

Preclinical Evaluation of ^{68}Ga - and ^{177}Lu -Labeled Integrin $\alpha_v\beta_6$ -Targeting Radiotheranostic Peptides

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The integrin $\alpha_v\beta_6$, an epithelium-specific cell surface receptor, is over-expressed on numerous malignancies, including the highly lethal pancreatic ductal adenocarcinomas. Here, we developed and tested a novel $\alpha_v\beta_6$ -targeting peptide, DOTA-5G (**1**) radiolabeled with ^{68}Ga , for PET/CT imaging and ^{177}Lu for treatment. With the goal to develop a radiotheranostic, further modifications were made for increased circulation time, renal recycling, and tumor uptake, yielding DOTA-albumin-binding moiety-5G (**2**). **Methods:** Peptides **1** and **2** were synthesized on solid phase, and their affinity for $\alpha_v\beta_6$ was assessed by enzyme-linked immunosorbent assay. The peptides were radiolabeled with ^{68}Ga and ^{177}Lu . In vitro cell binding, internalization, and efflux of ^{68}Ga -**1** and ^{177}Lu -**2** were evaluated in $\alpha_v\beta_6$ -positive BxPC-3 human pancreatic cancer cells. PET/CT imaging of ^{68}Ga -**1** and ^{68}Ga -**2** was performed on female nu/nu mice bearing subcutaneous BxPC-3 tumors. Biodistribution was performed for ^{68}Ga -**1** (1 and 2 h after injection), ^{68}Ga -**2** (2 and 4 h after injection), and ^{177}Lu -**1** and ^{177}Lu -**2** (1, 24, 48, and 72 h after injection). The ^{177}Lu -**2** biodistribution data were extrapolated for human dosimetry data estimates using OLINDA/EXM 1.1. Therapeutic efficacy of ^{177}Lu -**2** was evaluated in mice bearing BxPC-3 tumors. **Results:** Peptides **1** and **2** demonstrated high affinity (<55 nM) for $\alpha_v\beta_6$ by enzyme-linked immunosorbent assay. ^{68}Ga -**1**, ^{68}Ga -**2**, ^{177}Lu -**1**, and ^{177}Lu -**2** were synthesized in high radiochemical purity. Rapid in vitro binding and internalization of ^{68}Ga -**1** and ^{177}Lu -**2** were observed in BxPC-3 cells. PET/CT imaging and biodistribution studies demonstrated uptake in BxPC-3 tumors. Introduction of the albumin-binding moiety in ^{177}Lu -**2** resulted in a 5-fold increase in tumor uptake and retention over time. Based on the extended dosimetry data, the dose-limiting organ for ^{177}Lu -**2** is the kidney. Treatment with ^{177}Lu -**2** prolonged median survival by 1.5- to 2-fold versus controls. **Conclusion:** ^{68}Ga -**1** and ^{177}Lu -**2** demonstrated high affinity for the integrin $\alpha_v\beta_6$ both in vitro and in vivo, were rapidly internalized into BxPC-3 cells, and were stable in mouse and human serum. Both radiotracers showed favorable pharmacokinetics in preclinical studies, with predominantly renal excretion and good tumor-to-normal-tissue ratios. Favorable human dosimetry data suggest the potential of ^{177}Lu -**2** as a treatment for pancreatic ductal adenocarcinoma.

Key Words: integrin $\alpha_v\beta_6$; ^{68}Ga ; ^{177}Lu ; theranostics; albumin-binding moiety

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Despite exhaustive testing and some encouraging advances in first- and second-line treatments, pancreatic ductal adenocarcinoma (PDAC) remains the fourth leading cause of cancer-related deaths, with a 5-y survival below 10% (1,2). This dire reality is also in part due to diagnosis at an advanced stage of the disease and the poor reliability of current standard imaging approaches. Therefore, there clearly remains an urgent unmet clinical need for more effective molecularly targeted diagnostics and therapeutics.

The heterodimeric transmembrane receptor integrin $\alpha_v\beta_6$ has been identified as a potential molecular target; it is an epithelium-specific cell surface receptor that is undetectable in healthy adult epithelium but is significantly upregulated in a wide range of epithelium-derived cancers, including PDAC (3–10). In fact, $\alpha_v\beta_6$ was initially identified in PDAC with nearly uniform high expression among patient samples screened; moreover, metastatic lesions demonstrate further highly upregulated expression of $\alpha_v\beta_6$ when compared with the primary tumor, and $\alpha_v\beta_6$ is undetectable in normal pancreas (11). These traits further underscore the potential of $\alpha_v\beta_6$ as an attractive target for both early detection and targeted delivery of a therapeutic payload in PDAC.

Radiotheranostics combines molecular imaging with targeted radionuclide therapy, often using the same targeting ligand, and has shown efficacy in several cancers (12,13). Over the last few years, we have seen an exponential growth in the development and acceptance of radiotheranostics for applications in oncology. For example, ^{68}Ga -DOTATATE for imaging of neuroendocrine tumors and ^{177}Lu -DOTATATE for peptide receptor radionuclide therapy were the first radiotheranostic peptides to be approved by the Food and Drug Administration, in 2018 (14). More recently, ^{18}F -DCFPyL and ^{68}Ga -PSMA-11 gained approval for imaging, as did ^{177}Lu -PSMA-617 for treatment of prostate-specific membrane antigen-positive metastatic castration-resistant prostate cancer (15).

