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Imaging Cancer-Associated Fibroblasts (CAFs) with FAPi PET

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Abstract

The tumor microenvironment (TME) surrounding tumor cells is a complex and highly dynamic system that promotes tumorigenesis. Cancer-associated fibroblasts (CAFs) are key elements in TME playing a pivotal role in cancer cells' proliferation and metastatic spreading. Considering the high expression of the fibroblast activation protein (FAP) on cell membrane, CAFs emerged as appealing TME targets, namely for molecular imaging, leading to a pan-tumoral approach. Therefore, FAP inhibitors (FAPis) have been recently developed for PET imaging and radioligand therapy, exploring the clinical application in different tumor sub-types. The present review aimed to describe recent developments on radiolabeled FAP inhibitors and evaluate the possible translation of this pan-tumoral approach in clinical practice.

At present, the application of FAPi-PET has been explored mainly in single-center studies, generally performed in small and heterogeneous cohorts of oncological patients. However, preliminary results were promising, in particular in low FDG-avid tumors such as primary liver and gastro-entero-pancreatic cancer, or in regions with unfavorable tumor-to-background ratio at FDG-PET/CT (i.e. brain), as well as in radiotherapy planning of head and neck tumors. Further promising results have been obtained in the detection of peritoneal carcinomatosis, especially in ovarian and gastric cancer. Data regarding the theranostics approach are still limited at presents, and definitive conclusion about its efficacy cannot be drawn at present. Nevertheless, the use of FAPi-based radio-ligand to treat the TME has been evaluated in first-in-human studies and appears feasible.

Although the pan-tumoral approach in molecular imaging showed promising results, its real impact in day-to-day clinical practice has yet to be confirmed, and multi-center, prospective studies powered for efficacy are needed.

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1. INTRODUCTION

Targeting the tumor microenviroment

Tumors are composed of two interdependent compartments: the malignant cells and the stroma, or tumor microenvironment (TME), that may account for up to 90% of the mass in common malignancy as breast, stomach, and pancreatic carcinomas. It is a highly dynamic and heterogeneous system composed of immune cells, fibroblasts, precursor cells, endothelial cells, signaling molecules and extracellular matrix (ECM) components which interact closely with tumor cells, contributing to tumorigenesis [1].

The natural progression of TMEs can be described in this sequence [2]:

1) Small cluster of homogeneous cancer cells. These tumors evaded immune surveillance due to their very early stage of development or because they're a newly metastasized colony.
2) Tumors with lymphocyte infiltration that release cytokines and directly engage with cancer cells recruiting blood cells into the tumor. At the same time, nearby macrophages and fibroblasts are converted into tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs).
3) Tumors without infiltrating lymphocytes, encapsulated by CAFs with ECMs. They're filled with many stromal cells including TAMs, CAFs and myeloid-derived suppressor cells (MDSCs) and doesn't release cancer cells into the blood circulation.
4) Tumors with a subgroup of cancer cells undertaking epithelial-mesenchymal transition, which downregulates some genes (such as E-cadherin, β-catenin, cytkeratins 5 and 6) and upregulates other genes (such as E-cadherin, vimentin, Snail, Slug, Twist, ZEB1 and 2, S100A4, MMP2 and 3, α-smooth muscle actin), so these tumors become metastasized activating mobility-enhancing genes (such as S100 CBPP) releasing cancer cells into the blood circulation often chaperoned with stromal cells.

Accordingly, key elements in tumor stroma are CAFs: metabolically active, spindle-shaped cells with enhanced proliferative and migratory properties that release many growth factors and proinflammatory cytokines, such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6). The causes for transformation of fibroblast to CAF are not entirely understood. However, the transformation is driven by the occurrence of mutations, including inactivation of TP53 and PTEN [3] and loss of heterozygosity (LOH) [4].

Through this biochemical crosstalk with the surrounding cells of TME, CAFs play a pivotal role in cancer cell invasion, migration and growth, metabolic reprogramming, immunosuppression, and

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angiogenesis [5]. A distinguishing feature of CAFs is their high expression of fibroblast activation protein (FAP), a type II membrane-bound glycoprotein belonging to the dipeptidyl peptidase 4 (DPP4) family. FAP has both dipeptidyl peptidase and endopeptidase activity, has a large extracellular domain and is associated with the regulation of the extracellular matrix [6] (Fig. 1). This integral protein is coexpressed with DPP4 in the alpha cells of Langerhans-islets, in multipotent bone marrow stromal cells and is also slightly present in the cervix and uterine stroma (during the proliferative cycle) [7, 8, 9]. In contrast to CAFs, normal fibroblasts have no or very slight FAP expression and, therefore, FAP expression is low in normal adult human tissues. Like CAFs, FAP expression is instead increased in activated fibroblasts in the case of tissue damages, remodeling, or inflammation and, therefore, in benign conditions such as wound healing, arthritides and myocardial infarction [10, 11, 12].

Developing PET radiopharmaceuticals

The differential expression of the protein in normal tissue compared with tumors/inflammation makes FAP a promising target for molecular imaging of a large variety of tumors and for some non-oncological diseases. For this purpose, FAP-targeting radiopharmaceutical based on FAP-specific inhibitors (FAPis) such as ^{68}Ga-FAPi-02 and ^{68}Ga-FAPi-04 have been recently developed [13, 14]. These quinoline-based radiotracers bind to the enzymatic domain of FAP with very high specificity, and the complex is then rapidly internalized. Biodistribution studies on tumor-bearing mice and on mixed population of different cancers showed high intratumoral uptake of the tracers and fast renal clearance, with very low uptake in normal organs (especially brain, oral mucosa, and liver), leading to higher tumor-to-background ratio (TBR) and improving diagnostic performance of PET imaging [13, 15, 16].

The use of DOTA or other chelators offers the possibility of easily incorporating therapeutic isotopes such as ^{177}Lu or ^{90}Y in these compounds, allowing a theranostic approach [14]. Nevertheless, the therapeutic application of FAPi tracers is still impaired by their relatively short tumor retention time, even if FAPi-04 already obtained an improvement in tumor retention compared to FAPi-02 (75% washout) with a 50% of tumor uptake from 1 to 3 hours after injection. Lately was developed FAPi-46 which consent a theranostic approach due to its longer tumor residence time [17].

Attempts were initially made to label FAPi with covalently attached ^{18}F and there were favorable results with NOTA-containing FAPi-74 [18]. Indeed, due to the longer half-life of ^{18}F and lower positron energy than ^{68}Ga (half-life of 110’ and positron energy 0,65-MeV versus 68’ and 1,90-

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MeV), labelling FAPi with ^{18}F would reduce costs for production in case of on-site cyclotron and facilitate its distribution together with improved spatial resolution due to the lower positron energy [19]. In addition, local on-demand production in centers already equipped with $^{68}Ge/^{68}Ga$ generator would also be simplified by using NOTA chelator, allowing chelation with ^{68}Ga at room temperature. [18]. Conversely, the use of $^{68}Ge/^{68}Ga$ generators in clinical practice is impaired by the size of the generator and so by the maximum of patients that can be handled per-synthesis as well as by the increasing prices of generators [53].

