Fibroblast activation protein (FAP) has become an attractive target for diagnosis and therapy, and a series of FAP inhibitor (FAPI)-based radiotracers has been developed and had excellent performance for diagnosis outcomes in clinical applications. Yet, their fast clearance and insufficient tumor retention have hampered their further clinical application in cancer treatment. In this study, we developed 2 albumin binder-conjugated FAPI radiopharmaceuticals, TEFAPI-06 and TEFAPI-07. They were derived from FAPI-04 and were optimized by conjugating 2 types of well-studied albumin binders, 4-(p-iodophenyl)butyric acid moiety (TEFAPI-06) and truncated Evans blue moiety (TEFAPI-07), to try to overcome the above limitations at the expense of prolonging the blood circulation. Methods: TEFAPI-06 and TEFAPI-07 were synthesized and labeled with $^{68}$Ga, $^{89}$Y, and $^{177}$Lu successfully. A series of cell assays was performed to identify the binding affinity and FAP specificity in vitro. PET imaging, SPECT imaging, and biodistribution studies were performed to evaluate the pharmacokinetics in pancreatic cancer patient–derived xenograft (PDX) animal models. The cancer treatment efficacy of $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 were evaluated in pancreatic cancer PDX-bearing mice. Results: The binding affinities (dissociation constants) to FAP of $^{68}$Ga-TEFAPI-06 and $^{68}$Ga-TEFAPI-07 were $10.16 \pm 2.56$ nM and $7.81 \pm 2.28$ nM, respectively, which were comparable with that of $^{68}$Ga-FAPI-04. Comparative PET imaging of HT-1080-FAP and HT-1080 tumor–bearing mice and a blocking study showed the FAP-targeting ability in vivo of these 2 tracers. Compared with $^{177}$Lu-FAPI-04, PET imaging, SPECT imaging, and biodistribution studies of TEFAPI-06 and TEFAPI-07 demonstrated their remarkably enhanced tumor accumulation and retention, respectively. Notable tumor growth inhibition by $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 were observed, whereas the control group and the group treated by $^{177}$Lu-FAPI-04 showed a slight therapeutic effect. Conclusion: Two albumin binder-conjugated FAPI radiopharmaceuticals have been developed and evaluated in vitro and in vivo. Significantly improved tumor uptake and retention were observed, compared with the original FAPI tracer. Both $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 showed remarkable inhibition of PDX tumors, whereas the side effects were almost negligible, demonstrating that these radiopharmaceuticals are promising for further clinical translational studies.

**Key Words:** albumin binder; FAP inhibitor; radionuclide therapy

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molecules would improve the FAP-targeted radiotherapy efficacy at the expense of increased retention in blood.

In this study, 2 albumin binder–FAPI conjugates, TEFAPI-06 and TEFAPI-07, were developed by logistic fabrication of 3 functional components: a quinoline-based FAPI originating from FAPI-04, a chelator (i.e., DOTA group) that allows radionuclide labeling for imaging (68Ga or 86Y) or therapy (177Lu), and an albumin binder: 4-(p-iodophenyl) butyric acid moiety (TEFAPI-06) or truncated Evans blue moiety (TEFAPI-07). The purpose of the study was to evaluate whether the modification improves tumor retention in vivo and which albumin binder better matches FAPI molecules. A series of detailed experiments and comparisons, including cell binding assays, a PET imaging study, a biodistribution study, and a radiotherapy study, was performed. The results demonstrated these 2 albumin binder–conjugated FAPI radio tracers to have high FAP binding affinity and specificity, enhanced tumor retention, and improved radiotherapy efficacy.

MATERIALS AND METHODS

Ligands and Radionuclides
The synthesis route and chemical characterization of TEFAPI-06 and TEFAPI-07 are described in Supplemental Figures 1–20 (supplementary materials are available at http://jnm.snijnjongournals.org). 68Ga-Cl3 was eluted with a solution of 0.6 M hydrochloride from a 68Ge–68Ga generator (iThemba LABS). 86Y-Cl3 was produced with a 14.6-MeV cyclotron; the target design follows our previous report (27), and the purification procedure follows the previous protocol (28). 177Lu-Cl3 in a solution of 0.1 M hydrochloride was purchased from ITG.