Several groups, including our own, have developed molecular imaging agents to target the integrin $\alpha_v\beta_6$, and several promising agents have advanced to clinical trials for imaging cancer and fibrosis (16–20). Building on over a decade of work by the Sutcliffe laboratory to develop $\alpha_v\beta_6$ -targeted molecular imaging agents (17,21,22), we now propose to address the clear unmet need for new therapies for PDAC using a radiotheranostic strategy. We present a novel molecularly targeted radiotheranostic approach via the integrin $\alpha_v\beta_6$ for peptide receptor radionuclide therapy. DOTA-5G (**1**) was designed to selectively target the integrin $\alpha_v\beta_6$; in addition, with the goal to increase blood residence time, tumor uptake, and renal recycling, an albumin-binding moiety (ABM) was incorporated in

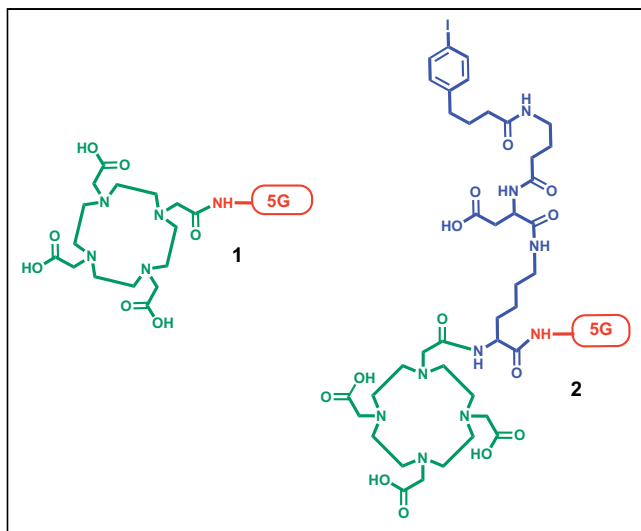


FIGURE 1. Chemical structures of **1** and **2**. The peptide is indicated in red, DOTA chelator in green, and ABM in blue.

the peptide to yield DOTA-ABM-5G (**2**) (Fig. 1). The peptides were synthesized on solid phase, labeled with ^{68}Ga (half-life, 68 min; E_{β^+} [max], 900 keV [23%]) for imaging and ^{177}Lu (half-life, 6.7 d; E_{β^-} [max], 490 keV; E_{γ} , 208 keV [11%]) for therapy, and evaluated in vitro (for cell binding, internalization, and efflux) in $\alpha_v\beta_6$ -expressing BxPC-3 human pancreatic cancer cells. Albumin binding and stability in mouse and human serum were determined. In vivo PET/CT imaging and biodistribution studies were performed on mice bearing BxPC-3 tumors xenografts, and $\alpha_v\beta_6$ -specific targeting was confirmed by blocking studies. Human dosimetry was estimated from the extended biodistribution data, and therapeutic efficacy was evaluated in mice bearing BxPC-3 tumors.

MATERIALS AND METHODS

Chemistry and Radiochemistry

Peptide synthesis on solid phase (23) and radiolabeling and formulation of ^{68}Ga - and ^{177}Lu -labeled peptides are described in Supplemental Section 2 (supplemental materials are available at <http://jnm.snmjournals.org>).

In Vitro Experiments

Enzyme-linked immunosorbent assays (ELISA) (23,24) and cell binding and internalization, albumin binding (23), and serum stability (25) tests followed previously published procedures as described in Supplemental Section 3.

In Vivo Imaging, Biodistribution, and Targeted Radionuclide Therapy

All animal studies were performed according to procedures approved by the University of California Davis Institutional Animal Care and Use Committee. BxPC-3 cells (5×10^6) were implanted subcutaneously into the left shoulder of 6- to 8-wk-old female nu/nu nude mice (Charles River Laboratories) and allowed to grow for 3 wk (imaging and biodistribution) or approximately 2 wk (therapy).

For imaging, the radiotracer (^{68}Ga -**1** or ^{68}Ga -**2**, 7.4–9.25 MBq) in phosphate-buffered saline (PBS) solution (150 μL , pH 7.2) was injected into the tail vein of mice ($n = 3/\text{time point}/\text{radiotracer}$) anesthetized with 3% isoflurane in medical-grade oxygen. After conscious uptake periods of 1 and 2 h, the animals were anesthetized and imaged 2 at a time, side by side in a feet-first prone position as previously described (26).

For biodistribution, the radiotracer (3–3.7 MBq in 100 μ L of PBS) was injected into the tail vein, followed by conscious uptake periods of

1 and 2 h (^{68}Ga -**1**), 2 and 4 h (^{68}Ga -**2**), or 1, 24, 48, and 72 h (^{177}Lu -**1** and ^{177}Lu -**2**). For blocking studies, the respective peptides **1** and **2** (48 mg/kg, 16 mg/mL solution in PBS) were injected 10 min before the radiotracer. At each time point, the mice ($n = 3/\text{time point/radiotracer}$) were anesthetized and sacrificed, tissues were collected and rinsed with PBS, and the radioactivity was measured with a γ -counter. Radioactivity concentrations were calibrated, decay-corrected, and expressed as percentage injected dose per gram of tissue (%ID/g).

An extended biodistribution study was performed for $^{177}\text{Lu-2}$. In 100 μL of PBS, 3.7–5.55 MBq were injected into the tail vein of male ($n = 4/\text{time point}$) and female ($n = 4/\text{time point}$) mice, followed by conscious uptake periods of 24 h, 48 h, 72 h, 1 wk, and 2 wk. The dosimetry values for $^{177}\text{Lu-2}$ were computed using OLINDA/EXM1.1 using a female or male model with organ mass scaling, and effective doses were reported as mSv/MBq.

For the therapeutic efficacy study, mice were randomly chosen and divided into 4 treatment groups: control saline (group 1, $n = 5$), control peptide **2** (group 2, $n = 6$), 74 MBq of ^{177}Lu -**2** (group 3, $n = 10$), and 2×37 MBq of ^{177}Lu -**2** (group 4, $n = 7$). Tumor volumes at the start of the treatment ranged from 14 to 218 mm³. Group 2 received 20 μg of peptide **2**, group 3 received a single dose of 74 MBq (20 μg of peptide **2**) on day 14 after tumor implantation (treatment day 0), and group 4 received 1 dose of 37 MBq (10 μg of peptide **2**) on days 14 and 21 after tumor implantation (treatment days 0 and 7). Body weights and tumor volumes were measured the day before treatment and twice a week after treatment throughout the study.

Statistical Analysis

All statistical data are reported as mean $\pm$ SD. Paired, 2-tailed Student *t* tests were used to evaluate statistical significance, with a *P* value of less than 0.05 being considered statistically significant.

