

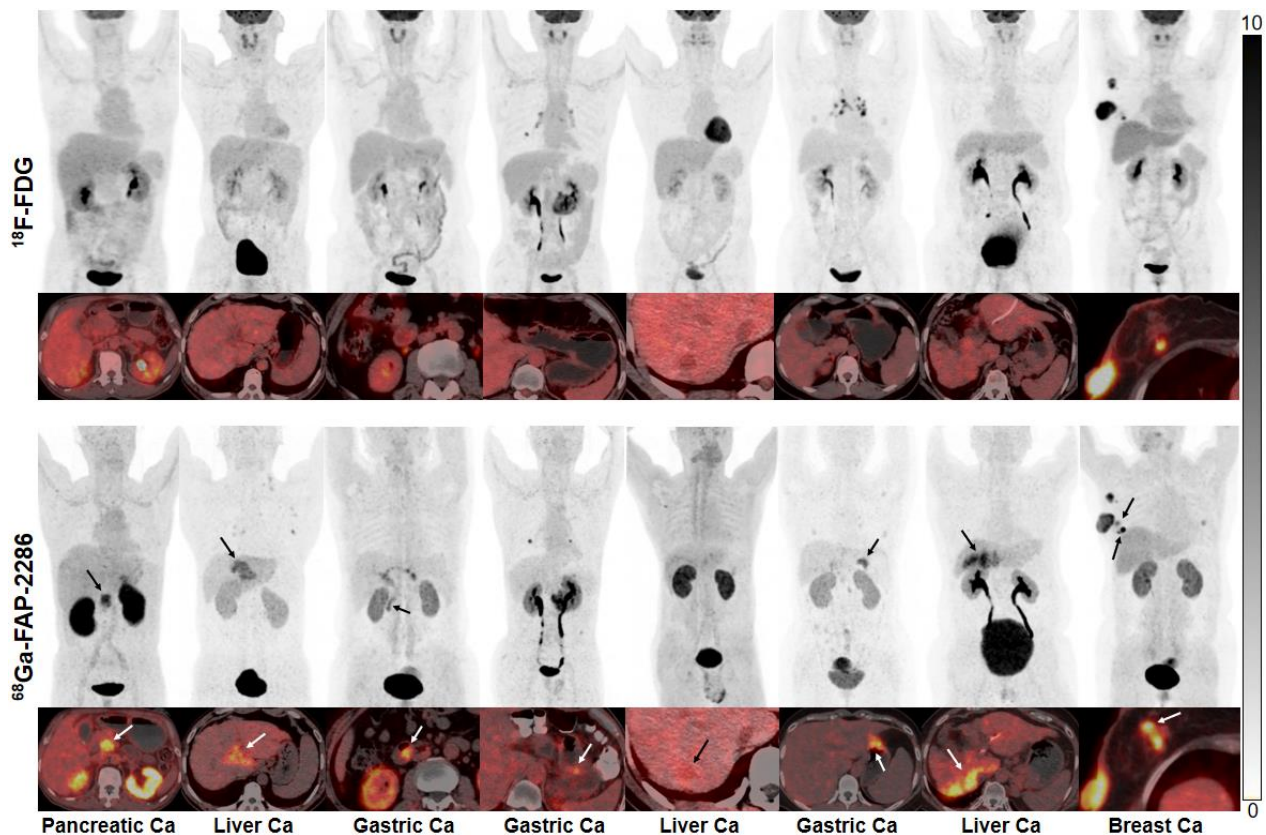
## Radiochemical Processing

$^{18}\text{F}$ -FDG was synthesized in-house by a cyclotron (MINItrace, GE Healthcare) in accordance with the standard methods, using the coincidence  $^{18}\text{F}$ -FDG synthesis module (TracerLab FxFN, GE Healthcare) (16). The precursor FAP-2286 and FAPI-46 were obtained from Yantai Dongcheng Pharmaceutical Group Co., Ltd (Shandong, China) and Jiangsu Huayi Technology Co., Ltd. (Jiangsu, China), respectively. Both compounds were used for research purpose. Radiolabeling of  $^{68}\text{Ga}$ -FAPI-46 was performed according to previously described protocols (17).  $^{68}\text{Ga}^{3+}$  was eluted from  $^{68}\text{Ge}/^{68}\text{Ga}$  generator (ITG, Germany). The elution volume of  $^{68}\text{Ga}^{3+}$  (925-1110 MBq in 0.6M HCl, 4 ml) was added to a solution of FAPI-46 (25  $\mu\text{g}$  [28.2 nmol] in sodium acetate, 1mL). The reactor vial was heated to 100°C for 10 min. After trapping of on a solid-phase cartridge (Sep-Pak C18 Plus Light Cartridge, Waters, USA), the cartridge was washed with water (20mL). The elution of  $^{68}\text{Ga}$ -FAPI-46 was performed by using 75% ethanol (1.0 ml) and the final formulation of  $^{68}\text{Ga}$ -FAPI-46 was diluted with normal saline (14 mL). The radiolabeling of  $^{68}\text{Ga}$ -FAP-2286 was performed in a similar protocol, with a reaction mixture of 25  $\mu\text{g}$  (17.0 nmol) FAP-2286 and 925-1110 MBq  $^{68}\text{Ga}$  solution. Quality control of the radiosynthesis was performed by ultraviolet and radio-high performance liquid chromatography (HPLC). The radiochemical purity was over 95% for both  $^{68}\text{Ga}$ -FAP-2286 and  $^{68}\text{Ga}$ -FAPI-46, and the final product was diluted with saline and sterilised by passing through a 0.22- $\mu\text{m}$  Millipore filter into a sterile multidose syringe. The final product was sterile and pyrogen-free.