FAPI radiopharmaceuticals demonstrated intense radioactivity in the urinary tract, with kidneys as the main excretory organs. Uptake of the radioactivity was also observed in the gallbladder and common bile duct, implying elimination via hepatobiliary system as well. Moderate uptake of radioactivity was observed in other organs, including the submandibular gland, thyroid, and pancreas. Only minimal or mild physiological uptake was observed in other organs and tissue, including brain, parotid, oral mucosa, lung, myocardium, liver, intestine, fat, spine, and muscle. Differences in biodistribution of [^{18}F]FAPI-42 and [^{68}Ga]Ga-FAPI-04 in normal organs might be due to the different lipophilicity of the NOTA-chelator and DOTA-chelator groups. This might influence the detection of lesions in specific regions, especially for pancreatic, gallbladder, and biliary tract tumors [54]. Drug-related pharmacologic effects or physiologic responses have not been never observed so far in clinical studies implying the use FAP inhibitors for PET imaging. The radiopharmaceuticals injection was well tolerated, and no side effects have been described.

2. MATERIALS AND METHODS

Search strategy

A comprehensive search was performed using the Ovid platform and comparing Embase and Medline databases. No time restrictions were applied. The following search strategy was used: (“cancer associated fibroblast” OR “cancer-associated fibroblast” OR “CAF” OR “CAFs”) AND (“fibroblast activation protein” OR “fibroblast activation protein inhibitor OR “FAPi” OR “FAP”) AND (“Positron Emission Tomography” OR “PET”). Web search was implemented with manual search (authors consultation and web-search included articles). Only studies in English were selected. The literature search was updated until December 10th, 2021.

Article selection and data extraction

According to PRISMA guidelines, from all studies, we selected for this review the most relevant articles, evaluating manuscript reporting about the use of radiopharmaceuticals for PET imaging

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targeting cancer associated fibroblast with fibroblast activation protein inhibitors, based on the following criteria: (1) original article or case series or case reports in the (2) English Language regarding the (3) use of PET other FAPi (either ^{68}Ga or ^{18}F). Two authors (LG and LSAF) independently reviewed abstracts and titles of the retrieved studies for inclusion in the review based on the inclusion criteria and removed duplicates. A second review was performed to delete additional studies outside the scope of this review. Disagreements were resolved through consultation with a third author (FC) or consensus. The authors tabulated and organized relevant studies and performed a comprehensive qualitative narrative synthesis of both tabulated studies and non-tabulated articles.

3. RESULTS

Oncological setting: Pan-tumoral radiotracer

Different tumor types might share common driver mutations: this assumption underlies the concept of pan-cancer analysis [20]. FAP-targeting radiopharmaceuticals might be potentially superior to ^{18}F-FDG and thus there is growing interest in its application as pan-tumoral radiotracer. This approach inspired a translational prospective exploratory study [21] evaluating the role of FAPi-PET as a pan-cancer imaging biomarker for FAP expression in 141 patients with 14 different types of cancer (bile duct, bladder, breast, esophagus, colon, liver, stomach, lung, ovary, uterus, oropharynx, prostate, pancreas, and kidney). All patients were eligible for surgery and, thus, immunohistochemical confirmation of FAP expression was obtained in all cases. The authors found that FAPi-PET was positive in more than 50% of cases from eleven cancer types, with variability in the intensity of FAP expression strongly associated with FAP expression assessed by immunohistochemistry in surgery specimen. FAP expression was higher in cancers of bile duct, bladder, colon, esophagus, stomach, lung, oropharynx, ovary and pancreas, average in breast and uterus cancer, whereas liver, prostate and renal cell cancer showed only low FAP expression. In addition, all four evaluated metastatic lesions were FAPi-avid, which has important implications in theranostics. Although this study showed how FAPi could play a central role in the context of pan-cancer analysis, the limited number of patients enrolled for each tumor sub-types affected the study reproducibility and, thus, broader consideration cannot be drawn at this stage.

A similar conclusion was obtained in a previous study reporting [19] about FAPi-PET uptake in 80 patients and 28 different tumor types. The primary aim was to assess if FAPi-PET might improve tumor delineation (e.g., for radiotherapy planning) in patients with inconclusive or clinically

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unsatisfactory standard of care imaging. The highest uptake was detected in patients with sarcoma, esophageal, breast, cholangiocarcinoma, and lung cancer, whereas pheochromocytoma, renal cell, differentiated thyroid and gastric cancers were the lowest. Furthermore, the authors also found that despite the high intratumoral and interindividual variability, the low background activity, resulting in high tumor-to-background ratios, leads to excellent image contrast (Table 1).

Oncological setting: Gliomas, Primary Liver Cancer and Gastro-Entero-Pancreatic cancers

First experiences in mixed populations demonstrated that FAPi, unlike FDG, had significant lower background distribution in brain, liver and oral/pharyngeal mucosa and very low unspecific gastric and intestinal/peritoneal uptake [13, 15]. These characteristics make FAPi a very promising radiopharmaceutical for the detection of moderate-to-low FDG-avid tumors and for the evaluation of primary and secondary lesions in regions with low TBR at FDG-PET/CT, such as the brain and the liver. In their pilot study [22], Röhrich and colleagues characterized the uptake of FAP ligands in 18 glioma patients, 13 with isocitrate dehydrogenase (IDH)-wildtype glioblastoma WHO grade IV and 5 with IDH-mutant glioma. The authors observed elevated tracer uptake in IDH-wildtype glioblastomas and WHO grade III/IV IDH-mutant astrocytomas, but not in WHO grade II astrocytomas. Therefore, if these findings will be confirmed in larger populations, FAPi-based PET/CT may be useful in non-invasive characterization of gliomas and of their malignant progression from grade II to higher tumor grades. Moreover, the same 13 patients with IDH-wildtype glioblastomas were evaluated for radiotherapy planning [23]. FAPi-PET-based gross tumor volumes (GTVs) were incongruent with MRI GTVs. Indeed, increases of GTVs were highly significant for all FAP-specific PET thresholds. The clinical and therapeutic impact of these results has yet to be addressed.

Primary hepatobiliary tumors showed different patterns of FDG-uptake. Hepatocellular carcinoma (HCC), the most frequent primary tumor of the liver, is characterized by an FDG-uptake equal to the normal liver tissue, leading to a high false-negative rate in FDG-PET detection. Due to its favorable distribution characteristics, FAPi could overcome these performance deficiencies.

Indeed, recent studies demonstrated higher FAPi uptake in HCC and intrahepatic cholangiocarcinoma (ICC) compared to FDG, with a significantly higher sensitivity [24]. Moreover, FAPi-PET/CT proved to have an equivalent sensitivity for the detection of primary tumors compared to contrast-enhanced CT and MRI and a significantly higher detection rate than FDG-PET for all malignant lesions, including extrahepatic disease [25]. Guo et al demonstrated also an increased ^{68}Ga-FAPI-04 uptake in liver parenchyma of patients with cirrhosis, with a significantly

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higher FAPi-TBR in patients without cirrhosis [25]. Further studies are needed to evaluate the impact of these result on the diagnostic performance of FAPi-PET, in terms of definition of the correct extension of the disease and the detection of small intrahepatic lesions. FAPi represents an ideal radiopharmaceutical to detect liver metastasis as well, due to the lower physiological uptake, leading to potential upstaging and exact identification of tumor locations. In addition, targeted and personalized treatments such as liver radio-guided surgery, image-guided radiotherapy, or radio-embolization might be proposed based on the information derived by FAPi-PET. However, several benign conditions including hepatic cirrhosis showed FAPi uptake, thus limiting the role of this radiotracer in primary liver cancer, since the presence of hepatic cirrhosis is a common risk factor especially in patients affected by HCC.