Radiolabeling and Stability In Vitro
The radiolabeling of 68Ga, 86Y, and 177Lu was performed by incubation with 50 nmol of precursor at pH 4.5–5.0 at 90°C for 10 min. The product was purified by C18 column extraction, and the radiochemical purity was determined by high-performance liquid chromatography equipped with a radioactivity detector. The stability of 177Lu-TEFAPI-06 and 177Lu-TEFAPI-07 in saline and human serum was monitored from 2 to 168 h using radio–high-performance liquid chromatography (13). More details about the radiochemistry, the quality control testing, and the stability assay can be found in the supplemental materials (section 3).

Cell Culture and Assay
The human fibrosarcoma cell line (HT-1080), and the HT-1080 cell line transfected with human FAP gene (HT-1080-FAP, from WuXi AppTec), were cultivated in Eagle minimum essential medium containing 10% fetal bovine serum, 1% antibiotic–antimycotic, and a 4 μg/mL concentration of blasticidin S at 37°C under conditions of 5% carbon dioxide. For competition assays, HT-1080-FAP cells were seeded in 6-well plates and cultivated until they reached about 1.2 × 105 cells per well. The cells were incubated simultaneously with unla beled FAPI-04, TEFAPI-06, or TEFAPI-07 (10^-5 to 10^-9 M) with 68Ga-FAPI-04 in 1 mL of fresh medium without fetal bovine serum for 1 h. The medium was removed, and the cells were washed twice with phosphate-buffered saline (PBS). Subsequently, the cells were lysed with 0.5 mL of 1 M NaOH and washed with 0.5 mL of PBS twice, and the NaOH (0.5 mL) and PBS (0.5 mL × 2) were collected to determine the uptake counts. For saturation binding assays, HT-1080-FAP and HT-1080 cells were seeded in 24-well plates and cultivated until they reached about 2 × 105 cells per well. 68Ga-FAPI-04, 68Ga-TEFAPI-06, or 68Ga-TEFAPI-07 was diluted to a concentration 0.01–200 nM in fresh medium without fetal bovine serum. The cells were incubated in the above solution for 1 h and then washed twice with PBS. The lysed cells and the PBS for washing were collected to determine the counts.

Tumor-Bearing Animal Models
All animal care and experimental procedures were performed by following the animal protocols (CCME-LiuZB-2) approved by the ethics committee of Peking University. The mice were from the Beijing Vital River Laboratory Animal Technology Co., Ltd. For cell-line-derived xenograft models, 5 × 106 HT-1080-FAP or HT-1080 cells were subcutaneously inoculated into the right shoulder of 6-wk-old female nu/nu mice. To establish the patient-derived xenograft (PDX) model, tumor specimens were obtained from patients who underwent presurgical 68Ga-FAPI-04 PET/CT imaging to confirm that the tumor was FAP-positive. After surgical resection, the tumor specimens were immediately placed in ready-to-use fresh tissue preservation solution (TM2701-100) and transported under refrigerated conditions within 2 h. The research protocol was approved by the Institutional Ethics Committee of Peking Union Medical College Hospital (JS-2628). Six-week-old female nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice were used to establish the PDX models. After being removed from the preservation solution, the tumor specimens were immediately immersed in sterile PBS solution and minced with scissors, and the fragments were then implanted subcutaneously into the left and right shoulders of the mice, which were anesthetized with isoflurane in advance. Engraftment efficiency was determined by 68Ga-FAPI-04 PET/CT imaging (Supplemental Fig. 21A). Immunohistochemical staining (Supplemental Fig. 21B) demonstrated that the pancreatic cancer PDX model used in this study was indeed FAP-overexpressed.