RESULTS

Synthesis of 1 and 2 and Respective ^{nat}Ga and ^{nat}Lu Analogs

Peptides **1** and **2** were synthesized by solid-phase peptide synthesis and obtained in high purity (>98%) after high-performance liquid chromatography purification, and ^{nat}Ga-**1**, ^{nat}Ga-**2**, ^{nat}Lu-**1**, and ^{nat}Lu-**2** were obtained in at least 98% purity. The analytic data are provided in Supplemental Section 2. ^{nat}Ga-**1**, ^{nat}Ga-**2**, ^{nat}Lu-**1**, and ^{nat}Lu-**2** were used in an ELISA for half-maximal inhibitory concentration determination and as reference standards for high-performance liquid chromatography coinjection to confirm the identity of the ⁶⁸Ga- and ¹⁷⁷Lu-labeled peptides.

Radiochemical Synthesis

⁶⁸Ga-1 and ⁶⁸Ga-2 were obtained in at least 98% radiochemical purity after semipreparative high-performance liquid chromatography purification. ¹⁷⁷Lu-1 and ¹⁷⁷Lu-2 were obtained in at least 97% radiochemical purity.

Half-Maximal Inhibitory Concentration Determinations

The half-maximal inhibitory concentration of ^{nat}Ga-1, ^{nat}Ga-2, ^{nat}Lu-1, and ^{nat}Lu-2 for integrin α_vβ₆ determined by ELISA were 33.2 ± 1.5 nM, 37.2 ± 3.5 nM, 50.0 ± 4.4 nM, and 29.0 ± 0.6 nM, respectively. The binding affinities for integrin α_vβ₃ were more than 100 μM for all peptides.

Cell Binding, Internalization, and Efflux

For ^{68}Ga -1, $16.1\% \pm 0.3\%$ of total radioactivity bound to the BxPC-3 cells, of which $71.5\% \pm 0.6\%$ internalized into the cells. For ^{177}Lu -2, $31.5\% \pm 0.6\%$ bound, of which $72.3\% \pm 1.0\%$ internalized, and minimal efflux was observed for ^{177}Lu -2 at 1 h ($\sim 20\%$ of internalized radioactivity) (Fig. 2).

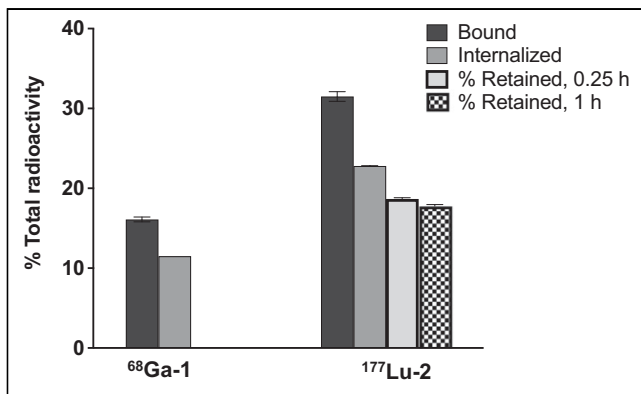


FIGURE 2. Cell binding and internalization of ⁶⁸Ga-1 and ¹⁷⁷Lu-2 at 1 h to BxPC-3 cells at 37°C, and percentage radioactivity of ¹⁷⁷Lu-2 retained in BxPC-3 cells after internalization. Internalization is expressed as fraction of total radioactivity ($n = 3$ /radiotracer; 1 h of incubation).

Albumin Binding

Albumin binding of ¹⁷⁷Lu-2 was $54.4\% \pm 2.8\%$ and $58.1\% \pm 0.4\%$ for mouse and human serum, respectively, compared with $18.1\% \pm 0.2\%$ and $16.2\% \pm 0.4\%$, respectively, for ¹⁷⁷Lu-1.

Serum Stability

⁶⁸Ga-1 and ⁶⁸Ga-2 were 100% intact at 2 h in both mouse and human serum. At 24 h, ¹⁷⁷Lu-1 and ¹⁷⁷Lu-2 were 72% and 78% intact, respectively, in mouse serum and at least 97% intact (both) in human serum.

In Vivo Imaging and Biodistribution

Imaging and Biodistribution of ⁶⁸Ga-1 and ⁶⁸Ga-2. Imaging and biodistribution were performed in the subcutaneous BxPC-3 tumor model at 1 and 2 h after injection for ⁶⁸Ga-1 and at 2 and 4 h after injection for ⁶⁸Ga-2 (Fig. 3; Supplemental Table 1). Uptake and retention in the tumor were evident at all time points for both ⁶⁸Ga-1 and ⁶⁸Ga-2. However, whereas ⁶⁸Ga-1 cleared from key organs such as the kidneys by 2 h, uptake of ⁶⁸Ga-2 continued to trend upward. Tumor uptake was $2.6\% \pm 0.8\%$ at 1 h and $2.0\% \pm 0.6\%$ at 2 h after injection for ⁶⁸Ga-1 and $9.4\% \pm 1.9\%$ at 2 h and $10.4\% \pm 2.6\%$ at 4 h after injection for ⁶⁸Ga-2.

Renal uptake of ⁶⁸Ga-1 was $23 \pm 3\% \text{ID/g}$ at 1 h, dropping to $14 \pm 4\% \text{ID/g}$ at 2 h ($P = 0.032$). For ⁶⁸Ga-2, renal uptake was $20 \pm 3\% \text{ID/g}$ at 2 h, increasing to $26 \pm 2\% \text{ID/g}$ at 4 h ($P = 0.039$). As depicted in Figure 3D, tumor-to-organ ratios at 2 h after injection for ⁶⁸Ga-1 and ⁶⁸Ga-2, respectively, were 0.75 ± 0.23 and 0.88 ± 0.25 for stomach, 1.6 ± 0.5 and 1.3 ± 0.3 for small intestine, 0.98 ± 0.26 and 1.2 ± 0.3 for large intestine, 20 ± 7 and 6 ± 0.9 for liver, 21 ± 2 and 7.2 ± 2.5 for pancreas, and 22 ± 3.9 and 1.1 ± 0.2 for blood. Collectively, these data suggest that ⁶⁸Ga-1 has the most favorable biodistribution properties for a PDAC imaging agent, clearing rapidly from key organs while being retained by the tumor.