Gastroenteropancreatic (GEP) tumors often disseminate to the liver. In this setting FAPi-PET was found to be superior to FDG-PET in detection of liver metastases in small studies including patients with gastric, colorectal, and other GEP tumors [26, 27]. Besides, FAPi-PET demonstrated higher detection rate for primary GEP tumors compared to FDG-PET, with higher TBR and clearer tumor identification [27], considering the lower physiological gastric or bowel uptake. In a retrospective, bicentric study focused on gastric cancer staging, FAPi-PET was superior to FDG-PET for the detection of the primary tumor, with a sensitivity of 100% vs 82%. In particular, the radiopharmaceutical FAPi outperformed FDG in signet ring cell carcinoma (7/7 vs 4/7), probably due to the low expression level of glucose transporter 1 in this histological type. SUVmax of T2-4 tumors was significantly higher than SUVmax of T1 tumors in FAPi-PET, highlighting the possibility of a non-invasive evaluation of the infiltration degree in primary gastric cancers [28] (Table 2). These preliminary and encouraging results show that, in the future, FAPi-PET may play an important role in brain, liver and GEP cancers, outperforming FDG, and need a validation in prospective clinical trials on larger samples.

Oncological setting: Head and neck cancers

Accurate tumor staging is crucial in head and neck cancer for adequate treatment choice, namely the identification of contralateral metastasis. Despite the use of contrast enhanced CT and MRI, radiological imaging often fails to correctly identify the extension of the disease, due to the diffuse tumor infiltration in complex structures and to the presence of underlying and concomitant inflammatory processes. FDG-PET is affected by several limitation as well, mainly related to high physiological uptake in healthy tissue such as salivary glands, brain and oral cavity, inflammatory uptake in cervical muscles or lymph nodes. Also, the presence of FDG-avid brown adipose tissue

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may impair image reading in FDG-PET scan. In this scenario, FAPi-PET could provide a potential solution, as highlighted by the first single-center studies performed in a cohort of nasopharyngeal carcinoma patients [29, 30].

In both papers, FAPI-PET proved to be superior to FDG for exact T staging. Low tracer uptake in normal structures adjacent to the tumor allowed imaging of primary tumors with higher TBR and better lesion delineation. Skull base and intracranial invasion are clearly visualized on ^{68}Ga-FAPI PET due to the low brain tissue uptake. Further studies [31, 32] confirmed that FAPi-PET lead to significant upstage of the disease compared to FDG-PET with consequent change in the therapy management. However, some concerns are still related to definition of positive lymph-node since inflammatory nodes might express low FAP overexpression (Table 3).

Nevertheless, the main clinical application for FAPi-PET in head and neck cancer is the planning of image-guided radiotherapy, especially considering the high TBR. Recently, improved target volume delineation has been reported in comparison with contrast enhanced CT and MRI [23, 33]. This image-guided approach might bring substantial implications in the planned target volume, (PTV) probably leading to better regional control of disease and less toxicity due to the more precise identification of the treatment field. Future studies are needed to define the optimal imaging time and thresholds of FAPi uptake for PTV delineation. Personalized radiotherapy approach can be drawn according to FAPi-PET images, with precise radiation dose escalation or de-escalation plans for tumor subvolumes or with plan adaptation due to microenvironment changes during treatment.

***Oncological setting*: Breast cancer**

Breast cancer (BC) is a heterogeneous disease in terms of pathological features, biological behaviors, therapeutic response, and prognosis. The tumors can be classified into subtypes distinguished by pervasive difference in their gene expression patterns and, consequently, phenotypes, where the key players are the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) [34, 35]. The different status of receptor expression serves as biomarker for the choice of specific and tailored treatment strategies. Although chemotherapy and targeted therapies have improved the survival of BC patients, many patients relapse or do not respond to first-line therapies. In the last years, it has become increasingly evident that breast cancer evolution is not solely dependent on the behavior of cancer cells, but also on the composition and biological function of TME and on the interactions between cancer cells and TME itself. CAFs represent the most abundant cell type of BC

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microenvironment [36] and, consequently, FAP is an excellent candidate as indirect tumor cell target.

To date, one prospective study assessed the role of FAPi-PET in patients with breast cancer, in comparison with FDG PET/CT [37]. Authors enrolled 20 patients with newly diagnosed or relapsed BC (15 and 5 patients, respectively). ^{68}Ga-FAPI-04 PET/CT was superior to FDG-PET in detecting primary tumors, with 100% and 95.6% of sensitivity and specificity, respectively. Moreover, FAPi-PET detected more lymph node, hepatic, bone, and brain metastases due to the lower background activity and higher uptake in subcentimetric lesions. Future studies should focus on the role of FAPi-PET in diagnosis of different molecular and histological subtypes of BC (as FDG-PET has known limitation in low-grade hormone-positive tumors and in lobular carcinomas) as well as on its potential in early detection of disease relapse, in the assessment of therapy response and of patient's prognosis.

Another attractive application in BC field concerns the use of FAPi-PET as a guide for the selection of patients for radio-ligand therapy (RLT) with FAP-specific inhibitors, using high energy β-emitters, like Lutetium-177. This theranostic approach could be of great value for patients with triple negative tumors, where currently used agents targeting ER, PR and HER2 are ineffective.

Oncological setting: Peritoneal carcinomatosis

Peritoneal carcinomatosis (PC) is a complication regarding several malignancies and generally associated with a poor outcome. Total peritonectomy and resection of involved tissue with intraperitoneal chemotherapy is a primary treatment of PC with curative intention [38]. Two of the most important prognostic factors are the extent and volume of the PC. Thus, the pre-operative evaluation of possible peritoneal involvement is crucial and, currently, exploratory surgery is the gold standard for PC detection [39]. Conventional imaging (CT or MRI) is obviously a less invasive procedure compared to surgery. FDG-PET showed good diagnostic accuracy to detect PC, even if is impaired by spatial resolution limitation, and pathological peritoneal thickness does not always show increased glycolytic metabolic activity [40]. Therefore, FAPi-PET might emerge as leading diagnostic procedure in this specific setting. Recently, in a retrospective analysis ^{68}Ga-DOTA-FAPi-04 PET/CT was compared to FDG-PET for evaluating PC in 46 patients (13 with gastric cancer, 10 colorectal, 9 ovarian, 6 pancreatic, 2 lung, 2 appendiceal, 1 cervical, 1 endometrial and 1 breast cancer and 1 primary PC). Authors observed a significant difference in standard uptake values (SUV) of lesions, namely in PC from gastric cancer. FAPi-PET showed a higher peritoneal cancer index (PCI) and better sensitivity than FDG PET [41]. Likewise, a case report about a 63-year-old

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man presented with rising carcinoembryonic antigen levels and unknown primary, who perform both FDG-PET and FAPi-PET, demonstrate that FAPi imaging was superior in the detection of primary gastric lesion (not FDG-avid) and a higher number of mesenteric nodules [42]. Another case report about a 60-year-old woman treated for BC showed that FAPI-PET detected more bone lesions and more favorable TBR for peritoneal nodules compared to FDG-PET [43]. Lastly a case report of a 55-year-old woman, who underwent FDG-PET and FAPi-PET, showed higher accuracy for FAPi imaging in the correct identification of mesenteric and omentum nodules, while both techniques correctly identified the primary pancreatic lesion. Follow-up FAPi-PET performed after three months, was able to assess the response to cytoreductive surgery [44].

