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Preclinical evaluation of peptide-based radiotracers for integrin αvβ6-positive pancreatic carcinoma

Präklinische Evaluation von Peptid-basierten Radiotracern für Integrin αvβ6-positive Pankreaskarzinome

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Key words
Integrins, αvβ6, peptides, pancreatic cancer, PET imaging, RGD motif

Schlüsselwörter
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ABSTRACT

Introduction Integrin αvβ6 shows a high expression rate in several cancer entities. As it is absent in most healthy adult tissues, it represents a promising target for tumor targeting with peptidic radiotracers. This study was performed to pave the way of the recently published αvβ6-binding peptide SFLAP3 for the clinical application in patients with pancreatic cancer.

Methods The expression of integrin αvβ6 on several pancreatic cancer cell lines was assessed using flow cytometry and cell binding assays. The affinity was determined in competition binding assays followed by internalization and efflux studies. To increase the affinity, the binding sequence was modified and trimerization of the SFLAP3 peptide was achieved by oxime ligation. PET and biodistribution assays were conducted in Capan-2 tumor bearing mice. Finally, a first pancreatic tumor patient was examined with 68Ga-DOTA-SFLAP3.

Results Flow cytometric analysis and in vitro cell binding revealed high expression of integrin αvβ6 on most pancreatic tumor cell lines. Modification of SFLAP3 led to compounds with improved in vitro binding properties. Unfortunately, these superior properties could not be transferred into improved pharmacokinetics. Consequently, the first pancreatic tumor patient was examined with 68Ga-DOTA-SFLAP3. The PET revealed specific accumulation (with SUV_{max} values in the metastases ranging from 5 to 10) and a long retention in the tumor.

Conclusion SFLAP3 showed high affinity to integrin αvβ6 on pancreatic cancer cell lines. The in vitro performance could be confirmed in tumor bearing mice and by PET imaging. These data suggest that DOTA-SFLAP3 is a promising tracer for targeting αvβ6-expressing pancreatic tumors.
Introduction

Pancreatic cancer was the third leading cause of cancer-related death in the USA in 2017. With 53,000 estimated new cases, 43,000 estimated deaths and only 8 percent 5-year survival rate this tumor entity has the worst prognosis among the widespread cancers [1]. The 5-year survival rate after resection of the primary tumor is 18% for patients that receive adjuvant therapy [2]. More than 70% of the surviving patients develop lymph node and distant metastases with palliative treatment as the only option [3]. Therefore, improved diagnosis, staging and therapy modalities are required.

The transmembrane receptor integrin αvβ6 is upregulated in many cancers, including squamous cell carcinoma, pancreatic and ovarian cancer, of which up to 80–100% are integrin αvβ6 positive [4, 5]. The protein is composed of one of the 18 α and one of the eight β subunits, which build up the 24 functional integrin heterodimers [6]. Integrin αvβ6 seems to be exclusively expressed in epithelial tissue mainly during embryonic development [7]. In healthy adults the expression of integrin αvβ6 is downregulated, except for wound healing processes [8]. In malignant lesions integrin αvβ6 plays an important role for tumor cell differentiation, migration and proliferation [9]. One of its main effects in cancer progression and metastasis is based on its interaction with transforming growth factor-β (TGF-β). TGF-β is normally found in an inactive form bound to a latency associated peptide (LAP). Together with the latent transforming growth factor-β binding protein (LTBP) it builds the large latency complex (LLC). Binding of LAP to the integrin αvβ6 leads to the release of the active form of TGF-β [10]. Thus, the LAP binding sequence represents a promising structure for novel integrin αvβ6 targeting tracers. In pancreatic ductal adenocarcinoma the functional relationship between TGF-β1 activation and cell invasion was shown to depend on presence of integrin αvβ6 [11]. Also, in immunohistochemistry studies one to two thirds of cervical, gastric and colon cancers were integrin αvβ6 positive, which additionally correlated with poor prognosis [12–14]. The low expression rate in healthy tissue makes this protein a suitable target for non-invasive diagnostics or for peptide receptor radionuclide therapy (PRRT).

