Journal of Nuclear Medicine, published on October 23, 2020 as doi:10.2967/jnumed.120.253062

Impact of ^{68}Ga-FAPI-PET/CT imaging on the therapeutic management of primary and recurrent pancreatic ductal adenocarcinomas

Running title: ^{68}Ga-FAPI - PET in PDAC

Manuel Röhrich[1], Patrick Naumann[2-4], Frederik L Giesel [1,5], Peter L Choyke[6], Fabian Staudinger[1], Annika Wefers[7,8], Dawn P Liew [1], Clemens Kratochwil[1], Hendrik Rathke[1], Jakob Liermann[2-4], Klaus Herfarth[2-4], Dirk Jäger[9,10], Jürgen Debus[2-5,11,12], Uwe Haberkorn[1,5,13,14], Matthias Lang[7[equal senior]] and Stefan A Koerber[2-4]

Affiliations:

1 Department of Nuclear Medicine, Heidelberg University Hospital, Heidelberg, Germany

2 Department of Radiation Oncology, Heidelberg University Hospital, Heidelberg, Germany

3 National Center for Tumor diseases (NCT), Heidelberg, Germany

4 Heidelberg Institute of Radiation Oncology (HIRO), Heidelberg, Germany 5 German Cancer Consortium (DKTK), partner site Heidelberg, Germany.

6 Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda/ Maryland, USA

7 Department of Neuropathology, Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany

8 Clinical Cooperation Unit Neuropathology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

9 Department of Medical Oncology and Internal Medicine VI, National Center for Tumor Diseases, University Hospital Heidelberg, Germany.

10 Clinical Cooperation Unit Applied Tumor Immunity, German Cancer Research Center, Heidelberg, Germany.

11 Heidelberg Ion-Beam Therapy Center (HIT), Department of Radiation

Oncology, Heidelberg University Hospital, Heidelberg, Germany
12 Clinical Cooperation Unit Radiation Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany
13 Clinical Cooperation Unit, Department of Nuclear Medicine, German Cancer Research Center (DKFZ), Heidelberg, Germany
14 Translational Lung Research Center Heidelberg, Member of the German Center for Lung Research DZL, Heidelberg, Germany

Corresponding Author:

Manuel Röhrich

Im Neuenheimer Feld 400 69120 Heidelberg
Telephone: 0049 6221 56 7731

Fax: 0049 6221 56 5473

Email: manuel.roehrich@med.uni-heidelberg.de

ORCID-ID: 0000-0001-7609-243X

Manuel Röhrich is resident at the Department of Nuclear Medicine, University Hospital Heidelberg, Germany.

Word Count: 5769 words

ABSTRACT:

Purpose:

Pancreatic ductal carcinoma (PDAC) is a highly lethal cancer and early detection and accurate staging are critical to prolonging survival. PDAC typically has a prominent stroma including cancer-associated fibroblasts (CAFs) that express Fibroblast Activation Protein (FAP). FAP is a new target molecule for PET imaging of various tumors. In this retrospective study we describe the clinical impact of PET/CT imaging using ^{68}Ga-labelled FAP-Inhibitors (68Ga-FAPI - PET/CT) in 19 patients with PDAC (7 primary, 12 progressive/recurrent).

Patients and Methods: All patients have undergone contrast enhanced Computed Tomography (ceCT) for TNM staging before they were subjected to ^{68}Ga-FAPI - PET/CT imaging. PET-scans were acquired 60 minutes after administration of 150- 250 MBq of ^{68}Ga-labelled FAP-specific tracers. In order to characterize ^{68}Ga-FAPI- uptake over time, additional scans after 10 minutes and/or 180 minutes were performed in six patients. SUVmax and SUVmean values of PDAC manifestations and healthy organs were analyzed. The tumor burden according to ^{68}Ga-FAPI - PET/CT was compared to TNM staging based on ceCT and changes in oncological management were recorded.

Results:

Compared to ceCT, ^{68}Ga-FAPI - PET/CT results led to changes in TNM staging in 10/19 patients. 8/12 patients with recurrent/progressive disease, were up-staged, 1 down-staged and 3 had no change. In newly diagnosed PDAC, 1/7 patients was up- staged, the staging of 6 patients did not change. Changes in oncological management occurred in seven patients. Markedly elevated uptake of ^{68}Ga-FAPI in PDAC manifestations after 1 hour was seen in most cases. Differentiation from pancreatitis based on static imaging 1 hour p.i. was challenging. With respect to imaging after multiple time points, PDAC and pancreatitis showed a trend for differential uptake kinetics.

Conclusion:

^{68}Ga-FAPI - PET/CT led to restaging in half of patients with PDAC and most patients

with recurrent disease compared to standard of care imaging. The clinical value of ^{68}Ga-FAPI - PET/CT should be further investigated.

Keywords: Fibroblast Activation Protein, FAP, Pancreatic Ductal Adenocarcinoma, PDAC, Positron Emission Tomography, TNM, Staging

Declarations

Funding: This work was funded by the Federal Ministry of Education and Research, grant number 13N 13341.

Conflicts of interest: Uwe Haberkorn, Clemens Kratochwil and Frederik Giesel have filed a patent application for quinoline based FAP targeting agents for imaging and therapy in nuclear medicine. No other potential conflict of interest was reported.

Ethics approval: This retrospective study was approved by the local institutional review board (study number S-115/2020).

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive types of cancer with a dismal 5 - year survival rate of less than 10% (*1*). Optimal imaging of PDAC is crucial for accurate initial staging and selection of the primary therapy as well as for follow-up examinations in order to accurately detect local recurrence and/or metastatic spread as early and as completely as possible.

Standard of care imaging techniques for PDAC include transabdominal ultrasonography (*2*), computed tomography (*3*)**,** magnetic resonance imaging (*4,5*) and endoscopic ultrasonography (*6*), with contrast enhanced CT considered the gold standard for TNM staging in pre-operative and follow-up settings (*7,8*)**.** Positron Emission Tomography with ^{18}F-Fluorodeoxyglucose (FDG) is not part of the clinical routine, but is sensitive for initial TNM-staging (*9*), evaluation of treatment response

(*10*) and detection of recurrence (*11*). Furthermore, FDG-PET/CT imaging parameters may predict treatment efficacy and clinical outcome for PDAC (*12*). However, FDG-PET/CT is clearly not an ideal imaging agent for PDAC due to its variable detection of metastatic lymph nodes (*13*) and possible false-positive findings in inflammation (*14*).

Histologically, PDAC is characterized by its prominent desmoplastic stroma (*15,16*). In general, tumor stroma is composed of extracellular matrix proteins and specialized connective-tissue cells, including activated cancer-associated fibroblasts (CAFs) (*17,18*). CAFs in PDAC are derived from pancreatic stellate cells (*19*) and transform their tumor-promoting biological properties through crosstalk with neoplastic cells (*19,20*). CAFs are thought to promote tumor growth, invasion, metastasis and therapy resistance both in PDAC (*18,21*) and other tumor entities (*22*). CAFs - in contrast to normal fibroblasts - express Fibroblast Activation Protein (FAP) on their surface (*23*). FAP is a type II membrane-bound glycoprotein with dipeptidyl peptidase and endopeptidase activity (*24*). FAP is a promising new target molecule for PET imaging of various epithelial tumors, among them PDAC (*25-28*). Biologically, the application of PET/CT using radioactive labelled FAP-Inhibitors (FAPI-PET/CT) in PDAC is of special interest as this new imaging modality depicts tumor-stroma interaction, which is crucial for the tumorigenesis of PDAC and cannot be visualized by morphological or metabolic imaging. Although previous studies have included single cases of PDAC (*27,29*), the

clinical value of FAPI-PET for PDAC has not yet been systematically investigated.

The purpose of this investigation is to explore the clinical impact (TNM staging) of ^{68}Ga-FAPI-PET/CT compared to standard of care imaging in patients with primary and recurrent PDAC.

PATIENTS AND METHODS

Patient cohort

This cohort consists of 19 patients with histologically confirmed PDAC. Written informed consent was obtained from all patients on an individual-patient basis following the regulations of the German Pharmaceuticals Act §13(2b). All patients were referred for the experimental diagnostics by their treating oncologists to assist diagnostic decision making. For example, in cases where the results of standard imaging were inconclusive, more information was sought on tumor extension and possible involvement of regional lymph nodes for target-volume segmentation before radiotherapy, or there was a need to select target-positive patients for experimental last-line therapy with therapeutic FAPI conjugates. Clinical characteristics and outcomes were collected through electronic patient records. This retrospective study was approved by the local institutional review board (study number S-115/2020).