Radioligand therapy targeting the tumor micro-environment

Theranostics is a neologism that merges the words therapeutics and diagnostic, defining the presence of a specific target that can be equally used both for PET imaging and radio-ligand therapy (RLT). Theranostics is part of nuclear medicine since decades, starting from the use of radioactive iodine-131, to the application in neuroendocrine tumors labeling somatostatin analogues with ^{68}Ga-, ^{177}Lu- or ^{90}Y-labeled somatostatin analogs and, more recently, in prostate cancer targeting the prostate-specific membrane antigen (PSMA). The concept of "TME targeted RLT" is, at present, only a theoretical approach. Nevertheless, FAP inhibitors can be proposed as theranostics agent, as FAPi-ligands are chelator-based containing DOTA, which can be labeled to different isotopes. All solid tumors require stroma to grow beyond a minimal size of 1-2 mm and generate it activating host's wound-healing response. Given the long-held notion of tumors as "wounds that do not heal" [45], and the increasing knowledge about the role of TME in tumorigenesis, the innovative approach of FAPi-based RLT consists in the treatment of the supporting system that allows cancer cells to grow and reproduce. First in-human studies evaluated RLT with ^{90}Y-FAPI-46, ^{177}Lu-FAPI-46 and ^{177}Lu-FAP-2286 on 9 [45], 18 [46] and 11 patients [47] with different cancers, respectively, and demonstrated the feasibility of this therapeutical approach. RLT was well tolerated, with acceptable side effects (predominantly haematological) and low radiation doses absorbed by non-target tissues, including kidneys. For ^{177}Lu-FAP-2286, kidney absorbed dose was comparable to that of ^{177}Lu-PSMA-617 and to that delivered by ^{177}Lu-DOTATATE with renal protection through amino-acid administration. Whole-body and bone marrow absorbed dose were similar, too [47]. The studies cohort were too small and heterogeneous (and most of all composed by heavily pre-treated patients and/or metastatic high-burden cases). Thus, any reliable conclusion regarding FAPi-based RLT efficacy cannot be

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drawn at this stage. At present the clinical application of RLT targeting the TME is only theoretical, and dedicated studies powered by efficacy are needed, since different tumor subtypes responding to TME targeted RLT, as well as the injected doses, types of radionuclides (α and/or β emitters) and the development FAP inhibitors with prolonged retention are under still investigation.

Non-oncological disease

FAP overexpression is observed in many healing processes and benign conditions like fibrosis, thus open to a hypothetical application of FAPi-PET even outside oncology. Fibroblasts play a pivotal role in cardiac tissue remodeling and wound healing [11] and the expression of FAP by activated cardiac fibroblasts increases after myocardial infarction and then declines over time [48]. A reduction in cardiac fibrosis and a restoration of systolic function have been achieved by ablation of FAP-positive cells in mice subjected to angiotensin II and phenylephrine [49]. Moreover, a retrospective study analyzing scans of 229 patients showed an association between a high FAPi signal intensities and the presence of a metabolic risk factor (such as arterial hypertension, diabetes mellitus and obesity) and an increased focal uptake could be suggestive of an underlying cardiovascular disease [50].

FAPi-PET is also showing a role in IgG_4-related diseases (IgG_4-RD). A case report of a patient with IgG_4-RD undergoing both FDG-PET and FAPi-PET showed that this quinoline-based radiotracer had an abnormal uptake in the uncinate process of the pancreas, in addition to sites indicated by FDG-PET/CT, except for some supra- and subdiaphragmatic lymph nodes [51]. Another similar case report demonstrated increased uptake in the lacrimal glands in FAPi-PET that was negative on FDG-PET but, as in the previous case, FDG-avid lymph nodes were negative on FAPi-PET [52].

CONCLUSION

Exploring the TME with molecular imaging is an attractive field of investigation and PET imaging with FAPi-PET is now gaining attention as “pan-tumoral” radiopharmaceuticals since CAFs are activated in many tumor subtypes. However, at present information regarding the feasibility and efficacy of FAPi-PET derive by single-center studies only, enrolling small and heterogenous cohorts of patients. The preliminary results are promising, as FAPi-PET seems to allow better evaluation of tumors with low FDG-avidity, leading to tumor upstage through the detection of unknown distant metastases (especially in case of peritoneal carcinosis) and an improved target volume delineation for radiotherapy planning. Furthermore, FAPi-PET allows the *in vivo* visualization of TME, leading to a better comprehension of tumor heterogeneity, namely when performed together with FDG-

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PET. Finally, first in-human studies highlighted the feasibility of radioligand therapy with FAPi inhibitors, targeting the TME in different tumor subtypes. Multi-center studies in larger cohorts, together with histological validation of FAPi-PET findings, are needed to assess the efficacy of FAPi based imaging and to understand if, and when, the translation into clinical practice of this new promising approach would be feasible.

Compliance with ethical standards

Not applicable.

Informed consent

Not applicable.

Conflicts of interest

The authors declare that they have no conflict of interest in relation to this work.

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TABLES AND FIGURES

Figure 1 - Tumor microenvironment consists of tumor cells and nonmalignant cells such as lymphocytes, macrophages, NK killer cells, normal epithelial cells and activated fibroblasts (CAFs).