Small-Animal PET Imaging
All PET scans were performed on a Mediso nanoScan PET 122S small-animal PET/CT imaging system. For the 60-min dynamic PET

FIGURE 1. (A) Chemical structures of TEFAPI-06 and TEFAPI-07. (B) Competition assays of TEFAPI-06 and TEFAPI-07. (C) Saturation binding assays of radiolabeled TEFAPI-06 and TEFAPI-07. (D) Cellular uptake assays of 68Ga-TEFAPI-06 and 68Ga-TEFAPI-07 in HT-1080-FAP and HT-1080 cells. IC50 = half-maximal inhibitory concentration.
scan, 29.6–37.0 MBq of 68Ga-TEFAPI-06 or 68Ga-TEFAPI-07 were given to healthy NOD/SCID mice through tail-vein injection. The static PET imaging was performed on mice bearing pancreatic PDX tumors, HT-1080-FAP tumors, and HT-1080 tumors at the indicated time points after intravenous injection of 7.4–11.1 MBq of 86Y-FAPI-04, 86Y-TEFAPI-06, or 86Y-TEFAPI-07.

Small-Animal SPECT Imaging
SPECT scans were performed on a Mediso nanoSPECT/CT imaging system. 177Lu-TEFAPI-06 or 177Lu-TEFAPI-07 SPECT imaging was performed on pancreatic cancer PDX–bearing mice at the indicated time points after intravenous injection of 37 MBq of 177Lu-TEFAPI-06 or 177Lu-TEFAPI-07, respectively.

Biodistribution Study
PDX-bearing mice were injected with 925.0 kBq of 177Lu-TEFAPI-06 or 177Lu-TEFAPI-07 for an ex vivo biodistribution study. The mice were killed at 24 h and 96 h after injection, the counts of the different organs were measured with a γ-counter, and the data were normalized to percentage injected dose (%ID)/g using 1% of total counts.

Radiotherapy Study
68Ga-FAPI-04 PET imaging was performed to evaluate the tumor volume, and the mice were treated when their average tumor volume reached 35 mm³. The PDX-bearing mice (6 groups, 7–9 mice per group) were treated by saline, 3.7 MBq of 177Lu-FAPI-04, 1.85 MBq of 177Lu-TEFAPI-06, 3.7 MBq of 177Lu-TEFAPI-06, 1.85 MBq of 177Lu-TEFAPI-07, or 1.85 MBq of 177Lu-TEFAPI-07. Tumor volume and body weight were monitored every 2 or 3 d, and the animals were euthanized when the tumor volume exceeded 1,000 mm³. Histopathologic staining was performed with an antihuman FAP monoclonal antibody (ab207178; Abcam), and hematoxylin and eosin staining was performed as previously described (29).

RESULTS
Radiochemistry and Stability In Vitro
The radiolabeling yield of TEFAPI-06 and TEFAPI-07 (Fig. 1A) was over 90%, and the radiochemical purity was over 99% (n > 20). The specific activity of 68Ga-FAPI-04, 68Ga-TEFAPI-06, and 68Ga-TEFAPI-07 was 5.2–6.7 GBq/μmol. The specific activity of 86Y-FAPI-06 and 86Y-TEFAPI-07 was 2.2–3.4 GBq/μmol. The specific activity of 177Lu-FAPI-04, 177Lu-TEFAPI-06, and 177Lu-TEFAPI-07 was 2.9–4.4 GBq/μmol. Stability of 177Lu-TEFAPI-06 and 177Lu-TEFAPI-07 in saline and human serum was analyzed using radio–high-performance liquid chromatography, as shown in Supplemental Figure 22. The radiochemistry purity of both 177Lu-TEFAPI-06 and 177Lu-TEFAPI-07 was still over 90% after incubation in saline and human serum for 7 d.