CT imaging and transabdominal ultrasonography

All patients underwent multi-phase contrast enhanced CT imaging for staging. CT scans were performed in average 17.6 days before FAP-specific PET/CT. Patients in a preoperative setting underwent additional transabdominal ultrasonography.

Radiopharmaceuticals and ^{68}Ga-FAPI-PET/CT imaging

Synthesis and labeling of both, ^{68}Ga-FAPI-4 and ^{68}Ga-FAPI-46, followed the methods described by Lindner et al.(*25*) and Loktev et al. (*26*). A Biograph mCT Flow scanner (Siemens) was used for PET imaging. Scans were performed according to scan protocols as previously published (*27,28*). In brief, after low-dose CT without contrast, PET scans were acquired in 3D-mode (matrix 200 x 200), emission data were corrected for attenuation and reconstructions were performed. The injected activity for the ^{68}Ga-FAPI examinations ranged from 167 to 293 MBq.

For all patients, PET scans were obtained 1h after injection of the radiotracer. This time point of image acquisition has been shown to be suitable for tumor imaging as we have

observed high tumor to background ratios for various tumor entities including pancreatic carcinomas in our previous studies with FAPI-PET (*25,27,28,30*).
In order to characterize ^{68}Ga FAPI-Uptake over time, multiple time point imaging was acquired in 6 patients (the other patients did not undergo imaging at multiple imaging timepoints due to reduced general condition and/or limited compliance and/or limited imaging capacities).

Image evaluation

The tracer biodistribution in patients was quantified by mean and maximum standardized uptake values (SUVmean and SUVmax, as widely accepted standard parameters for quantification and clinical evaluation of PET imaging) at 1 h post injection. For SUV calculation, circular regions of interest were drawn around the tumors on transaxial slices and automatically adapted to a three-dimensional VOI with e.soft software (Siemens) at a 60 % isocontour. The normal organs were evaluated with a 1 cm diameter (for the small organs thyroid, parotid gland, myocardium, oral mucosa, spinal cord) or 2 cm diameter (brain, muscle, liver, pancreas, spleen, kidney, fat, aortic lumen content, lung) sphere placed inside the organ parenchyma. ^{68}Ga-FAPI - PET/CT scans were evaluated by one board- certified radiologist (FLG), one board-certified radiation oncologist (SAK) and two board-certified nuclear medicine physicians (FLG, CK) in consensus. Contrast enhanced CT imaging was interpreted by two board-certified radiologists (PN, SAK) in consensus without knowledge of ^{68}Ga-FAPI-PET/CT results thus establishing the pre-^{68}Ga-FAPI-PET/CT staging. The staging was coded according to the 8th edition of the TNM classification of malignant tumors of the Union for International Cancer Control (UICC) (*31*).

For all patients, changes in TNM stage, localization of metastases and oncological management were recorded. This was done by documenting stage/ tumor localization and oncological management based on ceCT and based on ^{68}Ga- FAPI imaging by two nuclear medicine physicians and two radiation oncologists. All findings and changes were interpreted in consensus. Changes of oncological management (68Ga-FAPI-PET/CT based versus ceCT based) were graded by the level of impact: Fundamental

changes with regard to alteration of treatment type and/ or treatment intent and relevant changes within a treatment regime were classified as major and minor, respectively.

FAP Immunohistochemistry

In 5 of the patients examined by ^{68}Ga-FAPI-PET/CT sufficient material for immunohistochemistry was available. All specimens were from the archives of the Department of Pathology, Institute of Pathology, University Hospital Heidelberg, Germany. The primary anti-FAP antibody used was ab207178 (EPR20021; Abcam, Cambridge, UK) diluted 1:100. Immunohistochemistry was done on 0.5-µm-thick formalin-fixed, paraffin-embedded (FFPE) tissue sections mounted on Superfrost Plus slides (Thermo Scientific, Waltham, MA, USA) followed by drying at 80 °C for 10 min. Stainings were done on a Ventana BenchMark XT Immunostainer (Ventana Medical Systems, Tucson, AZ, USA). The slices were pretreated with cell conditioner 1 (pH 8) for 92 min, followed by incubation with the primary antibody at 37 °C for 32 min. The incubation was followed by Ventana standard signal amplification, UltraWash, counterstaining with one drop of hematoxylin for 4 min and one drop of bluing reagent for 4 min. For visualization, the ultraView™ Universal DAB Detection Kit (Ventana Medical Systems, Tucson, AZ, USA) was used. Negative controls were obtained by omitting the primary antibody (data not shown). Images were scanned and digitalized using NanoZoomer S60 Digital slide scanner (Hamamatsu Photonics, Hamamatsu, Japan).

Statistical analysis

We performed descriptive analyses for patients and their tumor characteristics. For determination of standardized uptake values, median and range were used. The correlation of FAPI-uptake within or outside the tumor was determined by using two-sided t-test, a p-value of < 0.05 was defined as statistically significant. All statistical analyses were performed using Microsoft Excel 2010.

RESULTS

Patient cohort

Table 1 depicts the demographic data as well as the previous therapies of all 19 patients. Median patient age was 64.0 years (range: 52 – 80 years). 7 patients underwent FAP-specific PET/CT preoperatively as initial staging (4 of them were treatment-naïve, 3 of them had undergone neoadjuvant chemotherapy), 11 patients presented with local tumor recurrence and 1 patient suffered from progressive disease after neoadjuvant chemotherapy.

Biodistribution of ^{68}Ga-FAPI-tracers

1h after injection overall SUVmax and SUVmean for all PDAC were 13.37 ± 5.45 and 7.32 (± 3.13), whereas preoperative tumors showed higher SUVmax and SUVmean values (17.41 ± 7.40 and 10.25 ± 4.10) than local recurrences (11.90 ± 3.31 and 6.34 ± 2.02). Similarly, elevated SUVmax and SUVmean values were observed in lymph node metastases (14.13 ± 8.50 and 7.62 ± 3.69) and distant metastases (7.34 ± 2.48 and 3.92 ± 1.57). All normal organs including the uninvolved pancreas showed low ^{68}Ga-FAPI-uptake, which resulted in high tumor-to background ratios (e.g. SUVmax tumor / bloodpool 8.31, SUVmax tumor / muscle 8.72 and SUVmax tumor / fat 23.34). Figure 1 provides an overview of the biodistribution of tumor uptake in PDAC manifestations and the background activity of normal organs. Of note, both tracer variants (^{68}Ga-FAPI-4 and ^{68}Ga-FAPI-46) showed a similar biodistribution (Supplemental figure 1).

Changes in TNM staging and oncological management after ^{68}Ga-FAPI-PET/CT

^{68}Ga-FAPI-PET/CT resulted in new findings in 10/19 patients, specifically, 8/12 patients with recurrent/progressive disease were upstaged and 1/12 patients downstaged (see Table 2). In patients with primary disease 1/7 patients was upstaged and no patients were downstaged. In all cases changes in staging were caused by the detection of new or additional distant metastases in one or more organ systems. Moreover, for two patients, there was a detection of new lymph node metastases. ^{68}Ga-FAPI-PET/CT led

to an upstaging to stage IV disease by detection of progression to distant metastatic disease in 1/7 and 3/12 patients with newly diagnosed or recurrent PDAC, respectively. In one patient with local recurrence, the lymph node status could not be evaluated definitely based on contrast enhanced CT. Here, the absence of FAPI-positive lymph nodes led to more certainty.

Furthermore, in one patient ^{68}Ga-FAPI-PET/CT led to downstaging. This patient had undergone Whipple operation followed by FLOFIRINOX chemotherapy. During follow-up, the patient was classified as having local recurrence by CT imaging (T2). Additional ^{68}Ga-FAPIPET/CT revealed no suspicious FAPI-uptake (T0). Based on these results, the planned oncological treatment for this patient was reassessed and a decision was made for watchful waiting instead of an initially considered local radiotherapy.