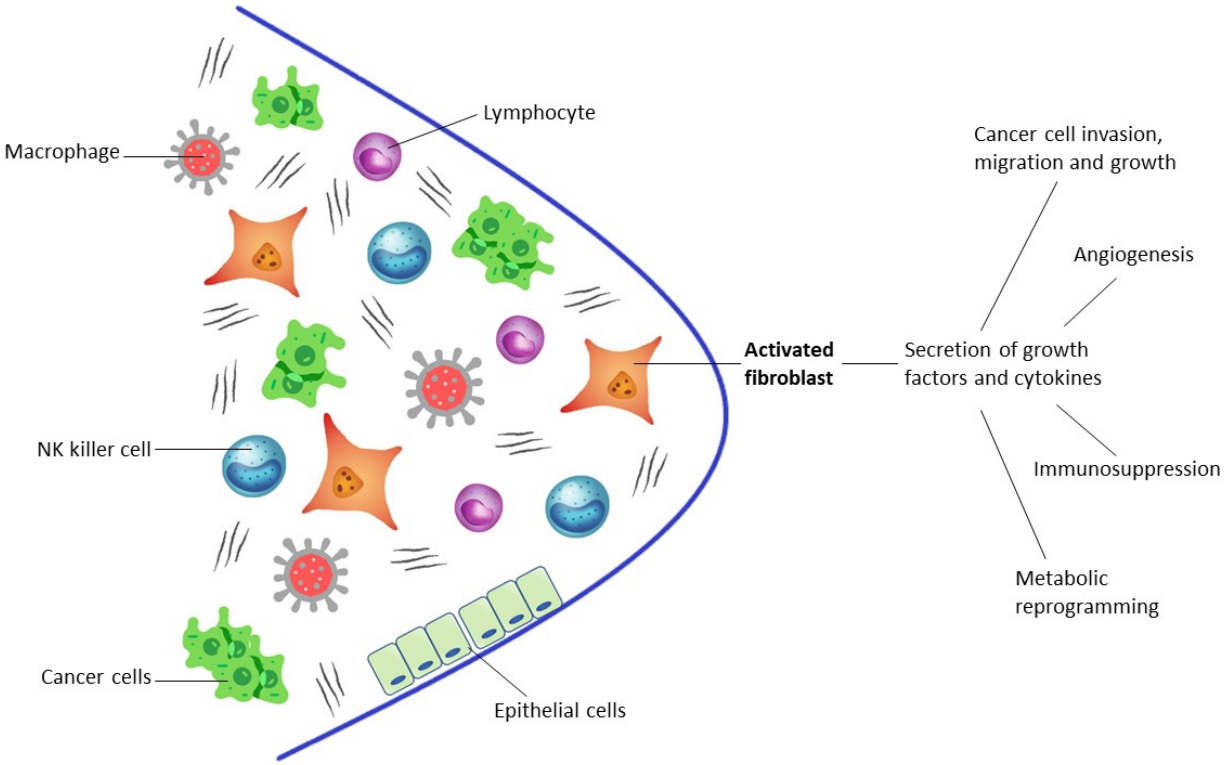

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Figure 2 40-year-old man with gastric signet cell cancer underwent gastrectomy 6 months before. Patient referred for re-staging after completion of adjuvant treatment. ^{18}F-FDG PET/CT (a) did not show any FDG-avid lesions, while there were suspicious lesions in the peritoneum and bone on the CT images (d, e). ^{68}Ga-FAPi-04 PET/CT was performed 11 days later (j) showing multiple abdominal, mediastinal, and supraclavicular lymph nodes (f, g) and multiple sclerotic bone lesions (i) with increased uptake, consistent with metastasis. FAPi-PET also demonstrated multiple peritoneal nodules with increased FAP expression (h, j), consistent with peritoneal carcinomatosis. A, Maximum intensity projection (MIP) image of ^{18}F-FDG PET/CT; b-e, axial PET and CT fusion images of ^{18}F-FDG PET/CT; f-i, axial PET and CT fusion images of ^{68}Ga-FAPI-04 PET/CT; j, MIP image of ^{68}Ga-FAPI-04 PET/CT.

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Figure 3 - 40-year-old woman with metastatic breast cancer status post total mastectomy and axillary lymph node dissection referred for ^{18}F-FDG PET/CT due to increased Ca 15-3 levels. The ^{18}F-FDG PET/CT (a) did not show any FDG-avid lesions. A ^{68}Ga-FAPI-04 PET/CT was performed 5 days later. FAPİ-PET showed multiple liver (e), lymph nodes (f), lung and bone (g,h) lesions with increased uptake, consistent with metastasis. A, Maximum intensity projection (MIP) image of ^{18}F-FDG PET/CT; b-d, axial PET and CT fusion images of ^{18}F-FDG PET/CT; e-g, axial PET and CT fusion images of ^{68}Ga-FAPI-04 PET/CT; h, MIP image of ^{68}Ga-FAPI-04 PET/CT.

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Figure 4 - 76-year-old woman with recurrent ovarian granulosa cell tumor who underwent hysterectomy and bilateral salpingo-oophorectomy approximately two years before. ^{18}F-FDG PET/CT (a) revealed a semisolid mass lesion without FDG uptake (c) and a non-FDG avid cystic lesion (d) in the peritoneal cavity. ^{68}Ga-FAPI-04 PET/CT was performed 10 days later. FAPİ-PET showed increased FAPI uptake in the two lesions (f, g) together with multiple FAPI-avid peritoneal nodules (e, h). A, Maximum intensity projection (MIP) image of ^{18}F-FDG PET/CT; b-d, axial PET and CT fusion images of ^{18}F-FDG PET/CT; e-g, axial PET and CT fusion images of ^{68}Ga-FAPI-04 PET/CT; h, MIP image of ^{68}Ga-FAPI-04 PET/CT.

Table 1 - *Oncological setting: Pan-tumoral radiotracer*

Authors	N° of patients	Tumor type	Clinical setting	Injected activity	Acquisition timing	Image analyses	Reference Standard	^{68}Ga-FAPI performance	Highest FAPi uptake	Lowest FAPi uptake
Mona CE et al. [21]	141	Various cancer (14 types)	Biodistribution and kinetics	174-185 MBq	54-96 minutes	S	HP	SE 80.9%	Bile duct, bladder, colon, esophagus, stomach, lung, oropharynx, ovary and pancreas cancer	Liver, prostate and renal cell cancer
Kratochwil C et al. [15]	80	Various cancer (28 types)	Staging, Restaging, RT planning	122–312 MBq	60 minutes	S	HP, imaging follow-up	Low uptake (≤ 6): 7/28 TT; Medium uptake (6>x<12): 14/28 TT; High Uptake (≥ 12): 7/28 TT	Lung, breast and esophageal cancer, cholangiocellular carcinoma and sarcoma (SUVmax ≥12)	Pheochromocytoma, renal cell, differentiated thyroid, adenoid cystic and gastric cancer (SUVmax ≤6)
Chen H et al. [31]	68	Various cancer (13 types)	Staging, Restaging	1.8-2.2 MBq/Kg	60 minutes	V, S	HP, imaging and clinical follow-up	T: SE 86.4%	T: liver, gastric, pancreatic and cervical cancer	T: oro-esophageal and lung cancer
Chen H et al. [32]	75	Various cancer (12 types)	Staging, Restaging	1.8-2.2 MBq/Kg	60 minutes	V, S	HP	T: SE 98.2% N: SE 86.4%, SP 58.8% M: SE 83.8%, SP 41.7%	Pancreatic, liver and oro-esophageal cancers, sarcoma and cholangiocarcinoma (SUVmax ≥12)	Brain cancer

V, visual analyses; *S*, semi-quantitative analyses; *HP*, histopathology; *T*, primary tumor; *N*, lymph node(s); *M*, distant metastases; *TT*, tumor types; *SE*, sensitivity; *SP*, specificity.

Table 2 - *Oncological setting: Gliomas, Primary Liver Cancer and Gastro-Entero-Pancreatic cancers*

Authors	N° of patients	Tumor type	Clinical setting	Injected activity	Acquisition timing	Image analyses	Reference Standard	^{68}Ga-FAPI performance	^{18}F-FDG performance	MRI performance
Röhrich M et al. [22]	18	Gliomas	Staging, Restaging	150-250 MBq	30 min (FAPi04); 10, 60 and 180 min (FAPi-02)	S	MRI	SE 83.3%	-	SE 100%
Windisch P et al. [23]	13	GBM	RT planning	150-250 MBq	30 minutes	S	MRI	SE 100%	-	SE 100%
Guo W et al. [25]	34	Hepatic nodules	Staging	148-259 MBq	60 minutes	V, S	HP, imaging follow-up	SE 87.4%	SE 64.9%	-
Şahin E et al. [26]	31	GEP	Staging and follow-up after treatment	2–3 MBq/Kg	45 minutes	V, S	Imaging follow-up, tumor biomarker findings, HP	SE 93.5% (patient based)	SE 71% (patient based)	-
								SE 95.9% (lesion based)	SE 79.6% (lesion based)	-
Pang Y et al. [27]	35	GI tract	Staging, Restaging	1.8-2.2 MBq/Kg	60 minutes	V, S	HP	SE 100%	SE 43.8%	-
								T: SE 100%	T: SE 52.6%	-
								N: SE 78.6%, SP 82.1%	N: SE 53.6%, SP 89.3%	-
								M: SE 88.6%, SP 28.6%	M: SE 57.1%, SP 85.7%	-
Jiang D et al. [28]	38	Gastric cancer	Staging	111-185 MBq	60 minutes	S	HP	T: SE 100%	T: SE 75%	-
								N: SE 60%, SP 92.9%	N: SE 50%, SP 92.9%	-

V, visual analyses; *S*, semi-quantitative analyses; *HP*, histopathology; *T*, primary tumor; *N*, lymph node(s); *M*, distant metastases; *SE*, sensitivity; *SP*, specificity; *GBM*, glioblastoma; *GEP*, gastro-entero-pancreatic; *GI tract*, gastro-intestinal tract.