Biodistribution of ¹⁷⁷Lu-1 and ¹⁷⁷Lu-2.

Uptake and retention in the BxPC-3 tumor were at least 4-fold greater for ¹⁷⁷Lu-2 than for ¹⁷⁷Lu-1, with ¹⁷⁷Lu-2 remaining at $5 \pm 0.8\% \text{ID/g}$ at 72 h (Figs. 4 and 5; Supplemental Tables 2 and 3). Both ¹⁷⁷Lu-1 and ¹⁷⁷Lu-2 demonstrated washout from key organs over time, resulting in tumor-to-kidney ratios for ¹⁷⁷Lu-1 of 0.08 ± 0.05 at 1 h and 0.14 ± 0.05 at 72 h; for ¹⁷⁷Lu-2, the ratios were 0.22 ± 0.05 at 1 h and 0.60 ± 0.02 at 72 h (Fig. 5B). For the same time points, tumor-to-stomach ratios were, respectively, 0.31 ± 0.06 and 0.87 ± 0.28 for ¹⁷⁷Lu-1 and 0.50 ± 0.08 and 1.16 ± 0.30 for ¹⁷⁷Lu-2 (Fig. 5C). Likewise, tumor-to-pancreas ratios were 5 ± 3 and 9 ± 1.4 for ¹⁷⁷Lu-1 and 5 ± 0.06 and 16 ± 5 for ¹⁷⁷Lu-2 (Fig. 5D); tumor-to-large intestine ratios were 0.31 ± 0.02 and 1.74 ± 0.39 for ¹⁷⁷Lu-1 and 0.77 ± 0.25 and 1.77 ± 0.26 for ¹⁷⁷Lu-2. Collectively, these data suggest that ¹⁷⁷Lu-2 has the most favorable pharmacokinetics to advance to therapeutic efficacy studies.

Blocking Studies. Preadministration of the respective blocking peptide (1 or 2) 10 min before administration of ⁶⁸Ga-1, ¹⁷⁷Lu-1, or ¹⁷⁷Lu-2 resulted in reduced tumor uptake. Tumor uptake dropped from $2.6 \pm 0.8\% \text{ID/g}$

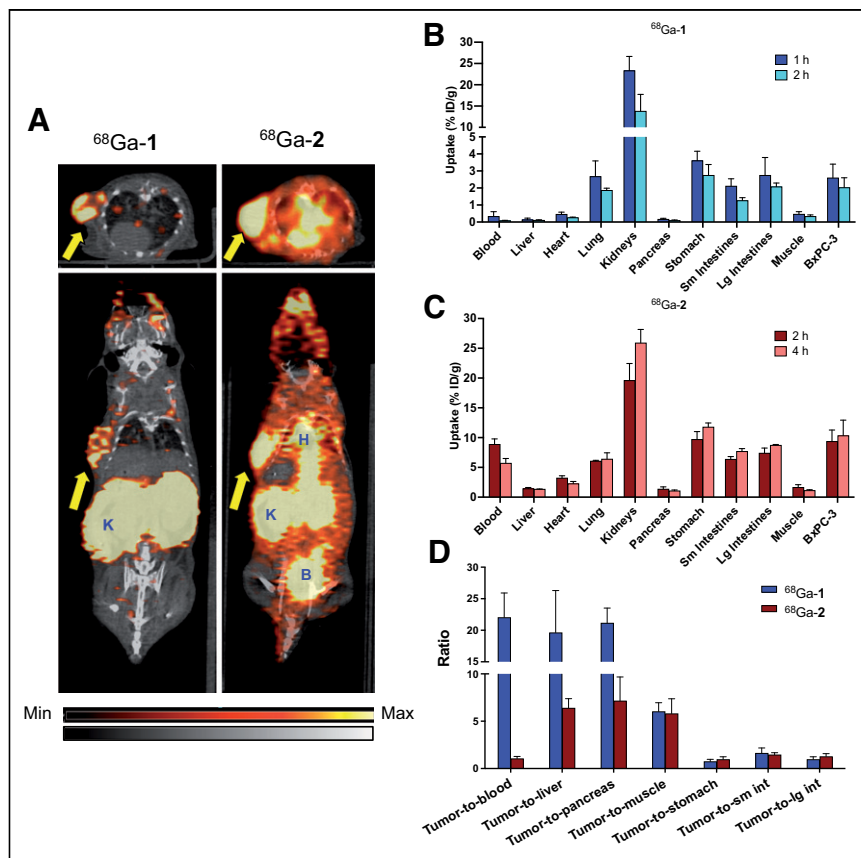


FIGURE 3. (A) Representative transaxial (top) and coronal (bottom) PET/CT cross sections of mice bearing BxPC-3 tumors (arrows) obtained at 2 h after injection. Both images are presented on same scale. Red is PET, and gray is CT. (B–D) Biodistribution showing uptake (%ID/g) of ⁶⁸Ga-1 (B) and ⁶⁸Ga-2 (C) in selected organs and $\alpha_v\beta_6$ -positive BxPC-3 tumors ($n = 3$ /group/time point) and tumor-to-tissue ratios 2 h after injection (D). B = bladder; K = kidney; H = heart; int = intestines; lg = large; sm = small.