Currently, there are several integrin αvβ6 binding radiopharmaceuticals under investigation. When compared to other integrins, the targeting of αvβ6 is more specific due to the RGDXXXR recognition motif. In particular, pancreatic tumors show a high overexpression of this integrin [5]. The biological importance of integrin αvβ6 is also reflected by its role in TGF-β activation. TGF-β can play a dual role (positive and negative) in tumorigenesis and is therefore upregulated in a number of tumor entities [15]. For this reason, several working groups are currently searching for novel specific tracers. The most prominent ones are a cysteine knot peptide named S02, the cyclic nonapeptide Avebehexin and the twenty-mer peptide A20FMDV2. S02 was identified by yeast surface display. It showed a high affinity against integrin αvβ6 in the single digit nanomolar level [16]. Avebehexin, a TRAP conjugated αvβ6-binding radiotracer showed promising accumulations in tumor bearing mice and a high affinity with an IC50 of 0.26 nM [17]. The radiotracer A20FMDV2 is derived from the foot-and-mouth disease virus. Its high affinity against integrin αvβ6 with a Kd of 0.22 nM and the accumulation of the tracer into the tumor of xenograft mice led to a first in human trial of 18F-FB-A20FMDV2 with healthy patients showing no adverse effects [18, 19]. Still no clinical applications (in cancer patients) were performed up to now and there is an urgent need for better diagnostic and probably therapeutic tools of pancreatic cancer.

In previous studies, we identified by phage display a peptidic lead structure embedded in the sunflower trypsin inhibitor scaffold [20]. The binding loop of this structure has been modified by exchange of three amino acids to yield the binding sequence of SFLAP3 [21]. Both peptides have been applied in preclinical models of head and neck tumors and in patients with lung and head and neck cancer. In this paper we describe the use of the peptide SFLAP3.
in pancreatic adenocarcinoma as well as the structure-activity relationship of various modifications of the peptide.

**Materials and Methods**

**Peptide synthesis**

Peptides were synthesized using standard Fmoc/TBu solid phase peptide synthesis (SPPS) and an Applied Biosystem ABI 433 A Synthesizer. Rink amide aminomethyl polystyrene resin was used as solid phase. Amino acids were thereby used in 5-fold access. Cleavage of the resin and removal of the protection groups was done with 2.5 % TFA and 2.5 % trisopropylsilanilo-water. Disulfide bridge formation was performed by adding the same volume iodine (0.25 mg/ml) dissolved in acetic acid to dissolved peptide (1 mg/ml) in 80 % acetic acid. After evaporation of the solvents the peptides were purified on a HPLC (Waters XBridgeBEH130 PREP C18 column 5 µm, 19 × 150 mm). To obtain N-terminal DOTA conjugated peptides, peptides were incubated with 2 eq. of DOTA p-nitrophenyl-ester and 10 eq. DIPEA in NMP [20]. For the final oxime ligation of the trimer, the core peptide and the aminooxy-modified SFLAP3 was heated over night at pH 2–3 (further information in the supplementary). Labeling of peptides with iodine-125, lutetium-177 and was heated over night at pH 2–3 (further information in the supplementary).

**Cell lines**

All cell lines used were obtained from ATCC, cultured in RPMI 1640 medium (Gibco) supplemented with 10 % fetal calf serum (Gibco) and incubated at 37° C and 5 % CO2.