Binding Assay
As shown in Figure 1B and Supplemental Figure 23A, cellular uptake of 68Ga-FAPI-04 can be significantly inhibited by treatment with cold TEFAPI-06 and TEFAPI-07. The ligand concentrations required for 50% inhibition (half-maximal inhibitory concentration) of TEFAPI-06 and TEFAPI-07 are 12.24 ± 0.65 nM and 17.02 ± 1.43 nM, respectively. The dissociation constants of 68Ga-TEFAPI-06 and 68Ga-TEFAPI-07 were 10.16 ±
2.56 nM and 7.81 ± 2.28 nM (Fig. 1C), respectively, which are comparable to that of 68Ga-FAPI-04 (1.91 ± 0.62 nM, Supplemental Fig. 23B). As shown in Figure 1D, both 68Ga-TEFAPI-06 and 68Ga-TEFAPI-07 exhibited almost negligible uptake in HT-1080 cells but had significant uptake in HT-1080-FAP cells. We also performed the binding assays in 0.05% human serum albumin (HSA), with the following results. The half-maximal inhibitory concentrations of TEFAPI-06 and TEFAPI-07 were 11.39 ± 1.15 nM and 27.68 ± 5.00 nM, respectively, in the presence of albumin. The dissociation constants of TEFAPI-06 and TEFAPI-07 were 4.37 ± 0.81 nM and 19.12 ± 5.54, respectively, in the absence of albumin. The half-maximal inhibitory concentration and dissociation constant of TEFAPI-07 were slightly impacted by the presence of albumin, which may be the reason why blood clearance was faster than for TEFAPI-06.

Small-Animal PET Imaging

To evaluate the in vivo pharmacokinetics of these 2 radiotracers, dynamic PET imaging of 68Ga-TEFAPI-06 and 68Ga-TEFAPI-07 was performed on healthy NOD-SCID mice. The signal in heart peaked rapidly at about 2 min after injection and then declined gradually. For 68Ga-TEFAPI-06, the signal decreased by 35.70% ± 4.74% from 10 to 60 min after injection, a decrease that was greater than that of 68Ga-TEFAPI-07 (23.15% ± 2.16%), whereas from 60 to 240 min after injection, the signal decreased by 31.80% ± 1.15% and 40.56% ± 5.25% for 68Ga-TEFAPI-06 and 68Ga-TEFAPI-07, respectively, resulting in a similar proportion in the decrease of these 2 radiotracers from 10 to 240 min at 56.18% ± 2.50% and 54.28% ± 4.98% for 68Ga-TEFAPI-06 and 68Ga-TEFAPI-07, respectively. As shown in Figure 2, for both 68Ga-TEFAPI-06 and 68Ga-TEFAPI-07, most of the radioactivity was retained in the blood circulation during the monitoring period, and the uptake in other organs, such as the liver, spleen, and kidney, was lower than in the heart or main blood vessels.

To identify the tumor-targeting ability and monitor the in vivo pharmacokinetics quantitatively over a longer period, TEFAPI-06, TEFAPI-07, and FAPI-04 were labeled with the radionuclide 86Y, which has a half-life of 14.7 h, and the PET imaging was performed using pancreatic cancer PDX-bearing mice. As shown in Figure 3 and Supplemental Figure 24, for both 86Y-TEFAPI-06 and 86Y-TEFAPI-07, tumor was completely visible at 2 h after injection. The tumor SUV mean of 86Y-TEFAPI-06 peaked at 0.73 at 18 h after injection, and that of 86Y-TEFAPI-07 peaked at 0.81 at 8 h after injection. Then, the tumor SUV mean decreased slowly but still remained high until 36 h after injection, with a value of 0.602 and 0.606 for 86Y-TEFAPI-06 and 86Y-TEFAPI-07, respectively. However, the tumor SUV mean of 86Y-FAPI-04 peaked at 0.35 at 0.2 h after injection and then decreased rapidly, and the areas under the curve for TEFAPI-07 and TEFAPI-06 were 35.5-fold and 37.9-fold that for FAPI-04.

To further confirm the FAP specificity in vivo of these 2 radiotracers, PET imaging of HT-1080-FAP and HT-1080 tumor–bearing mice was performed. As shown in Figure 4, Supplemental Figure 25, and Supplemental Figure 26, the uptake of 86Y-TEFAPI-06 and 86Y-TEFAPI-07 in HT-1080-FAP tumors was consistently 2- to 6-fold higher than that in HT-1080 tumors. A blocking study was also performed, as shown in Supplemental Figure 27; tumor uptake decreased at 12 h and 24 h after treatment with cold TEFAPI-06 and TEFAPI-07.

