0.132 mGy/MBq, and the absorbed dose of the bladder was 1.852 ± 1.047 mGy/MBq. Furthermore, a systemic dose of 0.128 ± 0.013 mGy/MBq demonstrated the non-targeted systemic distribution of the radiopharmaceutical.

3.4. *Correlation of SUV and absorbed doses in normal organs*

Linear regression analysis showed a high positive correlation of ^{68}Ga-DOTA-3PRGD2 SUV_{mean} and ^{177}Lu-AB-3PRGD2 counts_mean in normal organs at different time points, with the highest R-value at 24 h ($R = 0.830$ at 3 h, $P < 0.001$; $R = 0.840$ at 24 h, $P < 0.001$; $R = 0.790$ at 48 h, $P < 0.001$; $R = 0.760$ at 72 h, $P < 0.001$; $R = 0.760$ at 96 h, $P < 0.001$; $R = 0.730$ at 120 h, $P < 0.001$; $R = 0.700$ at 168 h, $P < 0.001$) (Fig. 6).

The SUV_{max} and SUV_{mean} of ^{68}Ga-DOTA-3PRGD2 correlated with the absorbed doses of ^{177}Lu-AB-3PRGD2 in normal organs ($R = 0.360$ for SUV_{max}, $P < 0.001$; $R = 0.340$ for SUV_{mean}, $P < 0.001$) (Fig. 7A).

3.5. *Correlation of SUV and absorbed doses in tumor lesions*

From the 10 patients, a total of 40 tumors were measured, including 5 head and neck soft tissue lesions, 14 lung metastases, 11 bone metastases, 5 liver metastases, 3 pleural metastases, and 2 lymph node metastases, with no more than 2 lesions from each organ of each patient. The SUV_{max} and SUV_{mean} of ^{68}Ga-DOTA-3PRGD2 in the tumor lesions correlated significantly with the corresponding absorbed doses of ^{177}Lu-AB-3PRGD2 ($R = 0.780$ for SUV_{max}, $P < 0.001$; $R = 0.790$ for SUV_{mean}, $P < 0.001$) (Fig. 7B).

3.6. *Response assessment*

In the symptom assessment (Table 4), three patients with ACC experienced worsened symptoms, including one patient with worsening visual acuity, one with more oral bleeding, and one with decreased physical strength and worsening fatigue. Three patients felt lessened symptoms, including one ICC patient with better appetite and 3-kg body weight gain within 8 weeks, one ACC patient with reduced pain caused by bone metastases, and one ACC patient with less coughing caused by lung metastases.

Table 3 Residence time and absorbed doses in main organs and the effective dose.

Target organ	Residence time (h)	Absorbed dose (mGy/MBq) or effective dose (mSv/MBq)
Brain	0.14 ± 0.05	0.012 ± 0.003
Heart	2.06 ± 0.84	0.273 ± 0.074
Kidneys	1.30 ± 0.37 (left) 1.12 ± 0.18 (right)	0.684 ± 0.132
Liver	3.96 ± 0.55	0.204 ± 0.028
Lungs	1.13 ± 0.62 (left) 1.98 ± 2.70 (right)	0.232 ± 0.198
Pancreas	0.17 ± 0.09	0.120 ± 0.058
Red marrow	2.03 ± 0.80	0.157 ± 0.032
Spleen	0.95 ± 1.14	0.359 ± 0.162
Intestine	1.28 ± 0.32	0.092 ± 0.024
Urinary bladder	8.45 ± 5.01	1.852 ± 1.047
Total body	—	0.128 ± 0.013
Effective dose	—	0.251 ± 0.047

According to PERCIST 1.0[25], the partial response rate was 10% (1/10), the stable disease rate was 80% (8/10), and the progressive disease rate was 10% (1/10). According to RECIST 1.1[26], nine patients exhibited stable disease, and one patient showed disease progression. The images of the patient with partial response, according to PERCIST 1.0, are shown in Fig. 8A and B.

4. Discussion

In this prospective pilot study, a novel radionuclide drug candidate targeting integrin $\alpha_v\beta_3$ with PEGylated cyclic RGD dimmers and an albumin-binding motif, ^{177}Lu-AB-3PRGD2, was translated into a first-in-human clinical trial in patients with integrin $\alpha_v\beta_3$-avid tumors, mostly adenoid cystic carcinoma. The primary outcome was to obtain the pharmacokinetics and dosimetry of ^{177}Lu-AB-3PRGD2 in human beings and the second outcome was the evaluation of the preliminary safety data and possible responses to the single-dose treatment.