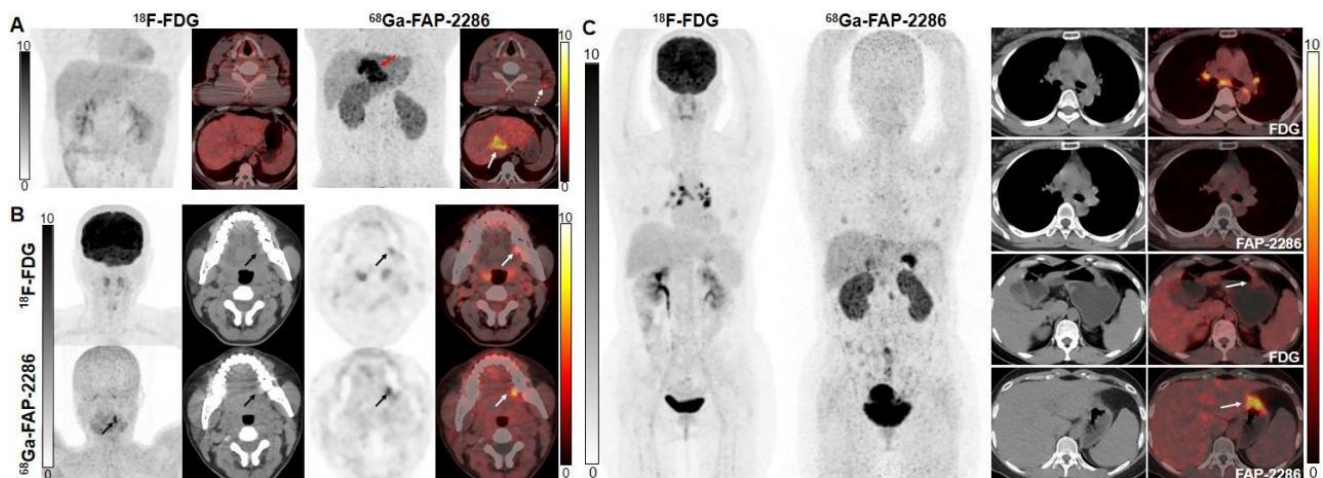
## PET/CT imaging and reconstruction

$^{18}\text{F}$ -FDG PET/CT was performed within 7 days of  $^{68}\text{Ga}$ -FAP-2286 PET/CT scan. Patients were instructed to fast for at least 6 h before  $^{18}\text{F}$ -FDG PET/CT scan and to drink 500 mL of water before the scan to stimulate  $^{18}\text{F}$ -FDG excretion from the renal calyces and subsequent voiding (18). A normal blood glucose level in the peripheral blood was ensured on  $^{18}\text{F}$ -FDG PET/CT imaging evaluation. In a certain group of patients,  $^{68}\text{Ga}$ -FAPI-46 PET/CT was performed within 7 days of  $^{68}\text{Ga}$ -FAP-2286 PET/CT for a direct comparison between the two FAPI derivatives. No specific preparation was required before  $^{68}\text{Ga}$ -FAPI-2286 and  $^{68}\text{Ga}$ -FAPI-46 PET/CT scans.