Figure 2 shows a case where ^{68}Ga-FAPI-PET/CT markedly changed primary TNM staging (upstaging) compared to contrast enhanced CT and FDG-PET/CT. Figure 3 shows an exemplary case of upstaging after ^{68}Ga-FAPI-PET/CT compared to contrast enhanced CT in the setting of local recurrence. Hybrid imaging caused changes in oncological management in seven patients. While minor changes occurred in two patients, five patients had major changes including cancellation of the planned therapy and watchful waiting instead (1 patient), cancelling of the planned pancreatectomy after confirmation of a FAPI-positive pulmonary metastasis by biopsy (1 patient, see figure 2), local irradiation of a single hepatic lesion (1) and FAPI-ligand therapy on individual patient basis (2 patients). Four patients with recurrent or progressive PDAC were selected for radiotherapy and the ^{68}Ga-FAPI- PET/CT data were used for target volume delineation.

^{68}Ga-FAPI-Uptake of PDAC and pancreatitis

Eleven of 19 patients had undergone no or only partial pancreatectomy before ^{68}Ga-FAPI-PET/CT. In 8 of these, significantly elevated tracer uptake was observed not only in the PDAC, but also homogenously within the rest of the pancreas. Four of these

patients had been diagnosed with chronic pancreatitis before ^{68}Ga-FAPI- PET/CT imaging. As tumor-related buildup of exocrine secretions and consequent pancreatitis is a typical finding in PDAC, we assumed that the patients without pre- diagnosed chronic pancreatitis were suffering from tumor-related pancreatitis. We considered pancreatic ^{68}Ga-FAPI uptake as a sign of pancreatitis in all 8 patients.

Although tumors showed higher average SUVmax and SUVmean values (15.64 ± 5.81 and 8.65 ±3.61) than pancreatitis (7.50 ± 3.52 and 4.07 ± 2.11) after 1 hour, we observed a certain overlap between tumor-related and inflammatory uptake, as illustrated by the boxplot graphs in figure 4a and 4b. In 1 patient with PDAC and pancreatitis, we performed ^{68}Ga-FAPI-PET/CT after 10, 60 and 180 minutes. Here, we observed slightly increasing uptake of the PDAC (SUVmax 11.48, 12.66 and 13.23 and SUVmean 6.65, 7.46 and 7.71 after 10,60 and 180 minutes) and decreasing uptake within the pancreatitis in the remaining pancreas (SUVmax 7.24, 6.55 and 5.63 and SUVmean 4.32, 3.01 and 2.96 after 10,60 and 180 minutes). Figure 4c shows exemplary images of PDAC and pancreatitis-related FAPI-uptake over time of this patient. Another patient with both PDAC and pancreatitis who underwent imaging after 60 and 180 minutes showed similar uptake kinetics (Tumor: SUVmax 12.05 and 12.43 and SUVmean 7.37 and 7.74 after 60 and 180 minutes and pancreatitis: SUVmax 6.12 and 5.56 and SUVmean 3.28 and 2.77 after 60 and 180 minutes).

The other 4 patients with imaging at more than one imaging time point had a local recurrence after pancreatectomy. Thus, we could evaluate only tumor lesions in these patients. Tumor lesions of all 6 patients with more than one imaging time point showed stable uptake between 10 and 60 minutes and a slight tendency to decreased uptake after 180 minutes. Figure 4D shows the summed SUVmax and SUVmean values in the PDAC lesions of all patients who have undergone imaging at more than one time point.

^{68}Ga-FAPI-Uptake related in other chronic inflammatory and reactive processes

Next to pancreatitis related FAPI-uptake we regularly found moderately elevated FAPI-uptake related to chronic inflammatory or reactive processes in other body sites, namely

joint associated, arthrosis related uptake (10 patients), in post-operative scars (2 patients), located in the mamma (1 patient, most likely due to mastitis), around a gamma nail implant (1 patient) and related to tendinopathy (1 patient).

FAP expression in pancreatic carcinomas

To further characterize FAP as a target structure within pancreatic carcinomas, we performed FAP-immunohistochemistry in 4 of the cases included. Supplementary Figure 2 shows Hematoxylin and eosin staining and FAP immunohistochemistry of two exemplary PDAC. The first PDAC (A-D) shows strong FAP expression in the tumor stroma, while the neoplastic cells were FAP-negative. The stroma of the second PDAC (E-H) was also FAP-positive, but less intinsively. In this case we observed FAP-positive cell clusters within the neoplastic cells, whereas FAP- positivity was pronounced in the peripheral zone of the cell clusters. These FAP- positive cell clusters most likely represent Langerhans islets, as it has been shown that the alpha cells of Langerhans islets express FAP(*32*).

DISCUSSION

This retrospective analysis of 19 patients with PDAC suggests that ^{68}Ga-FAPI- PET/CT is a promising new imaging modality in staging of PDAC that may help to detect new or clarify inconclusive results obtained by standard CT-imaging. Analyses of tracer biodistribution demonstrated a high FAPI uptake in primary PDAC as well as lymph nodes and distant metastases, whereas healthy tissues have negligible background activity. This leads to excellent tumor/background ratios for PDAC, similar to those shown by previous studies on FAPI-PET/CT in PDAC and other tumors (*27-29,33*).

PDAC is clinically challenging with very high mortality rates. Improvement in survival can only be achieved by effective treatment approaches customized to the individual patient's disease status. Thus, hybrid imaging using FAPI-tracer may open up new applications in staging and restaging of PDAC. To evaluate the potential impact of ^{68}Ga-FAPI-PET/CT on the clinical management of PDAC, we have documented that ^{68}Ga-FAPI-PET/CT resulted in changes of TNM staging of primary and recurrent/progressive PDAC compared to standard of care imaging. Clinically meaningful changes in TNM staging in a high percentage of recurrent tumors (9/12 cases) was seen and resulted in therapy changes. In staging for newly diagnosed PDAC upstaging occurred in 1 of 7 cases. These changes in TNM classification had a significant impact on oncological management. Apart from changes concerning systemic therapy, the use of ^{68}Ga-FAPI-PET/CT may also help to select patients for local treatment approaches like surgery or radiotherapy. In fact, four patients were considered candidates for irradiation according to standard staging. For all these patients the ^{68}Ga-FAPI-PET/CT data were used for radiotherapy planning. Radiotherapy was conducted as local treatment approach for unresectable disease, recurrent disease and in the setting of oligometastatic lymph nodes with either photons or carbon ions. For the management of local recurrences or unresectable PDAC the application of carbon ions is considered a promising radiotherapeutic modality (*34*).

FAPI-PET/CT may lead to significant changes in oncological management when compared to FDG-PET/CT. A recent study with head to head comparison of FDG-PET/CT and FAPI-PET/CT in various tumors showed a tendency towards upstaging

after FAPI-PET for four pancreatic carcinomas (*29*). Based on our comparison of FAPI-PET/CT and contrast enhanced CT we would speculate that there may be a similar discrepancy between FDG-PET/CT and FAPI-PET/CT based staging and resulting treatment decisions for PDAC. A study addressing this specific question in PDAC would be an important contribution.

With respect to the discrimination of PDAC and pancreatitis it must be emphasized that we have observed higher FAPI-uptake in PDAC than in pancreatitis. But there seems to be an overlap of the uptake intensities on static imaging after 1 hour. Repetitive imaging in two patients indicated that there may be differential uptake kinetics in PDAC (slightly increasing tracer uptake over time) and pancreatitis or fibrotic pancreatic tissue (decreasing tracer uptake over time). These findings are similar to our observations of dynamic FAPI-PET/CT in patients with pulmonary fibrosis and lung cancer (data unpublished). However, the results of the current study regarding the differential FAPI-uptake over time in PDAC and pancreatitis can only be considered preliminary. The hypothesis of differential uptake over time in PDAC and pancreatitis should be evaluated systematically in a larger patient cohort. Also, dynamic FAPI-PET imaging may provide useful additional parameters for this question.

Limitations of the study

The major limitation of this study is the relatively low number of patients which does not allow us to draw any definite conclusions on the diagnostic value of ^{68}Ga-FAPI-PET/CT. However, the high uptake values in PDAC suggest this will be a highly sensitive modality especially in recurrent and progressive disease. In our opinion, the results regarding the tracer biodistribution and the impact on TNM staging are promising and show great clinical potential for the future application of ^{68}Ga-FAPI- PET/CT for decision making on appropriate treatment options in PDAC. Moreover as serum screening methods become available for early PDAC detection, FAPI PET CT is a potential method of detecting these early tumors. Implementation of ^{68}Ga-FAPI- PET/CT in further clinical studies is recommended to gain further evidence of the value of this

new imaging modality. In our exemplary histological studies we observed strong stromal FAP expression in four primary PDAC. Systematic ^{68}FAPI-PET based bioptic studies of primary and metastatic lesions are needed to gain more diagnostic certainty regarding tumor-related FAPI-Uptake in PDAC and other malignancies.