According to this study, ^{177}Lu-AB-3PRGD2 was predominantly excreted through the renal-urinary pathway, with the most intense radioactivity observed and the highest absorbed doses in the bladder and followed by the kidneys, similar to the other RGD-based peptides in human being[27–29]. The established tolerance absorbed-dose limit for the kidneys was 23 Gy[30]. According to our dosimetry data 0.684 ± 0.132 mGy/MBq for kidneys, the maximum dosing activities in the kidneys would be 33.625 ± 6.489 GBq, which was 22.7 times the current administered dose. Moreover, the kidneys, once regarded as the critical organ for radiation toxicity, showed excellent tolerance to radiation doses as high as 50–60 Gy in selected cases[31]. ^{177}Lu-labeled peptides appeared to be well tolerated even in patients with preexisting chronic kidney disease stage 3, with a low incidence of permanent major nephrotoxicity[32]. No evidence of nephrotoxicity and no adverse event related to the urinary system was observed in this study. However, further researchers still need to pay attention to the nephrotoxicity of higher doses of ^{177}Lu-AB-3PRGD2 treatment with multiple courses.

Moderate physiological uptake was found in the liver, with a residence time of 3.96 ± 0.55 h and an absorbed dose of 0.204 ± 0.028 mGy/MBq. Among the 10 participants, two (20%) suffered from adverse events of liver function tests. One patient with grade 3 elevated ALT and AST levels had intrahepatic cholangiocarcinoma accompanied by liver metastases, who had been found with grade 1 elevation of ALT and AST levels before treatment. Another patient with grade 2 elevation of ALT and grade 1 elevation of AST levels had liver metastases of adenoid cystic carcinoma. His ALT and AST levels were transient and returned to normal in the 4th week after treatment

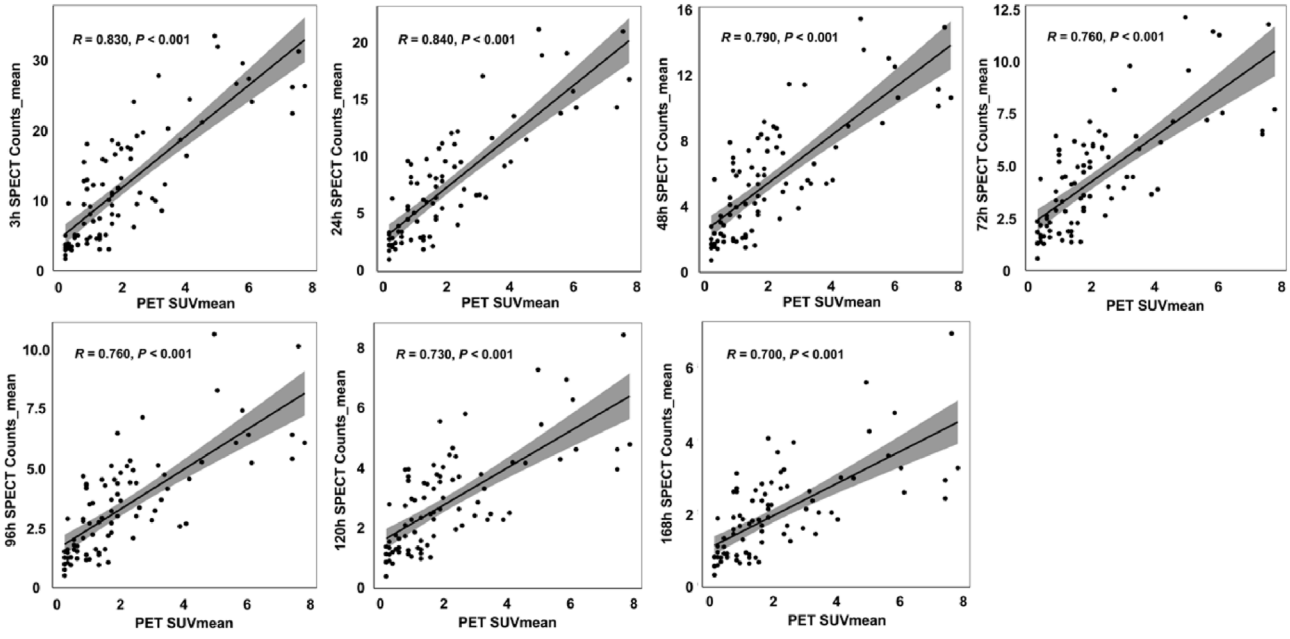

Figure 6 Correlation between the SUVmean of normal organs on ^{68}Ga-DOTA-3PRGD2 PET/CT and the corresponding counts_mean on ^{177}Lu-AB-3PRGD2 planar scans at different time points.