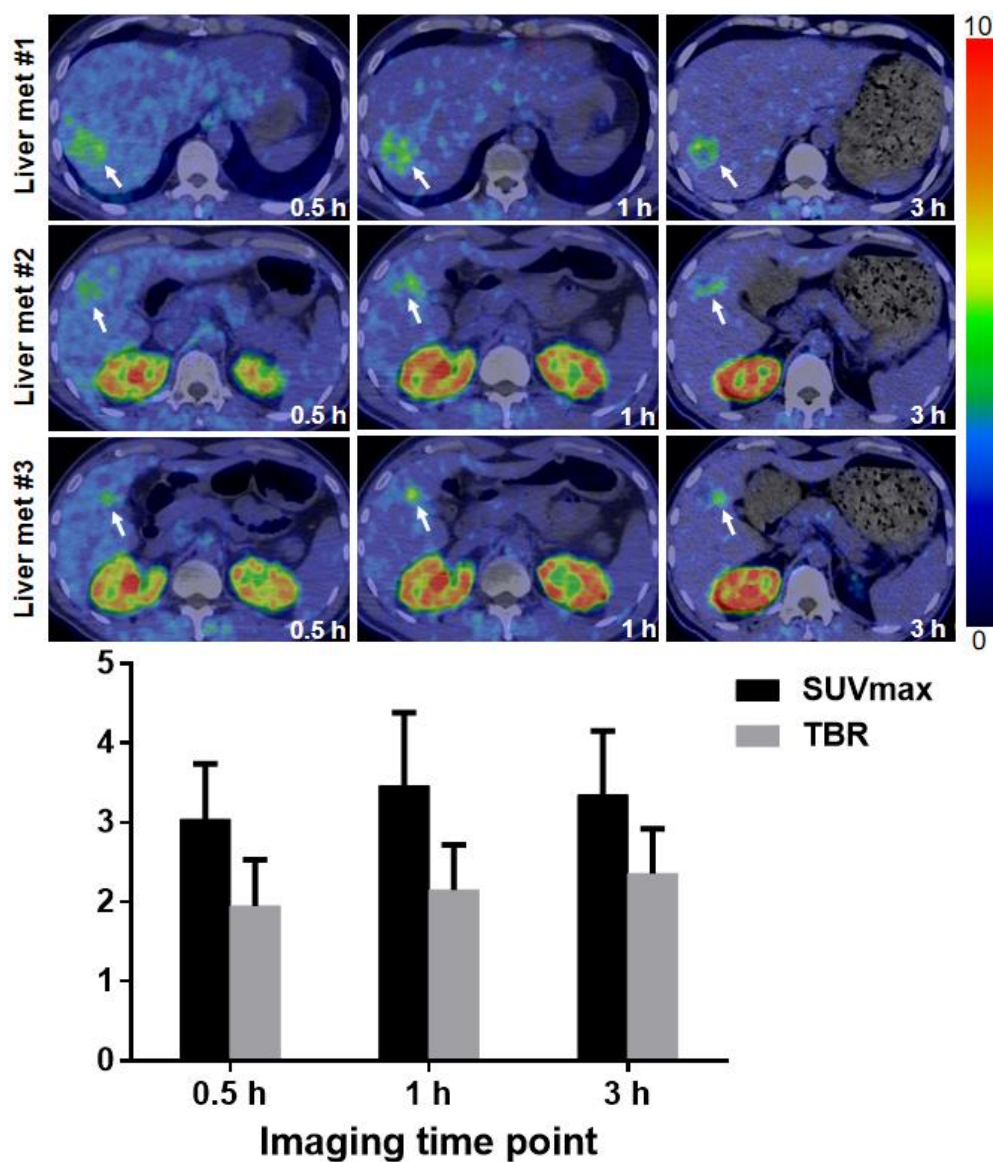
The injected activities for  $^{18}\text{F}$ -FDG were  $288.1 \pm 28.4$  MBq (range, 227.5-332.4),  $194.2 \pm 42.1$  MBq (range 141.8-264.5) for  $^{68}\text{Ga}$ -FAPI-46, and  $195.0 \pm 43.1$  MBq (range 143.7-272.4) for  $^{68}\text{Ga}$ -FAP-2286, respectively. Data were acquired using a hybrid PET/CT (Discovery MI, GE Healthcare) after 1 h of intravenous administration. A low-dose CT scan (100-120 keV; 80-120mA; slice thickness, 3 mm) was collected for attenuation correction and image fusion. All PET images were acquired in 3D mode and were reconstructed by the Bayesian penalized likelihood (BPL) reconstruction algorithm (Q.clear, GE Healthcare).