**In vitro binding assays**

For in vitro binding studies, 4 × 10^5 – 6 × 10^5 cells were seeded in 6 well plates and cultivated for 24 to 48 hours. At the start of the experiment the medium was replaced with 1 ml serum free medium with a definite amount of radioactive peptide per well (≈ 100 000 cpm from an approximately 30 GBq/µmol 177Lu-labeled peptide). For cell-binding assays the cells were incubated for 60 min at 37° C and afterwards washed 3 times with PBS (pH 7.4). Cells were lysed 2 times with 0.5 ml of a 0.3 M NaOH + 0.2 % SDS lysis-buffer. Competition assays were performed with 125I-labeled and unabeled (10^-6 to 10^-12 M) peptide which are coincubated for 60 min.

Cells were incubated for 10 to 240 min with 177Lu-labeled peptides in internalization assays. After the washing steps, the cells were incubated with 1 ml glycine – HCl (1 M, pH 2.2) buffer for 10 min to remove surface bound peptide. Prior to the lyses the cells were washed again. Values are given as percentage of applied dose per 10^6 cells. The efflux was tested with 177Lu-labeled peptides. After a 60 min incubation the radioactive medium was removed and 1 ml fresh serum-free medium was added. After additional incubation of 60, 120 or 240 min, cell bound activity was measured. Serum stability assays and flow cytometry were performed as described in the supplementary data.

**Animal studies**

All experiments were conducted in compliance with the German animal protection laws. 6 to 8 weeks old Balb c/c nude mice (Charles River Lab.) were inoculated with 5 × 10^6 Capan-2 cells in 100 µl Opti-MEM medium. After 4 to 6 weeks the tumor has grown 10 – 15 mm in diameter. For small animal PET imaging 68Ga-labeled peptides (approximately 25 GBq/µmol) were injected into the tail vein of an anesthetized (3–4 % isoflurane) animal. Images were acquired on an Inveon small animal PET scanner (Siemens) for 140 min as described [20, 21].

For biodistribution assays 177Lu-labeled peptides (approximately 1 GBq/µmol) were injected into the tail vein of tumor bearing mice. After different time points the animals were sacrificed. Peripheral blood, spleen, liver, intestine, kidney, heart, lung, brain, muscle, and tumor were removed and weighted. Radioactivity was measured in a gamma counter and expressed as injected dose per gram (%ID/g). The animals received comparable doses of radiotracer and radioactivity.

**PET/CT scans of a tumor patient**

Diagnostic imaging was conducted under the conditions of the declaration of Helsinki, § 37 and in accordance to the German Pharmaceuticals Law § 13 (2b). The patient signed a written informed consent which was approved by the institutional ethical review board of the University of Heidelberg. The patient was in final stage and the diagnostic a last resort in the setting of patient care and not part of prospective studies.

1 and 3 hours post administration of 304 MBq 68Ga-DOTA-SFLAP3 (approximately 43 GBq/µmol) PET/CT scans were performed on a Biograph mCT Flow™ PET/CT-Scanner (Siemens Medical Solution). The chosen parameters were a slice thickness of 5 mm, increment of 3–4 mm, soft tissue reconstruction kernel and care dose. Following the CT, a 3-D whole-body PET (matrix 200 × 200) was acquired in FlowMotion with 0.7 cm/min. The emission data were corrected for random, decay and scatter. The reconstruction was performed with an ordered subset expectation maximum (OSEM) algorithm with 2 iterations/21 subsets and Gauss-filtered to a transaxial resolution of 5 mm at full-width half-maximum (FWHM). Attenuation was performed with the low-dose non-enhanced CT data. The quantitative determination of standard uptake values (SUV) was accomplished using a region-of-interest technique with ellipsoidal regions.

**Results**

**Binding of SFLAP3 to pancreatic cancer cell lines**

SFLAP3 is an αvβ6 integrin binding ligand in which the LAP3 binding sequence GRGDLGRL is embedded in the sunflower trypsin inhibitor-1 (SFTI) scaffold (▶ Fig. 1).