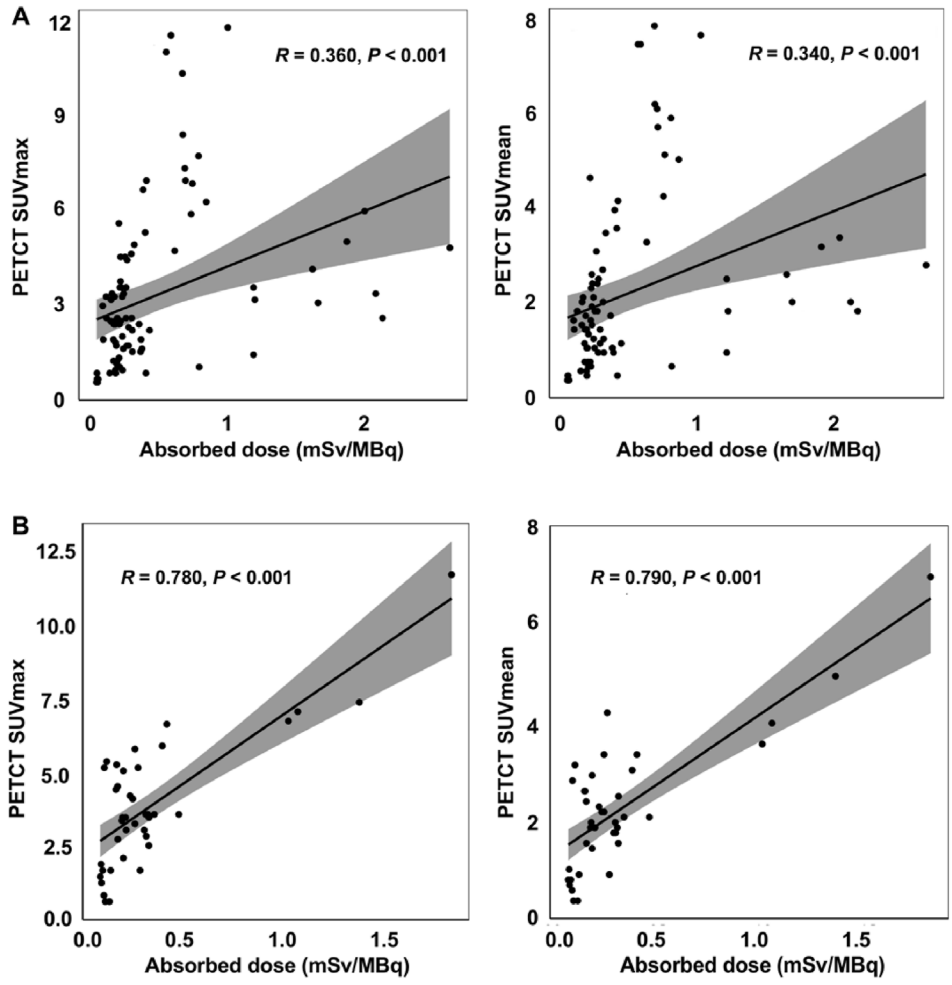

Figure 7 Correlation between the SUVmax (left) and SUVmean (right) of ^{68}Ga-DOTA-3PRGD2 and ^{177}Lu-AB-3PRGD2 absorbed dose of normal organs (A) and tumors (B).

and subsequent follow-ups. Although no confirmed hepatotoxicity has been established with the ^{177}Lu-AB-3PRGD2 treatment, it might be necessary to closely monitor liver function in further studies.

Radioactivity could also be observed in the intestine, which was changed over time, indicating partial hepato-intestinal excretion of ^{177}Lu-AB-3PRGD2. No adverse event related to the gastrointestinal system was observed in this study. The accurate percentage of excretion from the renal-urinary system and the hepato-intestinal route needed further studies to collect and measure radioactivity in urine and feces, respectively. The excretion of ^{99m}Tc-Maraciclatide in healthy volunteers was reported as 55% and 16% through the urinary pathway and intestinal tract, respectively[28].

Table 4 Response assessment of the participant patients.