Another limitation is that two different tracer molecules (FAPI-4 and FAPI-46) were used in this study. Molecules were switched during the investigation period of this study because FAPI-46 may open a theranostic perspective due to improved long-time tumor retention and tumor-to organ-ratios. For our analysis, we consider this switch of molecules a minor limitation as it has been shown that both substances do not significantly differ in their radiopharmaceutical properties at early time points (1-4 hours) (*30*), which we confirmed for this dataset by separate biodistribution analysis of both tracer molecules.

Conclusion

FAPI - PET/CT is a promising imaging modality for PDAC with high tracer uptake and excellent tumor to background ratios. FAPI - PET/CT based TNM staging differed in about half of all patients and nearly all patients with recurrent disease compared to staging obtained by contrast enhanced CT. In the primary setting dynamic FAPI-PET/CT imaging may be helpful for the discrimination of tumor versus inflammatory or fibrotic pancreatic lesions. The clinical value of FAPI - PET/CT should be further investigated.

KEY POINTS

QUESTION: Does ^{68}Ga-FAPI-PET/CT impact TNM staging and clinical management of PDAC?

PERTINENT FINDINGS: .FAPI-PET/CT led to significant changes in staging and clinical management of 19 patients with PDAC compared to ceCT, especially in the setting of local recurrence.

IMPLICATIONS FOR PATIENT CARE: Based on the encouraging results of this analysis, the clinical value FAPI-PET/CT in primary and recurrent PDAC should be further validated. FAPI-PET may be implemented in into the future clinical management of PDAC patients

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin.* 2019;69:7-34.

2. Conrad C, Fernandez-Del Castillo C. Preoperative evaluation and management of the pancreatic head mass. *J Surg Oncol.* 2013;107:23-32.

3. Montejo Ganan I, Angel Rios LF, Sarria Octavio de Toledo L, Martinez Mombila ME, Ros Mendoza LH. Staging pancreatic carcinoma by computed tomography. *Radiologia.* 2018;60:10-23.

4. Bowman AW, Bolan CW. MRI evaluation of pancreatic ductal adenocarcinoma: diagnosis, mimics, and staging. *Abdom Radiol (NY).* 2019;44:936-949.

5. Alabousi M, McInnes MD, Salameh JP, et al. MRI vs. CT for the Detection of Liver Metastases in Patients With Pancreatic Carcinoma: A Comparative Diagnostic Test Accuracy Systematic Review and Meta-Analysis. *J Magn Reson Imaging.* 2020.

6. Kitano M, Yoshida T, Itonaga M, Tamura T, Hatamaru K, Yamashita Y. Impact of endoscopic ultrasonography on diagnosis of pancreatic cancer. *J Gastroenterol.* 2019;54:19-32.

7. Zins M, Matos C, Cassinotto C. Pancreatic Adenocarcinoma Staging in the Era of Preoperative Chemotherapy and Radiation Therapy. *Radiology.* 2018;287:374-390.

8. Expert Panel on Gastrointestinal I, Qayyum A, Tamm EP, et al. ACR Appropriateness Criteria((R)) Staging of Pancreatic Ductal Adenocarcinoma. *J Am Coll Radiol.* 2017;14:S560-S569.

9. Yeh R, Dercle L, Garg I, Wang ZJ, Hough DM, Goenka AH. The Role of 18F-FDG PET/CT and PET/MRI in Pancreatic Ductal Adenocarcinoma. *Abdom Radiol (NY).* 2018;43:415-434.

10. Yoshioka M, Sato T, Furuya T, et al. Role of positron emission tomography with 2-deoxy-2- [18F]fluoro-D-glucose in evaluating the effects of arterial infusion chemotherapy and radiotherapy on pancreatic cancer. *J Gastroenterol.* 2004;39:50-55.

11. Sperti C, Pasquali C, Bissoli S, Chierichetti F, Liessi G, Pedrazzoli S. Tumor relapse after pancreatic cancer resection is detected earlier by 18-FDG PET than by CT. *J Gastrointest Surg.* 2010;14:131-140.

12. Wang L, Dong P, Shen G, et al. 18F-Fluorodeoxyglucose Positron Emission Tomography Predicts Treatment Efficacy and Clinical Outcome for Patients With Pancreatic Carcinoma: A Meta- analysis. *Pancreas.* 2019;48:996-1002.

13. Kauhanen SP, Komar G, Seppanen MP, et al. A prospective diagnostic accuracy study of 18F- fluorodeoxyglucose positron emission tomography/computed tomography, multidetector row computed tomography, and magnetic resonance imaging in primary diagnosis and staging of pancreatic cancer. *Ann Surg.* 2009;250:957-963.

14. Strobel O, Buchler MW. Pancreatic cancer: FDG-PET is not useful in early pancreatic cancer diagnosis. *Nat Rev Gastroenterol Hepatol.* 2013;10:203-205.

15. Leppanen J, Lindholm V, Isohookana J, et al. Tenascin C, Fibronectin, and Tumor-Stroma Ratio in Pancreatic Ductal Adenocarcinoma. *Pancreas.* 2019;48:43-48.

16. Spill F, Reynolds DS, Kamm RD, Zaman MH. Impact of the physical microenvironment on tumor progression and metastasis. *Curr Opin Biotechnol.* 2016;40:41-48.

17. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. *Nat Rev Clin Oncol.* 2018;15:366-381.

18. von Ahrens D, Bhagat TD, Nagrath D, Maitra A, Verma A. The role of stromal cancer- associated fibroblasts in pancreatic cancer. *J Hematol Oncol.* 2017;10:76.

19. Nielsen MFB, Mortensen MB, Detlefsen S. Typing of pancreatic cancer-associated

fibroblasts identifies different subpopulations. *World J Gastroenterol.* 2018;24:4663-4678.

20. Whittle MC, Hingorani SR. Fibroblasts in Pancreatic Ductal Adenocarcinoma: Biological Mechanisms and Therapeutic Targets. *Gastroenterology.* 2019;156:2085-2096.

21. Sun Q, Zhang B, Hu Q, et al. The impact of cancer-associated fibroblasts on major hallmarks of pancreatic cancer. *Theranostics.* 2018;8:5072-5087.

22. Kalluri R. The biology and function of fibroblasts in cancer. *Nat Rev Cancer.* 2016;16:582-598.

23. Sahai E, Astsaturov I, Cukierman E, et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer.* 2020;20:174-186.

24. McCarthy JB, El-Ashry D, Turley EA. Hyaluronan, Cancer-Associated Fibroblasts and the Tumor Microenvironment in Malignant Progression. *Front Cell Dev Biol.* 2018;6:48.

25. Lindner T, Loktev A, Altmann A, et al. Development of Quinoline-Based Theranostic Ligands for the Targeting of Fibroblast Activation Protein. *J Nucl Med.* 2018;59:1415-1422.

26. Loktev A, Lindner T, Mier W, et al. A Tumor-Imaging Method Targeting Cancer-Associated Fibroblasts. *J Nucl Med.* 2018;59:1423-1429.

27. Kratochwil C, Flechsig P, Lindner T, et al. (68)Ga-FAPI PET/CT: Tracer Uptake in 28 Different Kinds of Cancer. *J Nucl Med.* 2019;60:801-805.

28. Giesel FL, Kratochwil C, Lindner T, et al. (68)Ga-FAPI PET/CT: Biodistribution and Preliminary Dosimetry Estimate of 2 DOTA-Containing FAP-Targeting Agents in Patients with Various Cancers. *J Nucl Med.* 2019;60:386-392.

29. Chen H, Pang Y, Wu J, et al. Comparison of [(68)Ga]Ga-DOTA-FAPI-04 and [(18)F]

FDG PET/CT for the diagnosis of primary and metastatic lesions in patients with various types of cancer. *Eur J Nucl Med Mol Imaging.* 2020;47:1820-1832.

30. Loktev A, Lindner T, Burger EM, et al. Development of Fibroblast Activation Protein-Targeted Radiotracers with Improved Tumor Retention. *J Nucl Med.* 2019;60:1421-1429.