Since the binding capacity of the peptide to cancer cells depends on the number of receptor molecules expressed at their surface, different cell lines were analyzed with regard to their possible use in further experiments. Uptake assays on BxPC-3, Capan-1, Capan-2 and Mia PaCa-2 cells showed that Capan-2 cells had the highest 125I-SFLAP3 binding value with 17 percent applied dose per 10^6 cells (AD/10^6) (▶ Fig. 2b). Flow cytometry analysis also showed large differences in the integrin αvβ6 expression. BxPC-3 cells had a moderate (73.3 %) while Capan-1 and Capan-2 had a high integrin αvβ6 expression (92.2 % and 99.0 %) (▶ Fig. 3).
Improving the binding ability based on variations of the binding sequence

As demonstrated in previous studies, linear peptides comprising the LAP3 binding sequence HGRGLRGL show high affinities towards integrin αvβ6. In addition, the binding affinity is further increased by extending the peptide with amino acids of the native protein [22]. Based on that finding, five different SFLAP3 variations were created by adding or removing the surrounding amino acids of the native LAP3 protein (Fig. 2a).

After radiolabelling with iodine-125, the binding affinity of the peptides 125I-SFLAP3-(1–5) was assessed using different pancreatic carcinoma cell lines. As shown in Fig. 2b, addition of a histidine
or deletion of the glycine preceding the RGDXXL motif resulted in decreased binding values, whereas the attachment of a lysine (125I-SFLAP3-3 hereinafter referred to as 125I-SFLAP3K) led to an increased cellular uptake. As iodine-labeled peptides often show enzymatic deiodination, resulting in rapid loss of intracellular radioactivity, a N-terminal DOTA conjugated variant was developed. Compared to the original 125I-SFLAP3 peptide, 177Lu-DOTA-SFLAP3 showed decreased cell binding with 9.5 %AD/10^6 after 60 min (▶ Fig. 4a).

Trimerization of SFLAP3 leads to an increased tracer uptake

In comparison to monomeric peptides, ligand multimerization often leads to an increased apparent affinity due to additive binding effects. The trimerized version of SFLAP3 was produced using a DOTA-(GSGSK)3 linker, resulting in a two-and-a-half-fold increase of cell binding as compared to 177Lu-DOTA-SFLAP3 (22.7 %AD/10^6; p < 0.001) (▶ Fig. 4a).

DOTA-SFLAP3K and DOTA-SFLAP3-trimer show increased target affinities as compared to DOTA-SFLAP3

As shown in competition assays, all SFLAP3 variants demonstrated target affinities in the lower nanomolar range with IC50 values of 6.2 nM for 125I-DOTA-SFLAP3 and 2.1 nM for 125I-DOTA-SFLAP3K. The affinity of 125I-DOTA-SFLAP3-trimer was even further increased to an IC50 of 0.76 nM (▶ Fig. 4b and Supplementary ▶ Table 1).

For all three peptides, internalization between 25 – 50 % were measured (▶ Fig. 4c). Similar internalization of the peptides was observed during the first two hours of incubation. However, slightly larger differences were detectable after 4 hours, where 177Lu-DOTA-SFLAP3 had the highest internalization.

Efflux experiments showed that 50 % of the 177Lu-DOTA-SFLAP3 and the 177Lu-DOTA-SFLAP3K activity are released into the culture medium after one hour. In contrast, the trimerized peptide showed significantly reduced efflux of approximately 20 % (p < 0.01) during 4 hours incubation following exposure of the tumor cells to the radiolabeled peptide (▶ Fig. 4d).

DOTA-SFLAP3K and DOTA-SFLAP3-trimer show lower proteolytic stability in human serum

The proteolytic stability of 177Lu-DOTA-SFLAP3 was determined for up to 20 hours incubation in human serum. Slight degradations of 177Lu-DOTA-SFLAP3K were detected by radio-HPLC analysis after 2 hours of incubation. Degradation of 177Lu-DOTA-SFLAP3-trimer was already detectable after 2 hours of incubation (▶ Fig. 5 and ▶ Supplementary Fig. 1). The tumor uptake and primary elmi-
natin of peptides in vivo occur for the most part within the first 2 hours. Thus, serum stability of both peptides should be sufficient for in vivo assays.