**Supplemental Fig. 1** Among the 44 patients underwent PET/CT for initial staging, nine primary tumor lesions from 8 patients were not visualized on  $^{18}\text{F}$ -FDG PET/CT, but were well visualized on  $^{68}\text{Ga}$ -FAP-2286 PET/CT. The specific tumor entities include gastric cancer (n=3), liver cancer (n=3), breast cancer (n=2), and pancreatic cancer (n=1).



**Supplemental Fig. 2** Representative images of patients who underwent  $^{18}\text{F}$ -FDG and  $^{68}\text{Ga}$ -FAP-2286 PET/CT imaging. (A) A 47-year-old man with known hilar cholangiocarcinoma underwent PET/CT imaging for tumor staging.  $^{68}\text{Ga}$ -FAP-2286 demonstrated high tracer uptakes in the primary tumor (solid arrows) and metastatic cervical lymph nodes (confirmed via biopsy, dotted arrows), while  $^{18}\text{F}$ -FDG demonstrated no uptake. (B) A 47-year-old woman with tongue cancer who underwent surgical resection, underwent PET/CT imaging after 6 months for the detection of tumor recurrence. Compared to  $^{18}\text{F}$ -FDG,  $^{68}\text{Ga}$ -FAP-2286 demonstrated a higher tracer uptake in the recurrent tumor lesions (solid arrow). (C) A 40-year-old woman with known gastric signet ring cell carcinoma underwent PET/CT imaging for initial staging.  $^{68}\text{Ga}$ -FAP-2286 demonstrated a higher tracer uptake in the primary tumor than did  $^{18}\text{F}$ -FDG (solid arrows). Notably, high metabolic activity of the mediastinal lymph nodes was observed for  $^{18}\text{F}$ -FDG, while  $^{68}\text{Ga}$ -FAP-2286 demonstrated no abnormal activity. The patient subsequently underwent an endoscopic ultrasound fine-needle biopsy, and the pathological results revealed inflammatory lymph nodes.



**Supplemental Fig. 3** A 40-year-old man with a history of radical resection for colon cancer underwent  $^{68}\text{Ga}$ -FAP-2286 PET/CT imaging at different time points after injection. Rapid and stable radiotracer uptake was observed in the liver metastases. Semiquantitative analysis of the liver metastases demonstrated a stable  $^{68}\text{Ga}$ -FAP-2286 uptake but an increase in TBR from 0.5 to 3 h.

Abbreviation: FAP = fibroblast activation protein; Met = metastasis; TBR = tumor-to-background ratio.