Response	Symptomatic evaluation *n* (%)	PERCIST 1.0 *n* (%)	RECIST 1.1 *n* (%)
Complete response	0 (0%)	0 (0%)	0 (0%)
Partial response	3 (30%)	1 (10%)	0 (0%)
Stable disease	4 (40%)	8 (80%)	9 (90%)
Progressive disease	3 (30%)	1 (10%)	1 (10%)

With an albumin-binding motif reversibly binding to serum albumin to optimize the pharmacokinetics, the estimated half-life of ^{177}Lu-AB-3PRGD2 in the blood was 2.85 ± 2.17 h, which was much longer than the 8.6 min plasma half-life of a reported RGD dimmer [^{68}Ga]Ga-NODAGA-E[c(RGDyK)]$_2$ for PET imaging[29]. An albumin-binding motif could provide more chances for the target binding and remarkably increase the radioactivity accumulation in the tumor, as reported in our prior studies using Evan's blue and similar albumin binding moiety[31–33]. However, this may also increase the possibility of toxicity to normal organs, especially to red bone marrow causing hematological toxicity. In this study, only a patient with uterine leiomyosarcoma developed a grade 2 anemia two weeks after the treatment. She had borderline anemia before treatment and the changes were partly recovered 4 weeks after the therapy.

Many radionuclide-labeled RGD derivatives have been developed to specifically target integrin $\alpha_v\beta_3$, and several approaches have been investigated to improve the pharmacokinetics and tumor accumulation of these derivatives, including their combination with glycosyl, hydrophilic amino acids, PEGs, dimeric or polymeric derivative[5–9]. Reversibly binding to serum albumin is also one of the strategies to make it possible to produce long-acting therapeutic drugs to reduce the frequency and dosage of radiopharmaceuticals, which showed a significant increase of

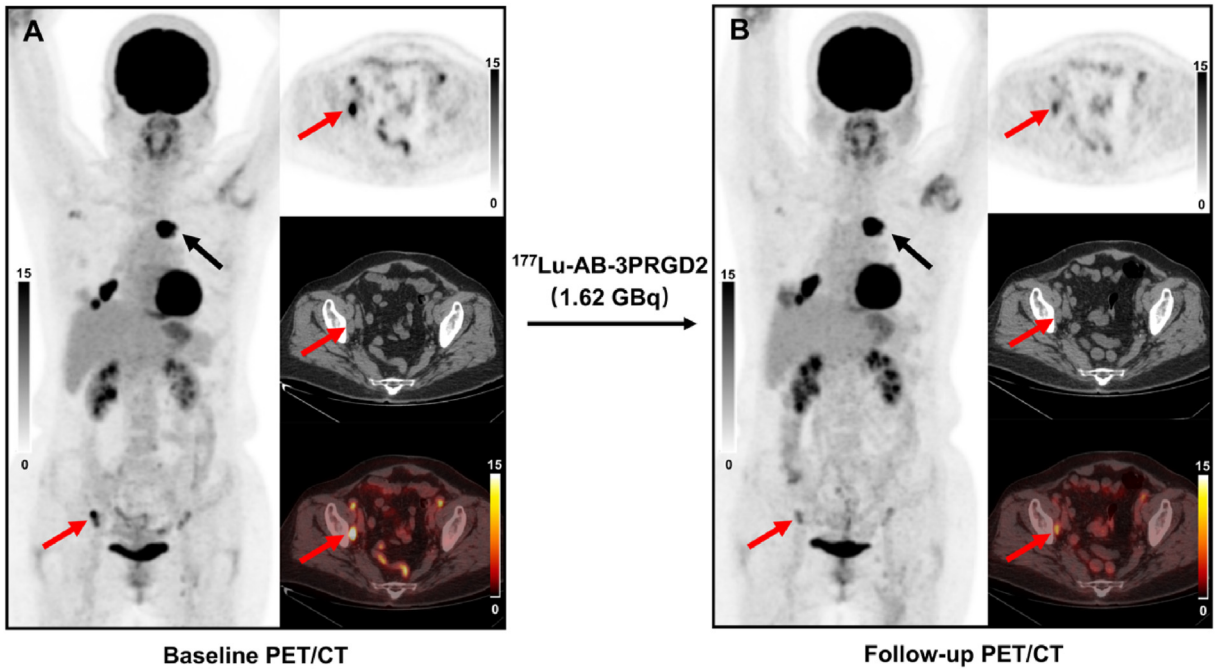

Figure 8 Comparison of ^{18}F-FDG PET/CT images with a single dose of 1.62 GBq (43.70 mCi) ^{177}Lu-AB-3PRGD2 treatment in a 70-year-old patient with uterine leiomyosarcoma. (A) The baseline images of maximum intensity projection (left) and pelvic transaxial view of PET, CT and PET/CT (right, from top to bottom); (B) The follow-up images 6 weeks after the treatment, with a remarkably decrease of the SUV_{max} from 12.0 to 4.6 in a pelvic lymph node metastasis (red arrow), but a mild change of the SUV_{max} from 22.7 to 22.4 in a larger lung metastasis (black arrow).