Small animal imaging of $^{68}$Ga-DOTA-SFLAP3K and $^{68}$Ga-DOTA-SFLAP3-trimer reveals no improvement as compared to the original ligand

Capan-2 xenografted balb/c nu/nu mice were injected with $^{68}$Ga-DOTA-SFLAP3 followed by small animal PET imaging. Tumor uptake was detectable over the entire course of the experiment (up to 140 min post radiotracer-injection) (Fig. 6a). Except for the kidneys and the intestine, the radiotracer was cleared rapidly from normal tissues and blood. The SUV(mean) values in the kidneys initially increased, followed by a decrease to a less than 2-fold higher SUV(mean) level in comparison to the tumor (1.8 fold higher after 120 min).

Accumulation of $^{68}$Ga-DOTA-SFLAP3K into the tumor was slightly better when compared to the original peptide (Fig. 6b). However, the clearance of the surrounding tissue was moderately slower and the uptake in the kidney seemed to stabilize at values which were 4 to 5 times higher than the activity in the tumor (Fig. 6c).

The $^{68}$Ga-DOTA-SFLAP3-trimer showed no improvement in tumor uptake but a continuous accumulation in the kidneys over the complete examined time (Fig. 6c). After 2 hours the SUV(mean) values are 20 – 30 fold higher than in the tumor (Fig. 6f). These findings indicate no benefit of $^{177}$Lu-SFLAP3-trimer compared to the original peptide.

Alteration of the position of DOTA influences the pharmacokinetics

Alteration of the DOTA-position from the N-terminus to the C-terminus via an amino-linker, resulted in the peptide SFLAP3-NH$_2$-DOTA-GA. This variant showed the highest tumor accumulation (SUV$_{\text{mean}}$ > 0.65) of all tested SFLAP3 variants. Unfortunately, an increased kidney accumulation was observed (Fig. 7a/ Fig. 7c). Additional conjugation of (4-iodophenyl)-acetic acid (IPAA), an albumin-binding moiety, at the N-terminus led to no prolonged circulation of IPAA-SFLAP3-NH$_2$-DOTA-GA in the blood but to a faster elimination from the kidneys (Fig. 7b/Fig. 7d).

Table 1 SUV(max) values of the metastases (patient in Fig. 9) 1 and 3 hours post administration of 304 MBq $^{68}$Ga-DOTA-SFLAP3.

<table>
<thead>
<tr>
<th>Number of examined metastases</th>
<th>Patient &gt; Fig. 9 SUV(max) 1h</th>
<th>Patient &gt; Fig. 9 SUV(max) 3h</th>
<th>Number of examined metastases</th>
<th>Patient &gt; Fig. 9 SUV(max) 1h</th>
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The biodistribution study of DOTA-SFLAP3 indicates the clinical potential of this radiotracer for pancreatic tumors

DOTA-SFLAP3 still offers the most balanced pharmacokinetic characteristics which is why further experiments were only performed with the original peptide. For the biodistribution experiments, Capan-2 bearing mice were injected with $^{177}$Lu-DOTA-SFLAP3. At 0.5, 1, 2, 4 and 6 hours p.i. the organs and tumors were removed, weighted and the radioactivity was measured. The results are presented as % injected dose/gram of tissue (%ID/g). As shown in Fig. 8a (Supplementary Table 2), the highest tumor activity was observed at 1 hour after injection (4 %ID/g). Tumor to tissue ratios were higher than 1 for all points in time except for the kidneys (Fig. 8b). However, higher activities were measured in the lung and the intestine compared to the other healthy organs (except the kidneys).