**Supplemental Table 1.** Comparison of  $^{18}\text{F}$ -FDG and  $^{68}\text{Ga}$ -FAP-2286 PET/CT-based TNM staging and recurrence detection in patients with additional findings

Initial Staging						
No.	Types of cancer	TNM Stage (FDG-based)	TNM Stage (FAP-2286-based)	Additional finding on FAP-2286 PET/CT (compared to FDG)		Staging change (compared to FDG)
Patient 2	Pancreatic Ca	IIA	IIA	Primary tumor detected		None
Patient 9	Liver Ca (ICC)	IIIA	IIIA	Primary tumor detected		None
Patient 12	Ovarian Ca	IVB	IVB	Larger disease extent of PM		None
Patient 13	HNC (NPC)	IVA	IVB	Bone mets		Upstaged
Patient 19	Gastric Ca	IV	IV	Primary tumor detected		None
Patient 21	Pancreatic Ca	IV	IV	More bone mets		None
Patient 25	Liver Ca (HCC)	II	II	Primary tumor detected; More liver mets		None
Patient 27	Pancreatic Ca	III	III	More abdominal LN mets		None
Patient 30	Liver Ca (HCC)	IVB	IVB	More abdominal LN mets and bone mets		None
Patient 31	Pancreatic Ca	IV	IV	Larger disease extent of PM		None
Patient 38	Esophageal Ca	IIIA	IIIB	Greater number of mediastinal LN mets		Upstaged
Patient 40	Breast Ca	IIIB	IIIB	More primary tumor detected		None
Patient 48	Gastric Ca	IIA	IIA	Primary tumor detected		None
Patient 49	Gastric Ca	IIA	IIB	Primary tumor detected; More abdominal LN mets		Upstaged
Patient 51	Liver Ca (ICC)	II	II	Primary tumor detected		None
Patient 62	Ovarian Ca	IVB	IVB	Larger disease extent of PM		None
Patient 63	HNC (NPC)	IVB	IVB	More bone mets		None
Patient 64	Pancreatic Ca	III	III	More abdominal LN mets		None
Recurrence detection						
No.	Types of cancer	Local recurrence detection		Distant metastases detection		Additional finding on FAP-2286 PET/CT
		FDG	FAP-2286	FDG	FAP-2286	
Patient 1	Glioblastoma	-	+	NA	NA	Local recurrence detected by FAP-2286 PET/CT
Patient 11	Gastric Ca	NA	NA	-	+	Abdominal LN mets detected by FAP-2286 PET/CT
Patient 22	Liver Ca (ICC)	-	+	+	+	Local recurrence detected by FAP-2286 PET/CT; More abdominal LN mets, subcutaneous mets, and bone mets detected by FAP-2286 PET/CT
Patient 24	Liver Ca (HCC)	-	+	NA	NA	Local recurrence detected by FAP-2286 PET/CT
Patient 28	Rectal Ca	NA	NA	+	+	Larger disease extent of PM detected by FAP-2286 PET/CT
Patient 34	Liver Ca (HCC)	NA	NA	-	+	Liver mets detected by FAP-2286 PET/CT
Patient 36	Gastric Ca	NA	NA	+	+	More bone mets and larger disease extent of PM detected by FAP-2286 PET/CT
Patient 43	HNC (Tongue Ca)	-	+	NA	NA	Local recurrence detected by FAP-2286 PET/CT
Patient 46	Renal Ca	NA	NA	+	+	More bone mets detected by FAP-2286 PET/CT
Patient 57	HNC (Tongue Ca)	+	+	-	+	Muscle mets and liver mets detected by FAP-2286 PET/CT
Patient 58	HNC (Tongue Ca)	-	+	NA	NA	Local recurrence detected by FAP-2286 PET/CT
Patient 59	Liver Ca (HCC)	-	+	NA	NA	Local recurrence detected by FAP-2286 PET/CT

Note. The clinical stage was assigned based on American Joint Committee on Cancer (AJCC) staging system (Eighth edition).

Abbreviations: Ca = cancer, ICC = intrahepatic cholangiocarcinoma, HNC = head and neck cancer, NPC = nasopharyngeal carcinoma, LN = lymph nodes, PM = peritoneal metastases, mets = metastases, HCC = hepatocellular carcinoma, NA = not applicable.