Table 3 - *Oncological setting: Head and neck cancers*

Authors	N° of patients	Tumor type	Clinical setting	Injected activity	Acquisition timing	Image analyses	Reference Standard	^{68}Ga-FAPI performance	^{18}F-FDG performance	MRI performance
Zhao L et al. [29]	45	Nasopharyngeal carcinoma	Staging, Restaging	1.8-2.2 MBq/Kg	40 minutes	V, S	HP, imaging follow-up	T: SE 86.7%	T: SE 84.4%	-
								N: SE 95%	N: SE 75.2%	N: SE 97.5%
Qin C et al. [30]	15	Nasopharyngeal carcinoma	Staging, Restaging	1.85-3.7 MBq/Kg	30-60 minutes	V, S	MRI	T: SE 100%	T: SE 100%	-
								N: SE 48%	N: SE 100%	-
								M: SE 100%	M: SE 0%	-
Chen H et al. [31]	68	Various cancer (13 types)	Staging, Restaging	1.8-2.2 MBq/Kg	60 minutes	V, S	HP, imaging and clinical follow-up	T: SE 86.4%	-	-
Chen H et al. [32]	75	Various cancer (12 types)	Staging, Restaging	1.8-2.2 MBq/Kg	60 minutes	V, S	HP	T: SE 98.2%	T: SE 82.1%	-
								N: SE 86.4%, SP 58.8%	N: SE 45.5%, SP 76.5%	-
								M: SE 83.8%, SP 41.7%	M: SE 59.5%, SP 58.3%	-

V, visual analyses; *S*, semi-quantitative analyses; *HP*, histopathology; *T*, primary tumor; *N*, lymph node(s); *M*, distant metastases; *SE*, sensitivity; *SP*, specificity.

REFERENCES

1. Dvorak HF (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 315:1650–9. https://doi.org/10.1056/NEJM198612253152606

2. Yamaguchi R, Perkins G (2018) Animal models for studying tumor microenvironment (TME) and resis- tance to lymphocytic infiltration. Cancer Biol Ther 18:1–10. https://doi.org/10.1080/15384047.2018.147 0722

3. Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C. Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. Nat Genet. 2002 Nov;32(3):355-7. https://doi.org/10.1038/ng1013. Epub 2002 Oct 15. Erratum in: Nat Genet 2002 Dec;32(4):681. PMID: 12379854.

4. Fukino K, Shen L, Patocs A, Mutter GL, Eng C. Genomic instability within tumor stroma and clinicopathological characteristics of sporadic primary invasive breast carcinoma. JAMA. 2007 May 16;297(19):2103-11. https://doi.org/10.1001/jama.297.19.2103. PMID: 17507346.

5. Liu T, Han C, Wang S, Fang P, Ma Z, Xu L and Yin R (2019) Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. J Hematol Oncol 12:86. https://doi.org/10.1186/s13045-019-0770-1

6. Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, Old LJ, and Rettig WJ (1994) Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. Proc Natl Acad Sci U S A. 91:5657–5661. https://doi.org/10.1073/pnas.91.12.5657

7. Busek, P., Hrabal, P., Fric, P. *et al.* Co-expression of the homologous proteases fibroblast activation protein and dipeptidyl peptidase-IV in the adult human Langerhans islets. *Histochem Cell Biol* **143,** 497–504 (2015). https://doi.org/10.1007/s00418-014-1292-0

8. Bae S, Park CW, Son HK, Ju HK, Paik D, Jeon CJ, Koh GY, Kim J, Kim H. Fibroblast activation protein alpha identifies mesenchymal stromal cells from human bone marrow. Br J Haematol. 2008 Sep;142(5):827-30. https://doi.org/10.1111/j.1365-2141.2008.07241.x Epub 2008 May 24. PMID: 18510677.

9. Schuberth, P.C., Hagedorn, C., Jensen, S.M. *et al.* Treatment of malignant pleural mesothelioma by fibroblast activation protein-specific re-directed T cells. *J Transl Med* **11,** 187 (2013). https://doi.org/10.1186/1479-5876-11-187

10. Hamson EJ, Keane FM, Tholen S, Schilling O, Gorrell MD (2014) Understanding fibroblast activation protein (FAP): substrates, activities, expression and targeting for cancer therapy. Proteomics Clin Appl 8:454–463. https://doi.org/10.1002/prca.201300095.

11. Tillmanns J, Hoffmann D, Habbaba Y, Schmitto JD, Sedding D, Fraccarollo D, Galuppo P, Bauersachs J. Fibroblast activation protein alpha expression identifies activated fibroblasts after myocardial infarction. *J Mol Cell Cardiol*. 2015; 87:194–203. doi: 10.1016/j.yjmcc.2015.08.016

12. Waumans Y, Baerts L, Kehoe K, Lambeir AM, De Meester I (2015) The dipeptidyl peptidase family, prolyl oligopeptidase, and prolyl carboxypeptidase in the immune system and inflammatory disease, including atherosclerosis. Front Immunol. 6:387. https://doi.org/10.3389/fimmu.2015.00387

13. Giesel FL, Kratochwil C, Lindner T, Marschalek MM, Loktev A, Lehnert W, Debus J, Jäger D, Flechsig P, Altmann A, Mier W, and Haberkorn U (2019) 68Ga-FAPI PET/CT: biodistribution and preliminary dosimetry estimate of 2 DOTA-containing FAP-targeting agents in patients with various cancers. J Nucl Med 60:386–392. https://doi.org/10.2967/jnumed.118.215913

14. Lindner T, Loktev A, Altmann A, Giesel F, Kratochwil C, Debus J, Jäger D, Mier W, and Haberkorn U (2018) Development of quinoline-based theranostic ligands for the targeting of fibroblast activation protein. J Nucl Med 59:1415–1422. https://doi.org/10.2967/jnumed.118.210443

15. Kratochwil C, Flechsig P, Lindner T, Abderrahim L, Altmann A, Mier W, Adeberg S, Rathke H, Röhrich M, Winter H, Plinkert PK, Marme F, Lang M, Kauczor HU, Jäger D, Debus J, Haberkorn U, Giesel FL (2019) ^{68}Ga-FAPI PET/CT: tracer uptake in 28 different kinds of cancer. J Nucl Med. 60:801–805. https://doi.org/10.2967/jnumed.119.227967

16. Loktev A, Lindner T, Mier W, Debus J, Altmann A, Jäger D, Giesel F, Kratochwil C, Barthe P, Roumestand C, Haberkorn U (2018) A tumor-imaging method targeting cancer-associated fibroblasts. J Nucl Med. 59:1423–1429. https://doi.org/10.2967/jnumed.118.210435

17. Loktev A, Lindner T, Burger EM, Altmann A, Giesel F, Kratochwil C, Debus J, Marmé F, Jäger D, Mier W, Haberkorn U. Development of Fibroblast Activation Protein-Targeted Radiotracers with Improved Tumor Retention. J Nucl Med. 2019 Oct;60(10):1421-1429. https://doi.org/10.2967/jnumed.118.224469. Epub 2019 Mar 8. PMID: 30850501; PMCID: PMC6785792.