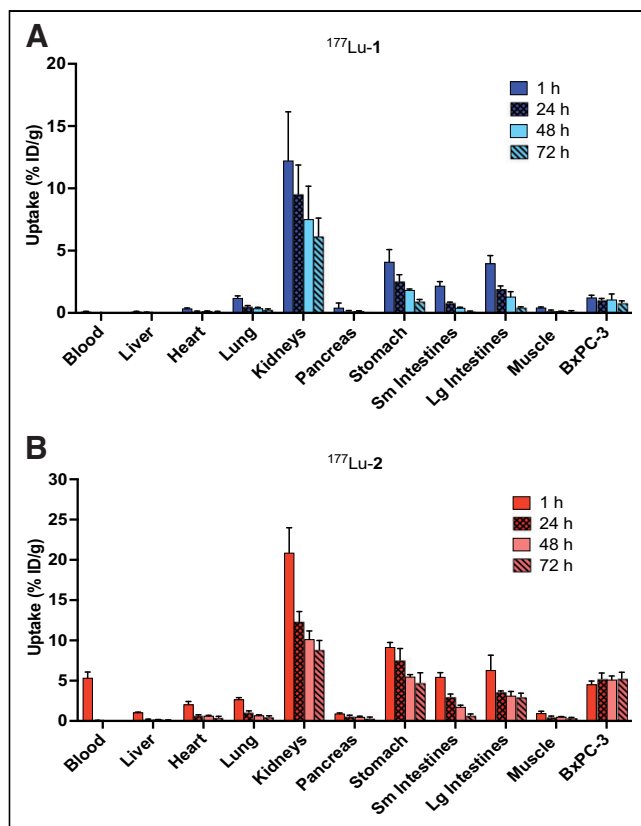


FIGURE 4. Biodistribution showing uptake (%ID/g) of $^{177}\text{Lu-1}$ (A) and $^{177}\text{Lu-2}$ (B) in selected organs and $\alpha_v\beta_6$ -positive BxPC-3 tumors ($n = 3/\text{group/time point}$). Lg = large; sm = small.

(unblocked) to $0.27 \pm 0.02\% \text{ID/g}$ for $^{68}\text{Ga-1}$ (1 h, -86% change); for $^{177}\text{Lu-1}$, tumor uptake dropped from $1.2 \pm 0.2\% \text{ID/g}$ to $0.3 \pm 0.02\% \text{ID/g}$ (1 h, -75% change); and for $^{177}\text{Lu-2}$, tumor uptake dropped from $7.2 \pm 3.0\% \text{ID/g}$ to $4.1\% \text{ID/g}$ (4 h, -42% change; Supplemental Figs. 1–3).

Therapeutic Efficacy

A significant delay in tumor growth was observed in treatment groups 3 (74 MBq $^{177}\text{Lu-2}$) and 4 ($2 \times 37 \text{ MBq } ^{177}\text{Lu-2}$) versus control groups 1 and 2 (Fig. 6A). All mice in groups 1 and 2 had met the endpoint criteria (tumor $\geq 2 \text{ cm}$ in any direction or ulceration) by days 63 and 68 from the start of treatment, respectively. In contrast, the mice in groups 3 and 4 had 30% and 43% survival rates, respectively, at the end of the study (120 d; Fig. 6B). The median survival was 56 d for mice in groups 1 and 2, versus 82 d for group 3 and 113 d for group 4. No treatment-related adverse effects (weight loss or signs of distress) were evident during the course of the study (Supplemental Fig. 4).

Dosimetry

The highest estimated dose, as expected from the imaging and biodistribution data, was to the kidneys. On the basis of the

extrapolated values obtained in OLINDA, the effective dose from $^{177}\text{Lu-2}$ to the kidneys would be 1.31 mSv/MBq and 1.14 mSv/MBq for a woman and man, respectively (Supplemental Tables 4 and 5).

DISCUSSION

There has been increasingly rapid growth in the field of radiotheranostics, fueled in part by the many successful clinical outcomes, significant investments by the pharmaceutical industry, and recent regulatory approvals (27). Numerous novel radiotheranostics for a wide range of clinically relevant targets are now under investigation (28). Here, we describe the development of peptide-based radiotheranostics to target the integrin $\alpha_v\beta_6$, an epithelium-specific receptor that has been identified as a relevant molecular target for both the detection and the treatment of cancer (29). Several $\alpha_v\beta_6$ -targeting imaging agents have demonstrated utility in the clinic for a range of carcinomas, including breast, colon, head and neck, lung, and pancreas (16–19). In view of the functional relevance of the integrin $\alpha_v\beta_6$, it is anticipated to become an important target for future radiotheranostics (30).

Building on over a decade of research and development of $\alpha_v\beta_6$ -targeted peptides for imaging by our group, we designed and report here a radiotheranostic strategy using ^{68}Ga for PET imaging and ^{177}Lu for both treatment and imaging with SPECT. We developed DOTA-5G (1), a peptide with high affinity and selectivity for the integrin $\alpha_v\beta_6$ and, with the goal to increase tumor uptake and reduce renal retention, further incorporated a 4-(*p*-iodophenyl) butyryl-containing ABM—based on prior literature showing favorable pharmacokinetics for targeting ligands such as folates, octreotides, and PSMA-targeting phosphoramidates (31–33)—to yield DOTA-ABM-5G (2). Given the notable presence of the integrin $\alpha_v\beta_6$ in PDAC, we evaluated 1 and 2 in a pancreatic mouse model (11,34,35).

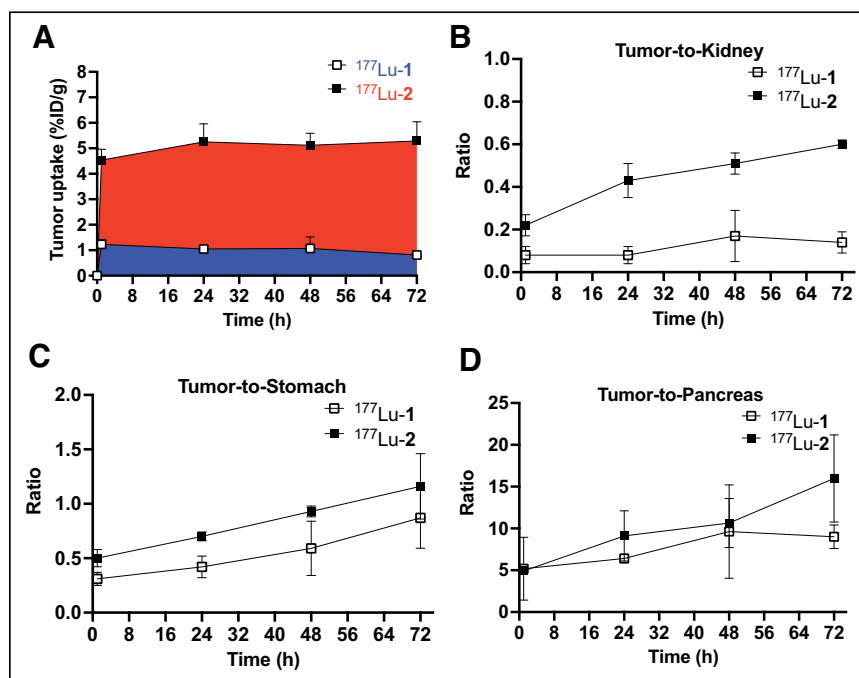


FIGURE 5. Uptake of $^{177}\text{Lu-1}$ and $^{177}\text{Lu-2}$ in BxPC-3 tumor (A) and tumor-to-organ ratios for kidney (B), stomach (C), and pancreas (D) derived from biodistribution data.