Diagnostic imaging in a human pancreatic cancer patient

A patient with metastasized pancreatic cancer was selected for PET/CT imaging using $^{68}$Ga-DOTA-SFLAP3. The patient had undergone a pancreatectomy followed by all guideline conform therapies and was in final stage of the disease. PET imaging showed multiple metastatic sites throughout the body with a high tracer accumulation in the tumor lesions up to three hours after administration (Fig. 9). Also, there was a high uptake into the kidneys, the thyroid and the intestine. The shift of activity in the intestine from the 1 h image to the 3 h image indicated localization of the tracer in the intestinal lumen. The general low signal background resulted in a good contrast of the metastases compared to the surrounding tissue.

Discussion

In previous experiments SFITGV6 and its improved variant SFLAP3 showed excellent binding affinities to αvβ6 positive tumors, especially to head and neck cancer [20, 21]. From the pancreatic cancer cell lines studied in this work, Capan-2 revealed the highest values in the flow cytometry analysis as well as the highest re-
results in in vitro binding assays. A strong correlation of the cellular SFLAP3 binding and the integrin αvβ6 expression (flow cytometry) on the different tumor cell lines was detectable. The measured $K_D$ of SFLAP3 with 7.4 nM to integrin αvβ6 using SPR spectroscopy analysis is comparable to the measured IC$_{50}$ on Capan-2 cells with 6.2 nM [21]. The specificity of the ligand for integrin αvβ6 was also ensured by SPR analysis [21]. There, SFLAP3 had a more than 10-time lower affinity for Integrin αvβ3 with a $K_D$ of 167 nM.

In order to improve the binding and uptake kinetics of SFLAP3, several variants of this molecule were designed and evaluated for their in vitro as well as in vivo properties. These included increase in length, variations in the position of the amino acids preceding and following the RGDXXXL motif and trimerization of the original peptide. The variations of the SFLAP3 sequence revealed that the target binding not only depends on the eight amino acids of the LAP3 binding motif, but also on the length of the binding loop.

The addition of lysine to the original eight amino acids (SFLAP3K) resulted in increased binding affinities to αvβ6-positive cells, whereas the αvβ6-negative cancer cell line MIA PaCa-2 showed no higher uptake values. This proofs that the increased binding properties of this variant are not based on unspecific binding.

Trimerization of the SFLAP3 peptide significantly enhanced the binding efficiency in vitro and increased the affinity against integrin αvβ6 which is most likely caused by the trivalent availability of the binding sequence.

Since the tracer kinetics is influenced not only by the affinity, but also by the stability of the ligand, measurements of serum stability were performed. The serum stability of DOTA-SFLAP3-trimer and DOTA-SFLAP3K was reduced compared to the original pep-
In both peptides one or three lysins are inserted in the sequence. Serine proteases with trypsin-like proteolysis, are able to cleave C-terminal to the lysine residue [23]. This could be responsible for the degradation. However, this rather slow degradation, which was detectable after 2 hours, should not interfere with the in vivo or in vitro assays.

In small animal imaging experiments ⁶⁸Ga-DOTA-SFLAP3 showed a considerable accumulation in the tumor. The clearance from the whole body occurred relatively fast and only the intestine and kidneys were visible in the PET images. As investigated in previous experiments an accumulation of SFLAP3 in HNSCC solid tumors could be confirmed by immunohistochemistry [21]. In vivo
The accumulation in the tumor after injection of $^{68}$Ga-DOTA-SFLAP3K was slightly higher compared to the original peptide. However, the clearance from healthy organs was moderately slower and the increased kidney signal is most likely based on the alteration in charge due to the lysine addition, which makes the peptide more hydrophilic. Gotthard et al. showed that the kidney accumulation correlated with the number of charged amino acids of the used peptide tracer [24]. The $^{68}$Ga-DOTA-SFLAP3-trimer showed no increase in tumor accumulation. Instead, the retention in the kidneys was higher with no reduction of activity for at least 140 min after administration. The introduction of the (GSGSK)$_3$ linker resulted in a higher hydrophilicity of the compound. Moreover, the trimerization increased the amount of total charged amino acids in the peptide, which may be the reason for enhanced renal accumulation and low kidney clearance. The observed tumor binding is in contrast to our in vitro results as well as the theoretical effects of multimerization. A compilation of monomers and TRAP-trimers of different tracers by Maschauer et al. showed that trivalent presence of the tracer leads to better tumor binding. The kidney accumulation also increases in all analyzed compounds which mostly leads to lower tumor/kidney ratios compared to the monomer.