18. Frederik L. Giesel, Sebastian Adeberg, Mustafa Syed, Thomas Lindner, Luis David Jiménez-Franco, Eleni Mavriopoulou, Fabian Staudinger, Eric Tonndorf-Martini, Sebastian Regnery, Stefan Rieken, Rami El Shafie, Manuel Röhrich, Paul Flechsig, Andreas Kluge, Annette Altmann, Jürgen Debus, Uwe Haberkorn and Clemens Kratochwil Journal of Nuclear Medicine February 2021, 62 (2) 201-207; DOI: https://doi.org/10.2967/jnumed.120.245084

19. Alejandro Sanchez-Crespo, Comparison of Gallium-68 and Fluorine-18 imaging characteristics in positron emission tomography, Applied Radiation and Isotopes, Volume 76, 2013, Pages 55-62, ISSN 0969-8043, https://doi.org/10.1016/j.apradiso.2012.06.034

20. Cancer Genome Atlas Research Network, Weinstein, J. N., Collisson, E. A., Mills, G. B., Shaw, K. R., Ozenberger, B. A., Ellrott, K., Shmulevich, I., Sander, C., & Stuart, J. M. (2013). The Cancer Genome Atlas Pan-Cancer analysis project. *Nature genetics*, *45*(10), 1113–1120. https://doi.org/10.1038/ng.2764

21. Mona CE, Benz MR, Hikmat F, Grogan TR, Lückerath K, Razmaria A, Riahi R, Slavik R, Girgis MD, Carlucci G, Kelly KA, French SW, Czernin J, Dawson DW, Calais J. Correlation of ^{68}Ga-FAPi-46 PET biodistribution with FAP expression by

immunohistochemistry in patients with solid cancers: a prospective translational exploratory study. J Nucl Med. 2021 Nov 5:jnumed.121.262426. https://doi.org/10.2967/jnumed.121.262426. PMID: 34740953.

22. Röhrich M, Loktev A, Wefers AK, Altmann A, Paech D, Adeberg S, Windisch P, Hielscher T, Flechsig P, Floca R, Leitz D, Schuster JP, Huber PE, Debus J, von Deimling A, Lindner T, Haberkorn U (2019) 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. 46:2569–2580. https://doi.org/10.1007/s00259-019-04444-y

23. Windisch P, Röhrich M, Regnery S, Tonndorf-Martini E, Held T, Lang K, Bernhardt D, Rieken S, Giesel F, Haberkorn U, Debus J, Adeberg S (2020) Fibroblast Activation Protein (FAP) specific PET for advanced target volume delineation in Glioblastoma. Radiotherapy and Oncology. 150:159–163
https://doi.org/10.1016/j.radonc.2020.06.040

24. Shi X, Xing H, Yang X, Li F, Yao S, Congwei J, Zhao H, Hacker M, Huo L, Li X (2021) Comparison of PET imaging of activated fibroblasts and ^{18}F-FDG for diagnosis of primary hepatic tumours: a prospective pilot study. Eur J Nucl Med Mol Imaging. 48:1593–1603. https://doi.org/10.1007/s00259-020-05070-9

25. Guo W, Pang Y, Yao L, Zhao L, Fan C, Ke J, Guo P, Hao B, Fu H, Xie C, Lin Q, Wu H, Sun L, Chen H (2021) Imaging fibroblast activation protein in liver cancer: a single-center post hoc retrospective analysis to compare [^{68}Ga]Ga-FAPI-04 PET/CT versus MRI and [^{18}F]-FDG PET/CT. Eur J Nucl Med Mol Imaging. 48:1604–1617.
https://doi.org/10.1007/s00259-020-05095-0

26. Şahin E, Elboğa U, Çelen YZ, Sever ÖN, Çayırlı YB, Çimen U (2021) Comparison of ^{68}Ga-DOTA-FAPI and ^{18}FDG PET/CT imaging modalities in the detection of liver metastases in patients with gastrointestinal system cancer. Eur J Radiol. 142:109867.
https://doi.org/10.1016/j.ejrad.2021.109867

27. Pang Y, Zhao L, Luo Z, Hao B, Wu H, Lin Q, Sun L, Chen H (2021) Comparison of ^{68}Ga-FAPI and ^{18}F-FDG Uptake in Gastric, Duodenal, and Colorectal Cancers. Radiology. 298:393–402. https://doi.org/10.1148/radiol.2020203275

28. Jiang D, Chen X, You Z, Wang H, Zhang X, Li X, Ren S, Huang Q, Hua F, Guan Y, Zhao J, Xie F (2021) Comparison of [^{68} Ga]Ga-FAPI-04 and [^{18}F]-FDG for the detection of primary and metastatic lesions in patients with gastric cancer: a bicentric retrospective study. Eur J Nucl Med Mol Imaging. https://doi.org/10.1007/s00259-021-05441-w

29. Zhao L, Pang Y, Zheng H, Han C, Gu J, Sun L, Wu H, Wu S, Lin Q, Chen H (2021) Clinical utility of [68Ga]Ga-labeled fibroblast activation protein inhibitor (FAPI) positron emission tomography/computed tomography for primary staging and recurrence detection in nasopharyngeal carcinoma. Eur J Nucl Med Mol Imaging. https://doi.org/10.1007/s00259-021-05336-w

30. Qin C, Liu F, Huang J, Ruan W, Liu Q, Gai Y, Hu F, Jiang D, Hu Y, Yang K, Lan X (2021) A head-to-head comparison of ^{68}Ga-DOTA-FAPI-04 and ^{18}F-FDG PET/MR in patients with nasopharyngeal carcinoma: a prospective study. Eur J Nucl Med Mol Imaging. https://doi.org/10.1007/s00259-021-05255-w

31. Chen H, Zhao L, Ruan D, Pang Y, Hao B, Dai Y, Wu X, Guo W, Fan C, Wu J, Huang W, Lin Q, Sun L, Wu H (2021) Usefulness of [^{68}Ga]Ga-DOTA-FAPI-04 PET/CT in patients presenting with inconclusive [^{18}F]FDG PET/CT findings. Eur J Nucl Med Mol Imaging. 48:73–86. https://doi.org/10.1007/s00259-020-04940-6

32. Chen H, Pang Y, Wu J, Zhao L, Hao B, Wu J, Wei J, Wu S, Zhao L, Luo Z, Lin X, Xie C, Sun L, Lin Q, Wu H (2020) Comparison of [68Ga]Ga-DOTA-FAPI-04 and [18F] FDG PET/CT for the diagnosis of primary and metastatic lesions in patients with various types of cancer. Eur J Nucl Med Mol Imaging. 47:1820–1832. https://doi.org/10.1007/s00259-020-04769-z