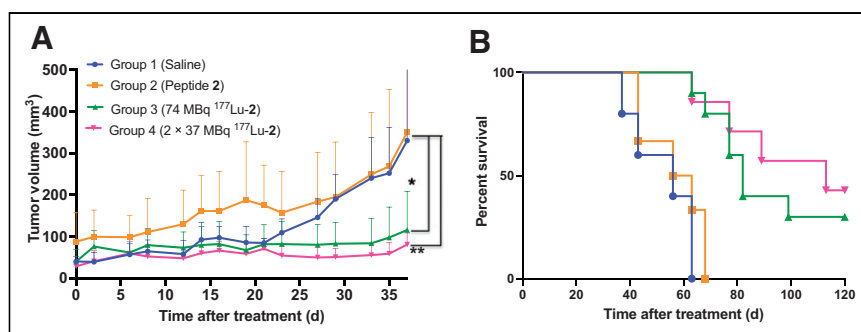


FIGURE 6. Therapeutic efficacy of ^{177}Lu -2 in mice bearing $\alpha_v\beta_6$ -positive BxPC-3 tumors, as determined by tumor growth (average tumor volume ≤ 37 d after treatment) (A) and survival data (B). * $P = 0.0077$. ** $P = 0.0064$.

$^{\text{nat}}\text{Ga}$ -1, $^{\text{nat}}\text{Ga}$ -2, $^{\text{nat}}\text{Lu}$ -1, and $^{\text{nat}}\text{Lu}$ -2 demonstrated high affinity and selectivity for the integrin $\alpha_v\beta_6$, similar to our previously published $\alpha_v\beta_6$ -targeting peptides (23,26). Tumors were clearly detected with ^{68}Ga -1 and ^{68}Ga -2 by PET (Fig. 3A) and showed uptake similar to other ^{68}Ga -peptides reported in the literature (19,36). Both ^{68}Ga -1 and ^{68}Ga -2 show predominantly renal excretion, as commonly reported for radiolabeled peptides (37,38). Compared with ^{68}Ga -2, ^{68}Ga -1 was cleared rapidly from the blood and other nontarget tissues, including healthy pancreas (Fig. 3D), resulting in improved contrast. These data reaffirmed our previous reports that although the inclusion of an ABM into targeting ligands increases circulation and tumor uptake, this might not be desirable for short-lived (diagnostic) radioisotopes such as ^{68}Ga (39) or ^{18}F (23). Overall, ^{68}Ga -1 compared favorably to other noteworthy examples, including ^{68}Ga -cycratide (36), ^{68}Ga -DOTA-SFITGv6 (16), and ^{68}Ga -trivehexin (19), and was therefore chosen as the clinical candidate for PET imaging.

In vitro studies showed $\alpha_v\beta_6$ -targeted cell binding and internalization with minimal efflux of the ^{177}Lu -2 from cells. ^{177}Lu -2 also demonstrated good stability ($>97\%$ at 24 h) in human serum. Compared with ^{177}Lu -1, the addition of the ABM expectedly resulted in longer circulation of ^{177}Lu -2, which, in combination with the long half-life of 6.7 d for ^{177}Lu , was highly beneficial as demonstrated by the biodistribution studies (Fig. 5). Lu-2 was rapidly taken up by and retained in the tumor over the 72-h window, along with significant washout from all key organs over this time frame. By the 72-h time point, only the kidneys showed higher uptake of ^{177}Lu -2 than the tumor; notably, no renal clearing agents were used in this preclinical study. To further mitigate the high kidney activity, other complementary approaches such as coinjection of positively charged amino acids such as lysine and arginine and pretargeting will be explored. Biodistribution data were extrapolated to obtain estimated human dosimetry using OLINDA/EXM1.1, showing the highest dose to be to the kidney—as expected from the imaging and biodistribution data—at 1.31 mSv/MBq (female) and 1.14 mSv/MBq (male). These values are considerably lower than those reported for other ^{177}Lu -compounds currently under investigation in the clinic (e.g., CTT1403, an agent for the treatment of prostate cancer, was reported as 5.18 mSv/MBq (40)). For ^{177}Lu -2, the estimated dose would equate to approximately a 1.21-Gy (female) and a 1.05-Gy (male) dose to the kidneys, based on a 925 MBq (25 mCi) starting injected dose for our proposed clinical trial, and is significantly lower than the current 23-Gy threshold.

On the basis of the in vitro and in vivo data, it was hypothesized that significant tumor killing could be achieved from selective

uptake and retention of ^{177}Lu -2 in $\alpha_v\beta_6$ -positive cells; therefore, the therapeutic efficacy of ^{177}Lu -2 was evaluated in the BxPC-3 tumor xenograft model. Two treatment doses were tested, a high single dose of 74 MBq and a fractionated dose of 2 times 37 MBq, 7 d apart. Both cohorts showed significantly increased survival above the control groups. While all mice in the control groups had reached endpoint criteria by day 68, the mice in the treatment groups had 80%–86% survival rates at that time point and showed 1.5- to 2.0-fold increased median survival times compared with the controls. Importantly, mice in both treatment groups maintained a healthy weight during the study, confirming no adverse effects from either the single dose (74 MBq) or the fractionated double dose (2×37 MBq) of ^{177}Lu -2 (Supplemental Fig. 4).

These results were highly encouraging and were contrary to the recently published data by Huynh et al., who reported severe weight loss and death due to kidney toxicity within the first 14 d in mouse cohorts receiving a 37 MBq dose of ^{177}Lu -IBA-DOTA-(PEG28)₂-A20FMDV2 (35).