31. Kamarajah SK, Burns WR, Frankel TL, Cho CS, Nathan H. Validation of the American Joint Commission on Cancer (AJCC) 8th Edition Staging System for Patients with Pancreatic Adenocarcinoma: A Surveillance, Epidemiology and End Results (SEER) Analysis. *Ann Surg Oncol.* 2017;24:2023-2030.

32. Busek P, Hrabal P, Fric P, Sedo A. Co-expression of the homologous proteases fibroblast activation protein and dipeptidyl peptidase-IV in the adult human Langerhans islets. *Histochem Cell Biol.* 2015;143:497-504.

33. Rohrich M, Loktev A, Wefers AK, et al. IDH-wildtype glioblastomas and grade III/IV IDH- mutant gliomas show elevated tracer uptake in fibroblast activation protein-specific PET/CT. *Eur J Nucl Med Mol Imaging.* 2019;46:2569-2580.

34. Liermann J, Shinoto M, Syed M, Debus J, Herfarth K, Naumann P. Carbon ion radiotherapy in pancreatic cancer: A review of clinical data. *Radiother Oncol.* 2020;147:145-150.

Figure 1

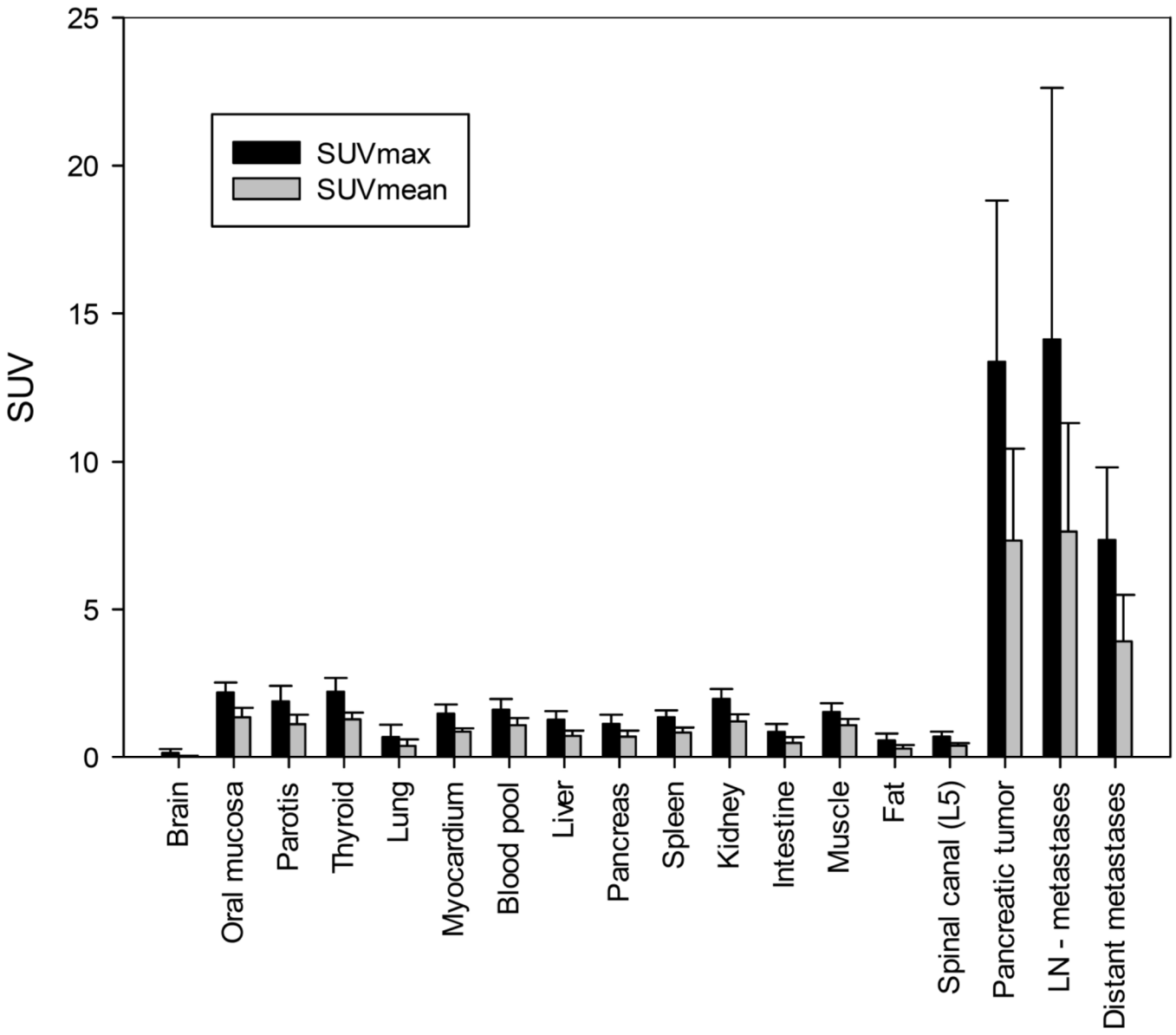


Figure 1

Biodistribution analysis (SUVmax and SUVmean) of 19 patients with PDAC based on PET/CT imaging 1 hour after injection of ^{68}Ga labelled FAPI tracer molecules (FAPI-4 in 16 patients and FAPI-46 in 3 patients)

Figure 2

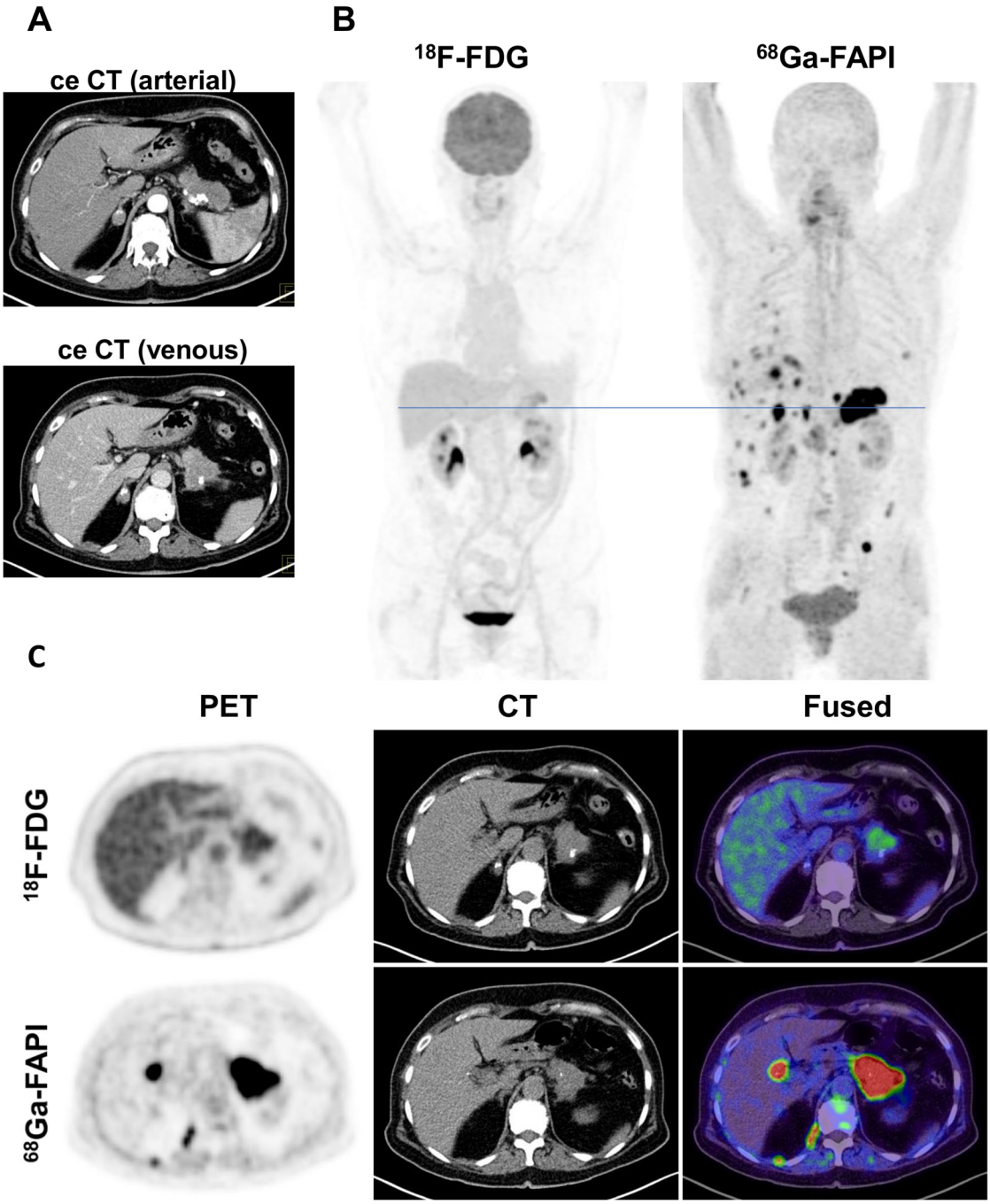


Figure 2

Primary staging of a patient with PDAC: **A** Axial images of the PDAC and the liver in arterial (upper image) and venous (lower image) contrast enhanced CT scan. **B** Mean