Small-Animal SPECT Imaging

To further characterize these 2 molecules, SPECT imaging was conducted on PDX tumor models for a longer time. As shown in Supplemental Figure 28, high tumor–to–nontargeted-tissue signal ratios were
observed for both $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 until 144 h after injection. The blood circulation properties of these 2 molecules were similar to those found in the previous PET study.

**Biodistribution Study**

To further evaluate the metabolic properties in vivo, biodistribution studies using the pancreatic cancer PDX–bearing mice were performed. As shown in Figure 5 and Table 1, the tumor uptake of $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 were, respectively, 8.68 ± 0.73 %ID/g and 7.87 ± 2.08 %ID/g at 24 h after injection, and the tumor-to-liver ratios were 2.91 and 2.45, respectively. The tumor uptake remained high until 96 h after injection, at 7.33 ± 2.28 %ID/g and 7.57 ± 2.68 %ID/g for $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07, respectively, and the tumor-to-liver ratios increased to 4.21 and 3.28, respectively. As is consistent with the results of PET and SPECT imaging, the kidney uptake of $^{177}$Lu-TEFAPI-07 remained high at both 24 and 96 h after injection, at 8.67 ± 2.30 and 10.16 ± 3.28 %ID/g, respectively, whereas the kidney uptake of $^{177}$Lu-TEFAPI-06 was much lower, at 2.66 ± 0.54 %ID/g, at 96 h after injection. By comparison, the blood clearance of $^{177}$Lu-TEFAPI-06 (24 h, 13.32 ± 1.33 %ID/g; 96 h, 2.25 ± 0.68 %ID/g) was slower than that of $^{177}$Lu-TEFAPI-07 (24 h, 5.64 ± 1.50 %ID/g; 96 h, 0.51 ± 0.24 %ID/g).

**Radiotherapy Study**

To make the assessment of therapeutic efficacy more relevant to the clinical setting, pancreatic cancer PDX–bearing mice were used for the indicated radiotherapy study (Fig. 6A). In a comparison to the group treated by saline or 3.7 MBq of $^{177}$Lu-FAPI-04, the groups treated with 1.85 MBq or 3.7 MBq of $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07, respectively, showed remarkable suppression of tumor growth (Fig. 6B). No statistical difference in treatment efficacy was observed between $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07. This result corroborates the PET imaging and biodistribution studies, as they shown equally high uptake in the tumors. Except for the control group

| Organ          | $^{177}$Lu-TEFAPI-06 | $^{177}$Lu-TEFAPI-07 |
|----------------|---------------------|---------------------|
|                | 24 h Mean | SD       | 96 h Mean | SD       | 24 h Mean | SD       | 96 h Mean | SD       |
| Blood          | 12.32 | 1.33   | 2.25 | 0.68   | 5.64 | 1.49   | 0.51 | 0.24   |
| Pancreas       | 2.01  | 0.26   | 0.75 | 0.42   | 2.16 | 0.69   | 0.53 | 0.22   |
| Spleen         | 3.00  | 0.66   | 3.66 | 1.22   | 2.69 | 0.87   | 1.88 | 0.34   |
| Small intestine| 2.55  | 0.64   | 0.61 | 0.28   | 1.19 | 0.26   | 0.16 | 0.09   |
| Large intestine| 1.66  | 0.27   | 0.53 | 0.35   | 1.15 | 0.33   | 0.10 | 0.15   |
| Liver          | 2.98  | 0.64   | 1.74 | 0.57   | 3.21 | 0.81   | 2.31 | 0.17   |
| Kidney         | 3.20  | 0.84   | 2.66 | 0.54   | 8.67 | 2.30   | 10.16| 3.28   |
| Stomach        | 1.75  | 0.17   | 0.54 | 0.21   | 1.20 | 0.45   | 0.29 | 0.14   |
| Fat            | 1.86  | 0.90   | 1.55 | 1.17   | 1.82 | 1.18   | 0.48 | 0.25   |
| Muscle         | 1.09  | 0.18   | 0.45 | 0.05   | 1.00 | 0.37   | 0.28 | 0.16   |
| Bone           | 1.32  | 0.30   | 0.35 | 0.20   | 1.71 | 0.63   | 0.18 | 0.28   |
| Lung           | 4.33  | 1.44   | 1.95 | 0.70   | 2.58 | 0.75   | 0.73 | 0.28   |
| Heart          | 3.70  | 1.59   | 1.51 | 0.42   | 2.53 | 1.19   | 0.62 | 0.17   |
| Brain          | 0.37  | 0.08   | 0.09 | 0.03   | 0.23 | 0.09   | -0.01| 0.04   |
| Tumor          | 8.68  | 0.73   | 7.33 | 2.28   | 7.87 | 2.08   | 7.57 | 2.68   |