Overall, the relevance of integrin $\alpha_v\beta_6$ in cancer, the high binding of ^{177}Lu -2 to the integrin $\alpha_v\beta_6$, and its excellent stability in human serum, along with the therapeutic efficacy and favorable estimated dosimetry, collectively suggest the utility of ^{177}Lu -2 as a therapeutic. ^{177}Lu -2 was therefore selected as the treatment candidate for our first-in-humans radiotheranostics study alongside ^{68}Ga -1 as the imaging agent (NCT04665947).

CONCLUSION

^{68}Ga -1 and ^{177}Lu -2 demonstrated high affinity (low nM) and selectivity for the integrin $\alpha_v\beta_6$ in both in vitro assays and in vivo mouse models, were rapidly internalized, and were stable in human serum. Both radiotracers showed favorable pharmacokinetics in preclinical studies, with predominantly renal excretion and good tumor-to-organ ratios. Favorable human dosimetry data calculated from the murine biodistribution data for ^{177}Lu -2 suggest the potential for this treatment. On the basis of these data, a first-in-humans study in patients with locally advanced or metastatic pancreas cancer is under way.

DISCLOSURE

This work was supported by Stand Up To Cancer and Lustgarten Foundation Pancreatic Cancer Collective (PCC) New Therapies Challenges (SU2C-AACR-PCC-06-18). Sven Hausner is a coinventor of intellectual property related to 1 and 2. Julie Sutcliffe is founder and chief executive officer of, and holds ownership interest (including patents) in, Luminance Biosciences, Inc., and is a coinventor of intellectual property related to 1 and 2. No other potential conflict of interest relevant to this article was reported.

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KEY POINTS

QUESTION: Can ^{68}Ga - and ^{177}Lu -radiolabeled integrin $\alpha_v\beta_6$ -targeting peptides detect and treat $\alpha_v\beta_6$ -positive cancers?

PERTINENT FINDINGS: ^{68}Ga -radiolabeled integrin $\alpha_v\beta_6$ -targeting peptides could detect $\alpha_v\beta_6$ -positive tumors, and the ^{177}Lu -radiolabeled $\alpha_v\beta_6$ -targeting peptide ^{177}Lu -2 demonstrated therapeutic efficacy in a pancreas cancer mouse model.

IMPLICATIONS FOR PATIENT CARE: This ^{68}Ga -/ ^{177}Lu -radiolabeled $\alpha_v\beta_6$ -targeting theranostic pair holds significant promise for patients with locally advanced or metastatic pancreas cancer or other $\alpha_v\beta_6$ -positive solid tumors.