Intensity Projection (MIP) images of FDG - and FAPI - PET/CT imaging. **C** Axial FDG - and FAPI - PET/CT images of the same patient on the level (see blue line in A) of the pancreatic tumor mass and another suspicious FAPI accumulation in projection on a perihepatic lymph node. The metastatic situation, which had been revealed by FAPI-PET/CT was confirmed by biopsy of a pulmonary lesion that was diagnosed as metastasis of the known PDAC.

(!)

Figure 3

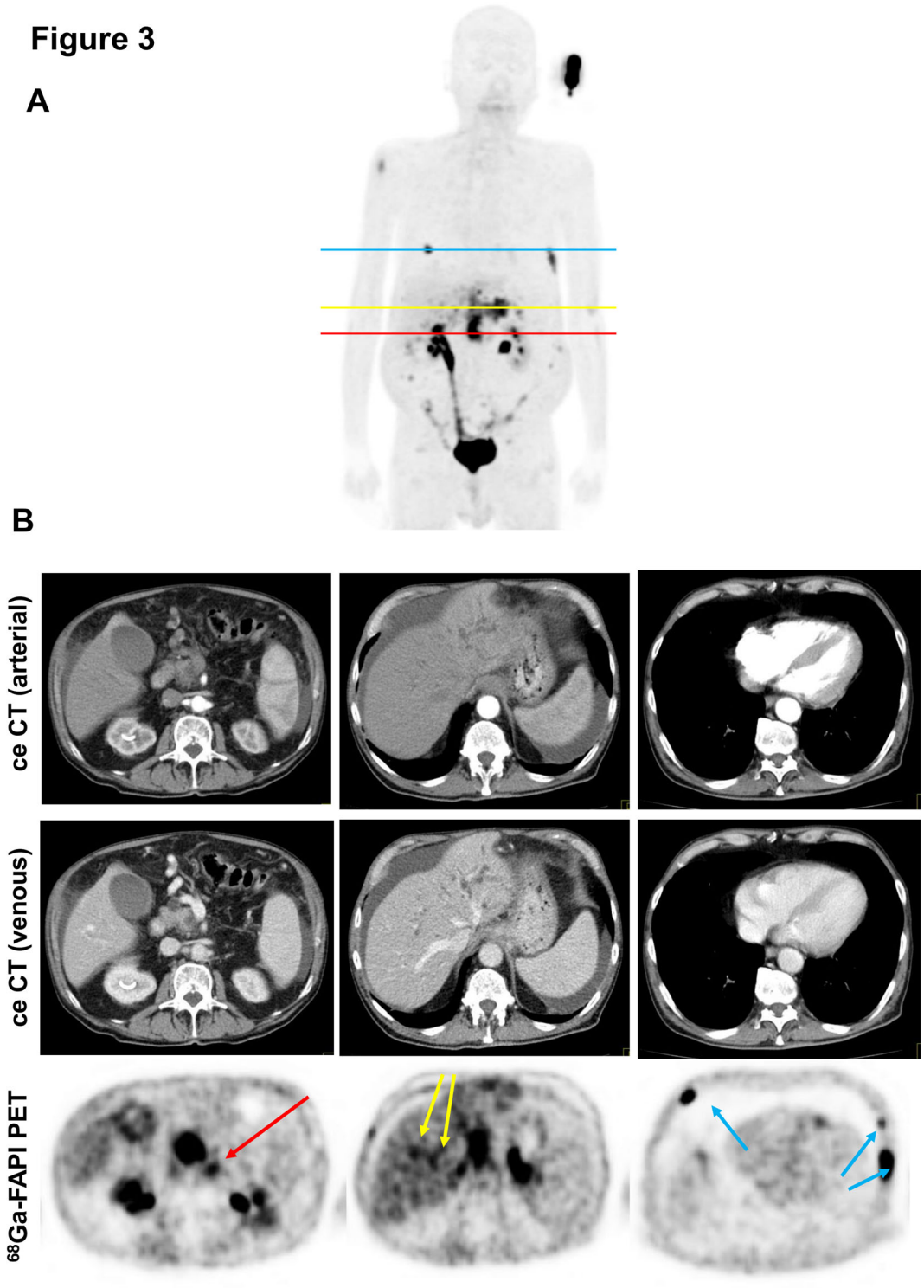


Figure 3

Staging of a patient with local recurrence of a PDAC: **A** Mean Intensity Projection (MIP) image of FAPI - PET/CT imaging. **B** Axial contrast enhanced CT and FAPI - PET/CT

images of the same patient on the level of the local recurrence (see red line in A), two metastasis suspicious intrahepatic foci (see yellow line in A) and the three suspicious osseous tracer accumulations (see blue line in A). In contrast to CT imaging, FAPI-PET/CT allows the discrimination of a metastatic lymph node from the local recurrence mass (red arrow). FAPI-PET/CT also revealed possible new liver (yellow arrows) and bone (blue arrows) metastases.