33. Syed M, Flechsig P, Liermann J, Windisch P, Staudinger F, Akbaba S, Koerber SA, Freudlsperger C, Plinkert PK, Debus J, Giesel F, Haberkorn U, Adeberg S (2020)

Fibroblast activation protein inhibitor (FAPI) PET for diagnostics and advanced targeted radiotherapy in head and neck cancers. Eur J Nucl Med Mol Imaging. 47:2836–2845. https://doi.org/10.1007/s00259-020-04859-y

34. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D (2000) Molecular portraits of human breast tumours. Nature. 406:747–52. https://doi.org/10.1038/35021093

35. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 98:10869–74. https://doi.org/10.1073/pnas.191367098

36. Kalluri, R (2016) The biology and function of fibroblasts in cancer. Nat Rev Cancer 16:582–598. https://doi.org/10.1038/nrc.2016.73

37. Kömek H, Can C, Güzel Y, Oruç Z, Gündoğan C, Yildirim ÖA, Kaplan İ, Erdur E, Yıldırım MS, Çakabay B (2021) ^{68}Ga-FAPI-04 PET/CT, a new step in breast cancer imaging: a comparative pilot study with the ^{18}F-FDG PET/CT. Ann Nucl Med. 35:744–752. https://doi.org/10.1007/s12149-021-01616-5

38. Glehen, O., Gilly, F.N., Boutitie, F., Bereder, J.M., Quenet, F., Sideris, L., Mansvelt, B., Lorimier, G., Msika, S. and Elias, D. (2010), Toward curative treatment of peritoneal carcinomatosis from nonovarian origin by cytoreductive surgery combined with perioperative intraperitoneal chemotherapy. Cancer, 116: 5608-5618. https://doi.org/10.1002/cncr.25356

39. Fagotti A, Fanfani F, Rossitto C, Lorusso D, De Gaetano AM, Giordano A, Vizzielli G, Scambia G. A treatment selection protocol for recurrent ovarian cancer patients: the role of FDG-PET/CT and staging laparoscopy. Oncology. 2008;75(3-4):152-8. https://doi.org/10.1159/000159266. Epub 2008 Oct 1. PMID: 18827492.

40. Kim SJ, Lee SW. Diagnostic accuracy of ^{18}F-FDG PET/CT for detection of peritoneal carcinomatosis; a systematic review and meta-analysis. Br J Radiol. 2018 Jan;91(1081):20170519. https://doi.org/10.1259/bjr.20170519. Epub 2017 Nov 21. PMID: 29099613; PMCID: PMC5966216.

41. Zhao L, Pang Y, Luo Z, Fu K, Yang T, Zhao L, Sun L, Wu H, Lin Q, Chen H. Role of [^{68}Ga]Ga-DOTA-FAPI-04 PET/CT in the evaluation of peritoneal carcinomatosis and comparison with [^{18}F]-FDG PET/CT. Eur J Nucl Med Mol Imaging. 2021 Jun;48(6):1944-1955. https://doi.org/10.1007/s00259-020-05146-6. Epub 2021 Jan 7. PMID: 33415432.

42. Guo W, Chen H. ^{68}Ga FAPI PET/CT Imaging in Peritoneal Carcinomatosis. Radiology. 2020 Dec;297(3):521. https://doi.org/10.1148/radiol.2020202469. Epub 2020 Oct 13. PMID: 33048036.

43. Pang Y, Zhao L, Chen H. 68Ga-FAPI Outperforms 18F-FDG PET/CT in Identifying Bone Metastasis and Peritoneal Carcinomatosis in a Patient With Metastatic Breast Cancer. Clin Nucl Med. 2020 Nov;45(11):913-915. https://doi.org/10.1097/rlu.0000000000003263. PMID: 32910045.

44. Zhao L, Pang Y, Wei J, Hao B, Chen H. Use of 68Ga-FAPI PET/CT for Evaluation of Peritoneal Carcinomatosis Before and After Cytoreductive Surgery. Clin Nucl Med. 2021 Jun 1;46(6):491-493. https://doi.org/10.1097/rlu.0000000000003611. PMID: 33782310.

45. Ferdinandus J, Fragoso Costa P, Kessler L, Weber M, Hirmas N, Kostbade K, Bauer S, Schuler M, Ahrens M, Schildhaus HU, Rischpler C, Grafe H, Siveke JT, Herrmann K, Fendler W, Hamacher R (2021) Initial clinical experience with ^{90}Y-FAPI-46 radioligand therapy for advanced stage solid tumors: a case series of nine patients. J Nucl Med. https://doi.org/10.2967/jnumed.121.262468

46. Assadi M, Rekabpour SJ, Jafari E, Divband G, Nikkholgh B, Amini H, Kamali H, Ebrahimi S, Shakibazad N, Jokar N, Nabipour I, Ahmadzadehfar H (2021) Feasibility and Therapeutic Potential of 177Lu-Fibroblast Activation Protein Inhibitor-46 for Patients With Relapsed or Refractory Cancers: A Preliminary Study. Clin Nucl Med. https://doi.org/10.1097/RLU.0000000000003810

47. Baum RP, Schuchardt C, Singh A, Chantadisai M, Robiller FC, Zhang J, Mueller D, Eismant A, Almaguel F, Zboralski D, Osterkamp F, Hoehne A, Reineke U, Smerling C, Kulkarni HR (2021) Feasibility, Biodistribution and Preliminary Dosimetry in Peptide-Targeted Radionuclide Therapy (PTRT) of Diverse Adenocarcinomas using ^{177}Lu-FAP-2286: First-in-Human Results. J Nucl Med. 2021 https://doi.org/10.2967/jnumed.120.259192

48. Varasteh Z, Mohanta S, Robu S, Braeuer M, Li Y, Omidvari N, Topping G, Sun T, Nekolla SG, Richter A, et al. Molecular imaging of fibroblast activity after myocardial infarction using a 68Ga-labeled fibroblast activation protein inhibitor, FAPI-04. *J Nucl Med*. 2019; 60:1743–1749. doi: 10.2967/jnumed.119.226993

49. Aghajanian H, Kimura T, Rurik JG, Hancock AS, Leibowitz MS, Li L, Scholler J, Monslow J, Lo A, Han W, et al. Targeting cardiac fibrosis with engineered T cells. *Nature*. 2019; 573:430–433. doi: 10.1038/s41586-019-1546-z

50. Heckmann MB, Reinhardt F, Finke D, Katus HA, Haberkorn U, Leuschner F, Lehmann LH. Relationship Between Cardiac Fibroblast Activation Protein Activity by Positron Emission Tomography and Cardiovascular Disease. Circ Cardiovasc Imaging. 2020 Sep;13(9):e010628. doi: 10.1161/CIRCIMAGING.120.010628. Epub 2020 Sep 11. PMID: 32912030; PMCID: PMC7497888. https://doi.org/10.1161/CIRCIMAGING.120.010628

51. Luo Y, Pan Q, Zhang W. IgG4-related disease revealed by 68Ga-FAPI and 18F-FDG PET/CT. *Eur J Nucl Med Mol Imaging*. 2019;46:2625–2626. doi: 10.1007/s00259-019-04478-2.

52. Pan Q, Luo Y, Zhang W. Recurrent immunoglobulin G4-related disease shown on 18F-FDG and 68Ga-FAPI PET/CT. *Clin Nucl Med.* 2020;45:312–313. doi: 10.1097/RLU.0000000000002919.