REFERENCES

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018; 68:7–30.
- Thomas JK, Kim MS, Balakrishnan L, et al. Pancreatic cancer database: an integrative resource for pancreatic cancer. *Cancer Biol Ther*. 2014;15:963–967.
- Berghoff AS, Kovanda AK, Melchardt T, et al. $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_6$ integrins in brain metastases of lung cancer. *Clin Exp Metastasis*. 2014;31:841–851.
- Allen MD, Marshall JF, Jones JL. $\alpha_v\beta_6$ expression in myoepithelial cells: a novel marker for predicting DCIS progression with therapeutic potential. *Cancer Res*. 2014;74:5942–5947.
- Yang GY, Guo S, Dong CY, et al. Integrin $\alpha_v\beta_6$ sustains and promotes tumor invasive growth in colon cancer progression. *World J Gastroenterol*. 2015;21:7457–7467.
- Niu J, Li Z. The roles of integrin $\alpha_v\beta_6$ in cancer. *Cancer Lett*. 2017;403:128–137.
- Peng C, Zou X, Xia W, et al. Integrin $\alpha_v\beta_6$ plays a bi-directional regulation role between colon cancer cells and cancer-associated fibroblasts. *Biosci Rep*. 2018;38: BSR20180243.
- Cantor DI, Cheruku HR, Nice EC, Baker MS. Integrin $\alpha_v\beta_6$ sets the stage for colorectal cancer metastasis. *Cancer Metastasis Rev*. 2015;34:715–734.
- Ahmed N, Pansino F, Clyde R, et al. Overexpression of $\alpha_v\beta_6$ integrin in serous epithelial ovarian cancer regulates extracellular matrix degradation via the plasminogen activation cascade. *Carcinogenesis*. 2002;23:237–244.
- Kawashima A, Tsugawa S, Boku A, et al. Expression of α_v integrin family in gastric carcinomas: increased $\alpha_v\beta_6$ is associated with lymph node metastasis. *Pathol Res Pract*. 2003;199:57–64.
- Reader CS, Vallath S, Steele CW, et al. The integrin $\alpha_v\beta_6$ drives pancreatic cancer through diverse mechanisms and represents an effective target for therapy. *J Pathol*. 2019;249:332–342.
- Herrmann K, Schwaiger M, Lewis JS, et al. Radiotheranostics: a roadmap for future development. *Lancet Oncol*. 2020;21:e146–e156.
- Jadvar H, Chen X, Cai W, Mahmood U. Radiotheranostics in cancer diagnosis and management. *Radiology*. 2018;286:388–400.
- Maqsood MH, Tameez Ud Din A, Khan AH. Neuroendocrine tumor therapy with lutetium-177: a literature review. *Cureus*. 2019;11:e3986.
- Sun M, Niaz MO, Nelson A, Skafida M, Niaz MJ. Review of ^{177}Lu -PSMA-617 in patients with metastatic castration-resistant prostate cancer. *Cureus*. 2020;12:e8921.
- Flechsigs P, Lindner T, Loktev A, et al. PET/CT imaging of NSCLC with a $\alpha_v\beta_6$ integrin-targeting peptide. *Mol Imaging Biol*. 2019;21:973–983.
- Hausner SH, Bold RJ, Cheuy LY, Chew HK, Daly ME, Davis RA. Preclinical development and first-in-human imaging of the integrin $\alpha_v\beta_6$ with [^{18}F] $\alpha_v\beta_6$ -binding peptide in metastatic carcinoma. *Clin Cancer Res*. 2019;25:1206–1215.
- Nakamoto R, Ferri V, Duan H, et al. Pilot-phase PET/CT study targeting integrin $\alpha_v\beta_6$ in pancreatic cancer patients using the cystine-knot peptide-based ^{18}F -FP-R01-MG-F2. *Eur J Nucl Med Mol Imaging*. 2022;50:184–193.
- Quigley NG, Steiger K, Hoberück S, et al. PET/CT imaging of head-and-neck and pancreatic cancer in humans by targeting the “Cancer Integrin” $\alpha_v\beta_6$ with Ga-68-Trivehexin. *Eur J Nucl Med Mol Imaging*. 2022;49:1136–1147.
- Lukey PT, Coello C, Gunn R, et al. Clinical quantification of the integrin $\alpha_v\beta_6$ by [^{18}F]FB-A20FMDV2 positron emission tomography in healthy and fibrotic human lung (PETAL Study). *Eur J Nucl Med Mol Imaging*. 2020;47:967–979.
- Hausner SH, Bauer N, Hu LY, Knight LM, Sutcliffe JL. The effect of bi-terminal PEGylation of an integrin $\alpha_v\beta_6$ -targeted ^{18}F peptide on pharmacokinetics and tumor uptake. *J Nucl Med*. 2015;56:784–790.
- Hausner SH, Abbey CK, Bold RJ, et al. Targeted in vivo imaging of integrin $\alpha_v\beta_6$ with an improved radiotracer and its relevance in a pancreatic tumor model. *Cancer Res*. 2009;69:5843–5850.
- Hausner SH, Bauer N, Davis RA, Ganguly T, Tang SYC, Sutcliffe JL. The effects of an albumin binding moiety on the targeting and pharmacokinetics of an integrin $\alpha_v\beta_6$ -selective peptide labeled with aluminum [^{18}F]fluoride. *Mol Imaging Biol*. 2020;22:1543–1552.
- Hausner SH, DiCara D, Marik J, Marshall JF, Sutcliffe JL. Use of a peptide derived from foot-and-mouth disease virus for the noninvasive imaging of human cancer: generation and evaluation of 4-[^{18}F]fluorobenzoyl A20FMDV2 for in vivo imaging of integrin $\alpha_v\beta_6$ expression with positron emission tomography. *Cancer Res*. 2007; 67:7833–7840.
- Hausner SH, Bauer N, Sutcliffe JL. In vitro and in vivo evaluation of the effects of aluminum [^{18}F]fluoride radiolabeling on an integrin $\alpha_v\beta_6$ -specific peptide. *Nucl Med Biol*. 2014;41:43–50.
- Ganguly T, Bauer N, Davis RA, Hausner SH, Tang SY, Sutcliffe JL. Evaluation of copper-64-labeled $\alpha_v\beta_6$ -targeting peptides: addition of an albumin binding moiety to improve pharmacokinetics. *Mol Pharm*. 2021;18:4437–4447.
- Bodei L, Herrmann K, Schöder H, Scott AM, Lewis JS. Radiotheranostics in oncology: current challenges and emerging opportunities. *Nat Rev Clin Oncol*. 2022;19:534–550.
- Rangger C, Haubner R. Radiolabelled peptides for positron emission tomography and endoradiotherapy in oncology. *Pharmaceuticals (Basel)*. 2020;13:22.
- Bandyopadhyay A, Raghavan S. Defining the role of integrin $\alpha_v\beta_6$ in cancer. *Curr Drug Targets*. 2009;10:645–652.
- Steiger K, Quigley NG, Groll T, et al. There is a world beyond $\alpha_v\beta_3$ -integrin: multimeric ligands for imaging of the integrin subtypes $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_v\beta_3$, and $\alpha_5\beta_1$ by positron emission tomography. *EJNMMI Res*. 2021;11:106.
- Rousseau E, Lau J, Zhang Z, et al. Effects of adding an albumin binder chain on [^{177}Lu]Lu-DOTATATE. *Nucl Med Biol*. 2018;66:10–17.
- Lau J, Jacobson O, Niu G, Lin KS. Bench to bedside: albumin binders for improved cancer radioligand therapies. *Bioconjug Chem*. 2019;30:487–502.
- Brandt M, Cardinale J, Giammei C, et al. Mini-review: targeted radiopharmaceuticals incorporating reversible, low molecular weight albumin binders. *Nucl Med Biol*. 2019;70:46–52.
- Tummers WS, Farina-Sarasqueta A, Boonstra MC, et al. Selection of optimal molecular targets for tumor-specific imaging in pancreatic ductal adenocarcinoma. *Oncotarget*. 2017;8:56816–56828.
- Huynh TT, Sreekumar S, Mpoy C, Rogers BE. Therapeutic efficacy of ^{177}Lu -labeled A20FMDV2 peptides targeting $\alpha_v\beta_6$. *Pharmaceuticals (Basel)*. 2022;15:229.
- Feng X, Wang Y, Lu D, et al. Clinical Translation of a ^{68}Ga -labeled integrin $\alpha_v\beta_6$ -targeting cyclic radiotracer for PET imaging of pancreatic cancer. *J Nucl Med*. 2020;61:1461–1467.
- de Jong M, Breeman WA, Bernard BF, et al. [^{177}Lu -DOTA₀Tyr₃] octreotate for somatostatin receptor-targeted radionuclide therapy. *Int J Cancer*. 2001;92:628–633.
- Notni J, Reich D, Maltsev OV, et al. In vivo PET imaging of the cancer integrin $\alpha_v\beta_6$ using ^{68}Ga -labeled cyclic RGD nonapeptides. *J Nucl Med*. 2017;58:671–677.
- Farkas R, Siwowska K, Ametamey SM, Schibli R, van der Meulen NP, Muller C. ^{64}Cu - and ^{68}Ga -based PET imaging of folate receptor-positive tumors: development and evaluation of an albumin-binding NODAGA-folate. *Mol Pharm*. 2016; 13:1979–1987.
- Ling X, Latoche JD, Choy CJ, et al. Preclinical dosimetry, imaging, and targeted radionuclide therapy studies of Lu-177-labeled albumin-binding, PSMA-targeted CTT1403. *Mol Imaging Biol*. 2020;22:274–284.