Figure 4

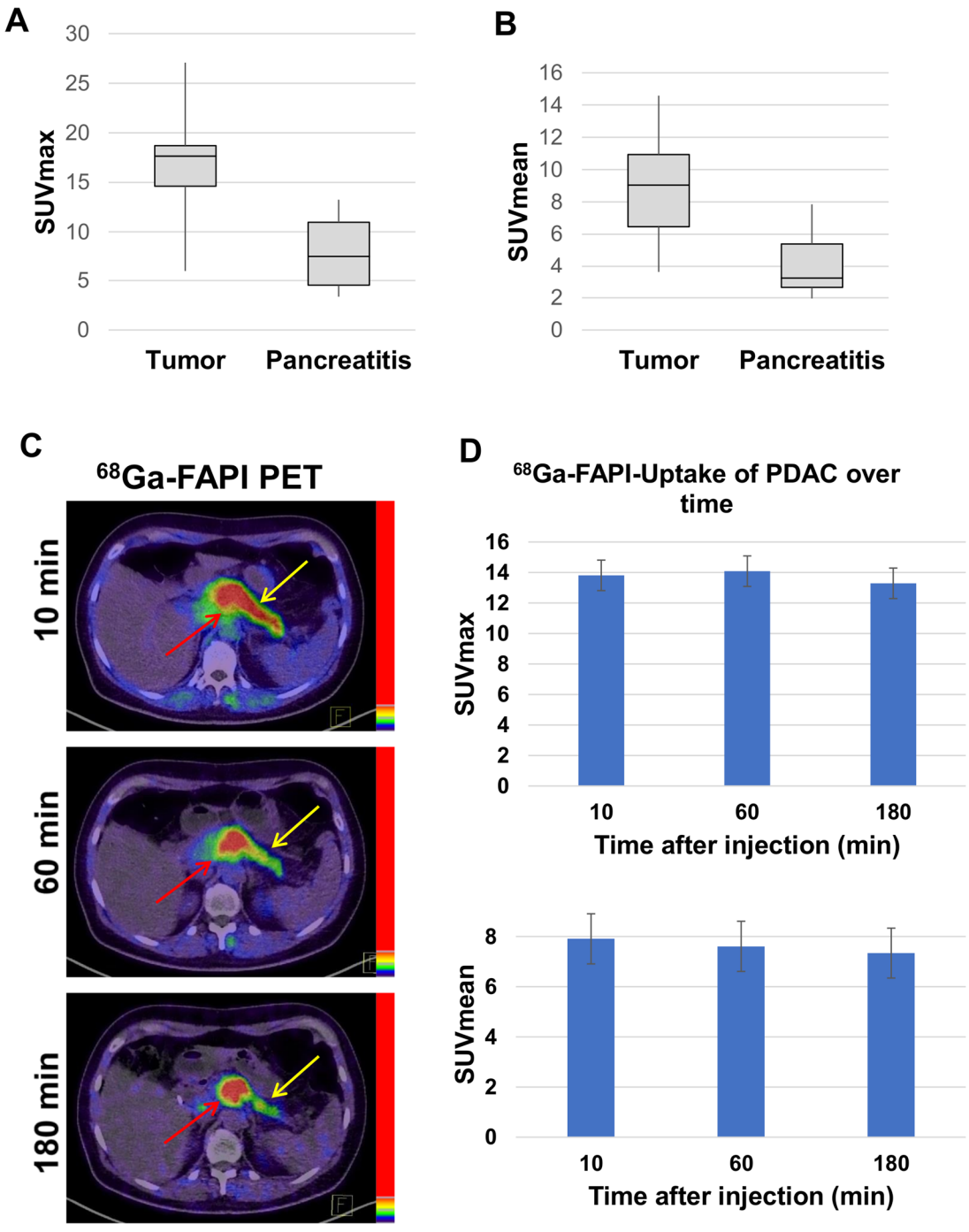


Figure 4

A, B Average SUVmax and SUVmean values 1 hour after injection of ^{68}Ga labelled

FAPI tracers in 8 PDAC and in accompanying pancreatitis in the rest of the pancreas. **C** Exemplary images of tumor related (red arrow) and pancreatitis related (yellow arrow) ^{68}Ga-FAPI uptake 10, 60 and 180 minutes after application. D ^{68}Ga-FAPI uptake 10, 60 and 180 minutes after application (SUVmax and SUVmean values) in PDAC lesions of 6 patients.

GRAPHICAL ABSTRACT

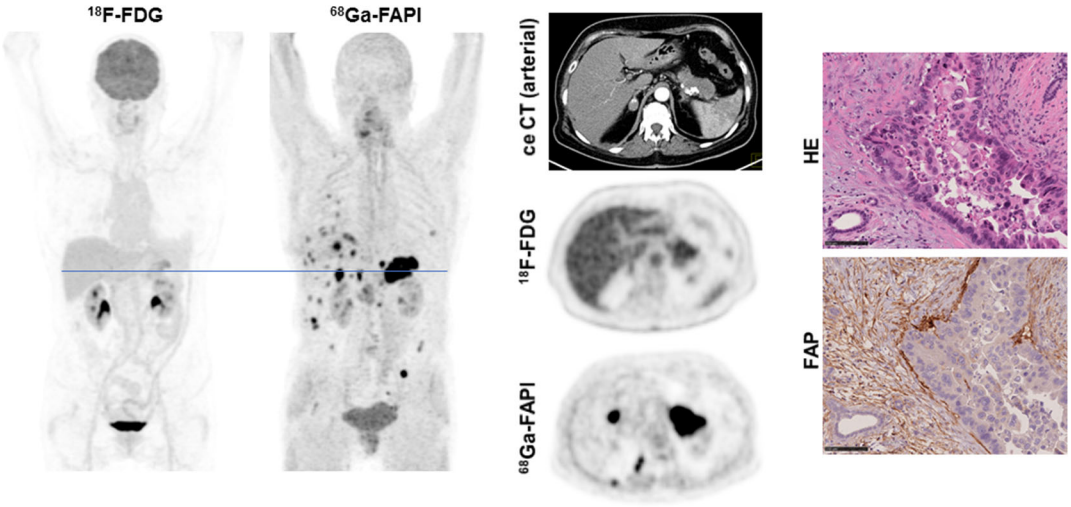


Table 1: Clinical data of 19 patients with PDAC examined by ^{68}Ga FAPI-PET/CT

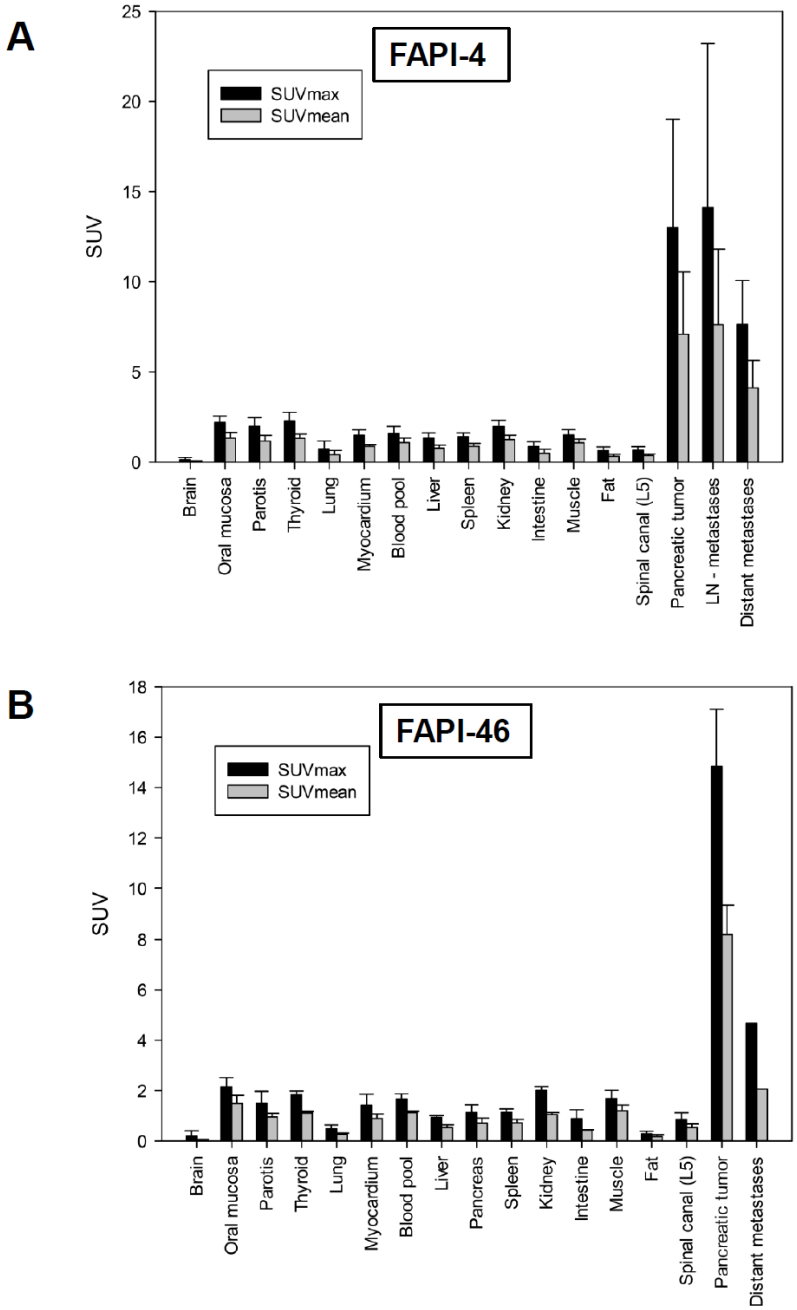
#	Age	Sex	Previous surgery	Previous chemotherapy	previous radiation	Clinical Indication	FAPI-Variant	Acquisition time (min. p.i.)
1	52	m	Whipple	FOLFIRINOX	mediastinal lymph nodes	Recurrence / PD	FAPI-4	60
2	52	m	Whipple	No	no	Recurrence / PD	FAPI-4	60
3	52	f	no	FOLFIRINOX	no	Recurrence / PD	FAPI-46	10/60/180
4	58	m	total pancreat-ectomy	FOLFIRINOX	no	Recurrence / PD	FAPI-4	60
5	58	m	no	No	no	Primary Staging	FAPI-4	60
6	59	f	distal pancreat-ectomy	Gem	no	Recurrence / PD	FAPI-46	10/60/180
7	60	m	no	FOLFIRINOX	no	Recurrence / PD	FAPI-4	60
8	60	m	no	No	no	Primary Staging	FAPI-4	60
9	61	f	Whipple	Gem + Cap	no	Primary Staging	FAPI-4	60
10	64	f	Whipple	FOLFIRINOX	no	Recurrence / PD	FAPI-4	60
11	64	m	distal pancreat-ectomy	FOLFIRINOX	no	Recurrence / PD	FAPI-4	60
12	65	f	Whipple	FOLFIRINOX	no	Primary Staging	FAPI-4	60
13	66	f	no	Gem	no	Primary Staging	FAPI-4	60
14	67	f	distal pancreat-ectomy	FOLFIRINOX	no	Recurrence / PD	FAPI-4	60
15	68	f	no	no	no	Primary Staging	FAPI-4	60
16	74	m	pp-Whipple	Cap + Ox	no	Recurrence / PD	FAPI-4	60
17	76	f	pp-Whipple	Gem + nab-paclitaxel	no	Recurrence / PD	FAPI-4	60/180
18	79	m	no	no	no	Primary Staging	FAPI-4	60
19	80	m	no	Gem + nab-paclitaxel	primary tumor	Recurrence / PD	FAPI-46	10/60/180