tumor uptake and retention, leading to enhanced efficacy of targeted therapy[22–24]. However, along with the increase of tumor uptake, the absorption of non-targeted organs such as the kidneys, liver, and red bone marrow may also increase[33–35]. Therefore, potential nephrotoxicity, hepatotoxicity, and hematological toxicity concerns still require careful attention in therapeutic research. In this study, intense to moderate physiological uptake of ^{177}Lu-AB-3PRGD2 was observed in the bladder, kidneys, liver, spleen, and intestines, the safety profile suggested that ^{177}Lu-AB-3PRGD2 was generally well tolerated by patients, with no significant acute adverse events or life-threatening toxicities. None of the patients experienced grade 4 or 5 toxicity, indicating that a dosage of 1.57 ± 0.08 MBq (42.32 ± 2.11 mCi) was relatively safe and in line with the therapeutic aim of providing an effective dose to the target tissue while preserving the normal organ system. However, the occurrence of possible drug-related adverse events in some patients, although not life-threatening, emphasizes the necessity of careful monitoring of these toxicities associated with the therapy in further studies with escalating doses and multiple treatment courses.

There were highly significant correlations between the ^{68}Ga-DOTA-3PRGD2 distribution on PET/CT and the ^{177}Lu-AB-3PRGD2 distribution on SPECT/CT. Furthermore, there were also positive correlations between the SUV_{max} or SUV_{mean} on ^{68}Ga-DOTA-3PRGD2 PET/CT and the absorbed doses of ^{177}Lu-AB-3PRGD2 in normal organs and tumors. These suggested that ^{68}Ga-DOTA-3PRGD2 PET/CT could be a robust and reliable method to select patients who might benefit from the ^{177}Lu-AB-3PRGD2 treatment.

Most patients in this study had stable conditions after treatment. However, three patients reported symptom improvement and one patient showed an ^{18}F-FDG uptake decrease, indicating positive responses to the current single low-dose treatment and providing a foundation for subsequent escalating and multi-dose treatments with ^{177}Lu-AB-3PRGD2.

This study had several limitations: First, the treatment dosage of ^{177}Lu-AB-3PRGD2 was relatively low as a pilot study mainly focusing on pharmacokinetics and dosimetry, making the safety evaluations insufficient for routine clinical radionuclide therapy with full dose. Second, only a single dose was administered to each patient, whereas 4–6 courses were usually adopted in a routine clinical setting. In addition, the number of patients was small and the disease types were limited in this study. Moreover, the follow-up period was only 6–8 weeks, an interval duration of two consecutive treatment courses. However, in light of the promising findings in this preliminary pilot study, further studies were warranted with escalated doses and multiple courses in a more diverse patient population to verify the safety and efficacy of ^{177}Lu-AB-3PRGD2. Furthermore, an extended follow-up period would be beneficial for assessing the long-term safety and overall survival of the new treatment strategy.

5. Conclusions

This prospective first-in-human pilot study of ^{177}Lu-AB-3PRGD2, a novel integrin $\alpha_v\beta_3$-targeting drug with an albumin-binding motif, preliminarily indicates its safety in human beings and demonstrates an optimal pharmacokinetics and dosimetry as a potential candidate drug for radionuclide therapy. ^{68}Ga-DOTA-3PRGD2 PET/CT can help to select patients who may benefit from the ^{177}Lu-AB-3PRGD2 therapy and evaluate the integrin $\alpha_v\beta_3$-avid tumors. The promising results of this study merit further investigations of ^{177}Lu-AB-3PRGD2 treatment in more patients with escalating doses and multiple courses, and subsequently, conduct long-term evaluations to determine its safety and efficacy.

Acknowledgments

This study was partly supported by Chinese Academy of Medical Science Innovation Fund for Medical Sciences (2022-I2M-2-002), the National High-Level Hospital Clinical Research Funding (2022-PUMCH-D-002, China), Clinical and translational medicine research project of the Chinese Academy of Medical Sciences (2022-I2M-C&T-A-008, China), the National Natural Science Foundation of China (82272046), and the Fundamental Research Funds for the Central Universities (3332023124, China).

Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

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