The addition of an albumin binding entity should lead to a longer circulation in the blood and therefore to a higher probability of tumor uptake. In a study of Choy et al. the longer circulation of an albumin binding modified PSMA tracer resulted in an increased tumor accumulation [25]. In our case the longer circulation was not detectable but compared to the variant with the N-terminal chelator the elimination of the tracer out of the kidneys was faster.

In biodistribution assays, DOTA-SFLAP3 uptake was not only observed in the tumor (4 %ID/g), but also in the kidneys, the lung and the intestine. The other healthy organs indicated very low accumulation of the radiotracer. Higher signals in the intestine and the lung are often observed in $\alpha\beta$6 tracer studies. As shown by Notni et al., $\beta$6 integrin is weakly expressed in bronchial and alveolar epithelial cells of the lung and in parietal cells of the glandular part of the mouse stomach [17]. $\beta$6 only dimerizes with the ubiquitously expressed $\alpha$ chain, thus its availability is limiting and indicative for the existence of the $\alpha\beta$6 heterodimer [26]. Slow excretion of $^{177}$Lu-DOTA-SFLAP3 from the parietal cells into the stomach and further into the intestine lumen might explain the higher intestine values.

In the pancreatic cancer patient, PET imaging revealed multiple metastatic sites, especially in the liver and the upper body region. The tracer accumulation in the metastases was clearly visible due to low signal background. However, an accumulation in the kidney and intestine was also observed. PET images showed transport of radioactivity along the gastrointestinal tract after 1 h and 3 h. It is not clear if the excretion into the intestine lumen occurs in the stomach or the duodenum. However, since the radioactivity is located in the lumen where it is transported from the proximal ileum to the terminal ileum in a short time this represents no obstacle for possible therapeutic applications. On the other hand the tumor lesions have comparably low tracer uptake with SUV$_{\text{max}}$ values generally ranging from 5 to 10 (Table 1). Over the time period of 3 hours the SUV$_{\text{max}}$ level of some metastasis increased, in others it decreased or remained constant. This strong heterogeneity in uptake and retention could lead to an underdosing of the lesions with a lack of an anti-tumor effect in therapy. Therefore, attempts are needed to further increase tumor uptake and retention.

### CONCLUSION

The high affinity integrin $\alpha\beta$6 binder SFLAP3 was shown to specifically accumulate in pancreatic cancer animal models. Several variants, i.e. SFLAP3K, a peptide comprising lysine in the binding loop, a peptide containing the albumin binding moiety IPAA and a trimer of the binding peptide, were synthesized. The novel compounds showed improved performance in vitro and the position change of the chelator improved the tumor accumulation in vivo. This illustrates the wide range of options to modify $\alpha\beta$6 binding peptides for clinical applications. As SFLAP3 still offers the most balanced characteristics, this compound was chosen to be studied in a pancreatic cancer patient. PET imaging with $^{68}$Ga-DOTA-SFLAP3 allowed delineation of the pancreatic tumor metastases with favorable dosimetric values and high tumor to background contrast. On the basis of these findings, $\alpha\beta$6-integrin-based tracers warrant further investigation as potential diagnostics and eventually therapeutics for pancreatic cancer.

### Conflict of interest

The authors declare that they have no conflict of interest.

### Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

### Availability of data and materials

Please contact authors for data request.

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