Abbreviations: Cap: capecitabine; FOLFIRINOX: 5-fluorouracil, leucovorin, irinotecan and oxaliplatin; Gem: gemcitabine; Ox: oxaliplatin; pp-Whipple: pylorus-preserving Whipple procedure

Table 2: Comparison of contrast enhanced CT-based and ^{68}Ga-FAPI-PET/CT based TNM staging of 19 patients with primary and recurrent/progressive PDAC

#	Clinical Indication	TNM stage (CT based)	TNM stage (FAPI-PET based)	Additional finding in FAPI-PET	staging change
1	relapse / progression	T1 N2 M1(LYM, **PUL**)	T1 **N0 M1 (LYM)**	(recurrent) mediastinal lymph node metastases	up
2	relapse / progression	T4 N0 M1(PER)	T4 N0 M1(PER)	none	none
3	relapse / progression	T3 N0 M0	T3 N0 **M1 (OSS)**	bone metastasis	up
4	relapse / progression	T4 N0 Mx	T4 N0 **M1 (PER)**	peritoneal carcinosis	up
5	Primary Staging	T1 N0 M0	T1 N0 M0	none	none
6	relapse / progression	T3 N0 M0	T3 N0 M0	none	none
7	relapse / progression	T4 N0 Mx	T4 N0 **M0**	none	none
8	Primary Staging	T4 N0 Mx	T4 N0 **M0**	none	none
9	Primary Staging	T4 N0 M0	T4 N0 M0	none	none
10	relapse / progression	T2 N0 M0	**T0** N0 M0	no local recurrence (T0)	down
11	relapse / progression	T4 N0 M1(LYM, HEP)	T4 **N2 M1**(LYM, HEP, **OSS**)	abdominal lymph node metastases, two more liver metastases, bone metastasis	up
12	Primary Staging	T4 N0 Mx	T4 N0 M0	none	none
13	Primary Staging	T3 N0 M0	T3 N0 M0	none	none
14	relapse / progression	T4 N2 M1(HEP)	T4 N2 **M1**(HEP, **PER**)	peritoneal carcinosis	up
15	Primary Staging	T4 N2 Mx	T4 N2 **M1(PER, PLE)**	pleural carcinosis, peritoneal carcinosis, liver metastases	up
16	relapse / progression	T0 N2 M1(LYM, HEP)	T0 N2 **M1(**LYM, HEP, **OSS)**	bone metastasis	up
17	relapse / progression	T2 N0 Mx	T2 N0 **M1(HEP, PUL)**	liver metastases without CT correlate, pulmonary metastasis	up
18	Primary Staging	T1 N0 Mx	T1 N0 M0	none	none
19	relapse / progression	T4 Nx M1(PER)	T4 **N1 M1(PER, OSS, HEP)**	lymph nodes definable from tumor conglomerate, bone metastases, liver metastases	up

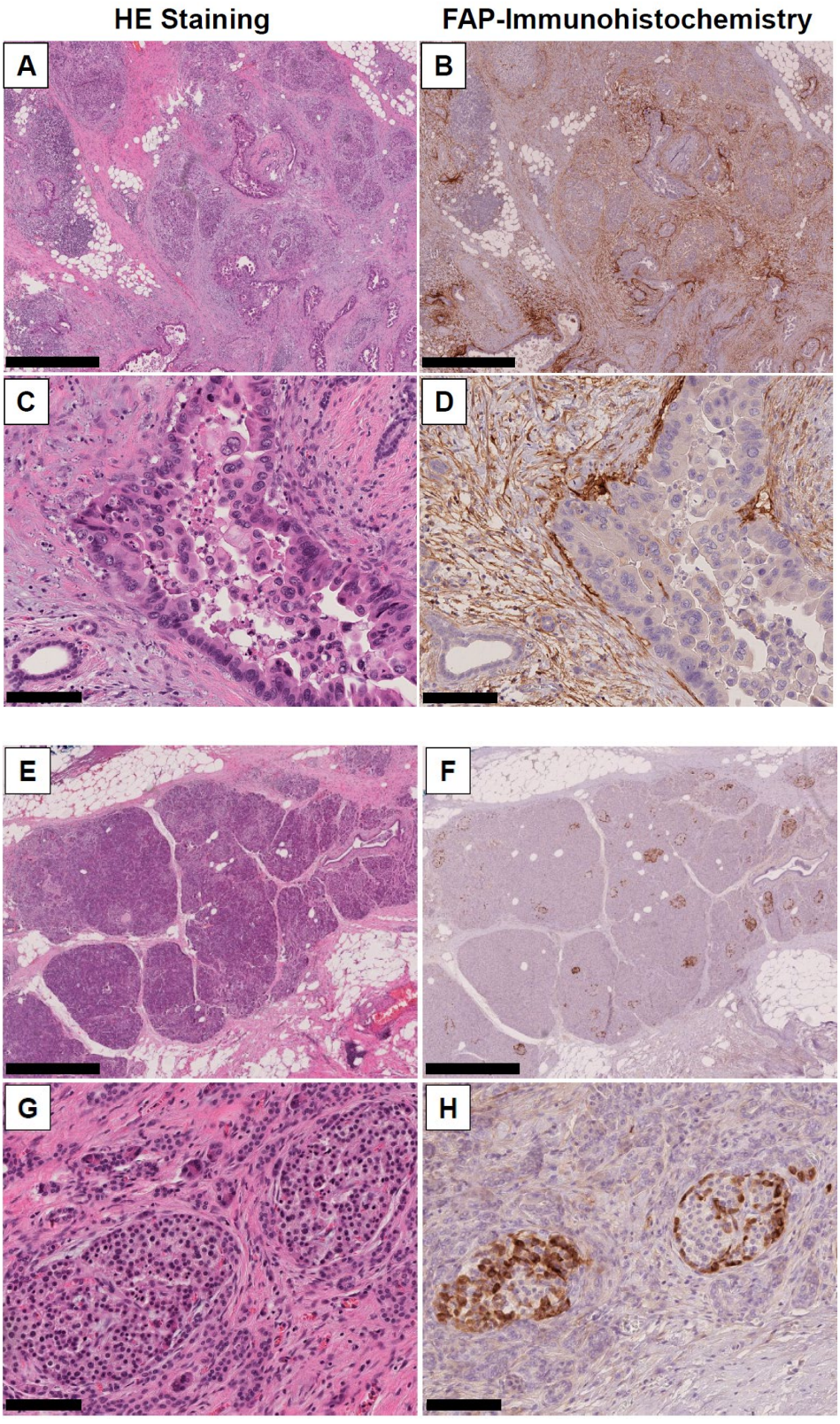
Supplementary Figure 1



Supplementary Figure 1

Separate Biodistribution analysis (SUVmax and SUVmean) of FAPI-4 (A, applied in 16 patients) and FAPI-46 (B, applied in 3 patients) in patients with PDAC based on PET/CT imaging 1 hour after injection of ^{68}Ga labelled tracer molecules.

Supplementary Figure 2



Supplementary Figure 2

Hematoxylin and eosin (HE) staining and FAP-immunohistochemistry of two exemplary PDAC. A-E shows a PDAC with strong stromal FAP expression and FAP-negative neoplastic cells. F-H shows a PDAC with less intensive stromal FAP-expression and FAP-positive islets within neoplastic cell clusters that may represent Langerhans islets. Scale bars: A, B: 1 mm, C, D: 100 mm, E, F: 1 mm, G, H: 100 mm