Data are %ID/g.
treated with only saline, transient weight loss was observed for all treatment groups, including $^{177}$Lu-FAPI-04, but then returned to the healthy level 7 d after the initial treatment. Hematoxylin and eosin staining of the main organs revealed that side effects from $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 treatment were almost negligible (Supplemental Fig. 29).

DISCUSSION

The purpose of this study was to develop FAPI-based radiopharmaceuticals that are more effective than the existing candidates for FAP-targeted radiotherapy. Two different albumin binders, 4-(p-iodophenyl)butyric acid and truncated Evans blue moieties, were chosen to be attached with FAPI-04. The resulting TEFAPI-06 and TEFAPI-07 were synthesized and radiolabeled with $^{68}$Ga, $^{86}$Y, and $^{177}$Lu. The radiolabeled TEFAPIs exhibited good stability in saline and human serum and high FAP binding affinity in vitro. In addition, SPECT imaging and biodistribution studies of $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 showed that tumor uptake was still notable even at 6 d after the injection. Meanwhile, almost no radioactive signal could be detected for $^{177}$Lu-FAPI-04 at 24 h after injection. We also wondered whether further modifications of the structure may lengthen the blood circulation and, thus, increase the tumor accumulation. However, it can be challenging to balance treatment efficacy against potential side effects from blood circulation.

With regard to the clearance pathway, there was no significant difference in uptake between $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 in tumor and main organs, except for the kidney. For TEFAPI-07, both the PET and the SPECT imaging results showed significantly higher kidney uptake than that of TEFAPI-06. Of note, imaging indicated that there was no obvious clearance of TEFAPI-07 from the kidneys over time, a finding that was consistent with the results of the biodistribution study. Besides, because both TEFAPI-06 and $^{177}$Lu-TEFAPI-07 have relatively longer blood circulation than the classic radiopharmaceuticals, the side effects may not be negligible. Therefore, a comprehensive hematoxylin- and eosin-staining study of major organs was performed, and no tissue damage was observed (Supplemental Fig. 29).

As reported in previous studies, the radiolabeled albumin binder may target the tumor because of enhanced permeability and retention of albumin (30,31). Thus, we were curious about whether the enhanced tumor uptake and retention of $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 are FAP-dependent. The PET imaging results of FAP-positive (HT-1080-FAP) and FAP-negative (HT-1080) tumor-bearing mice showed much higher uptake by FAP-positive tumors than by FAP-negative tumors, demonstrating that the higher tumor uptake was dependent on the FAP-targeting ability in vivo. For the blocking study, the tumor uptake of $^{68}$Ga-FAPI-04 decreased significantly when the mice were treated with cold TEFAPI-06 and TEFAPI-07 until 24 h after injection—a finding that supported the possibility that the prolonged tumor retention of these 2 radiotracers was also dependent mainly on their excellent FAP-targeting ability in vivo.

CONCLUSION

In this study, 2 albumin binder–conjugated FAPIs, denoted as TEFAPI-06 and TEFAPI-07, were developed to optimize the pharmacokinetics of current FAPI radiopharmaceuticals for cancer radiotherapy. Compared with $^{177}$Lu-FAPI-04, both $^{177}$Lu-TEFAPI-06 and $^{177}$Lu-TEFAPI-07 showed enhanced uptake and retention in tumors. The tumor accumulations were highly FAP-selective and resulted in remarkable inhibition of PDX tumor growth, with negligible side effects. Their promising pharmacokinetics warrant further investigations toward clinical translation for the treatment of FAP-positive cancers.

DISCLOSURE

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