SUPPLEMENTAL APPENDIX A

Eligibility Criteria

Inclusion Criteria

- Patients have inflammatory diseases with strong clinical or laboratory evidence of inflammatory focus/foci in defined anatomical regions preferably visible on ^{18}F-FDG PET/CT scans.
- Before intravenous injection with ^{18}F-FSPG, no antiinflammatory or antibiotic therapy was started and/or no changes were made in drug substance, dose, or route of administration of an ongoing antiinflammatory or antibiotic therapy.
- Age ≥35 and ≤75 years.
- Eastern Cooperative Oncology Group performance status of 0 to 2.
- Adequate function of major organs.

Exclusion Criteria

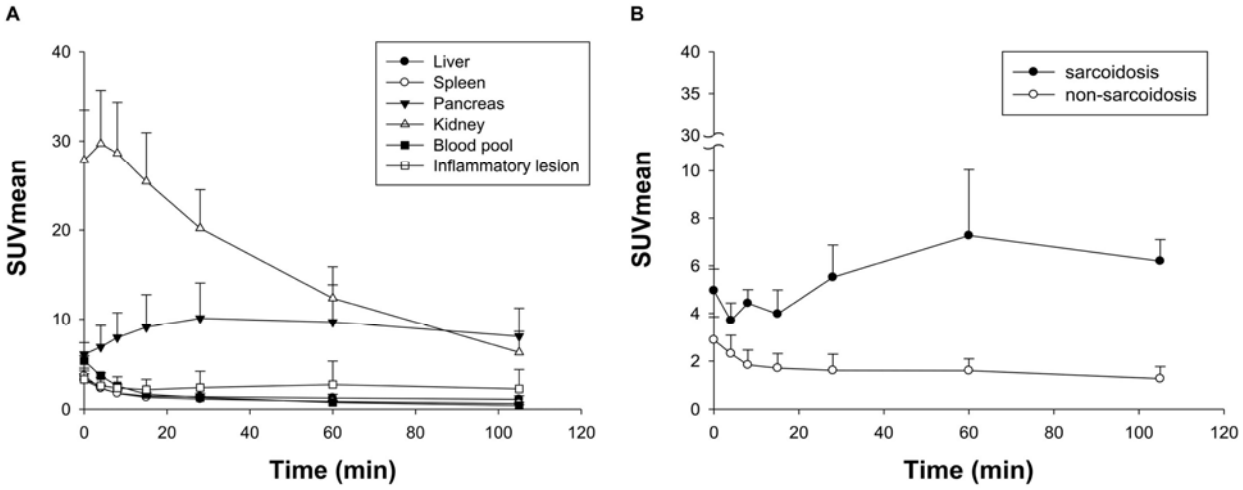
Patients were excluded from the study if any of the following conditions applied to them:

- Being pregnant or lactating.
- Having a concurrent, severe, uncontrolled, or unstable medical disease other than cancer.
- Having a lifetime history of alcohol or drug abuse.
- Being a relative or student of the investigator or otherwise being a dependent.
- Participating or having participated in another clinical study involving the administration of an investigational drug during the preceding 4 wks.

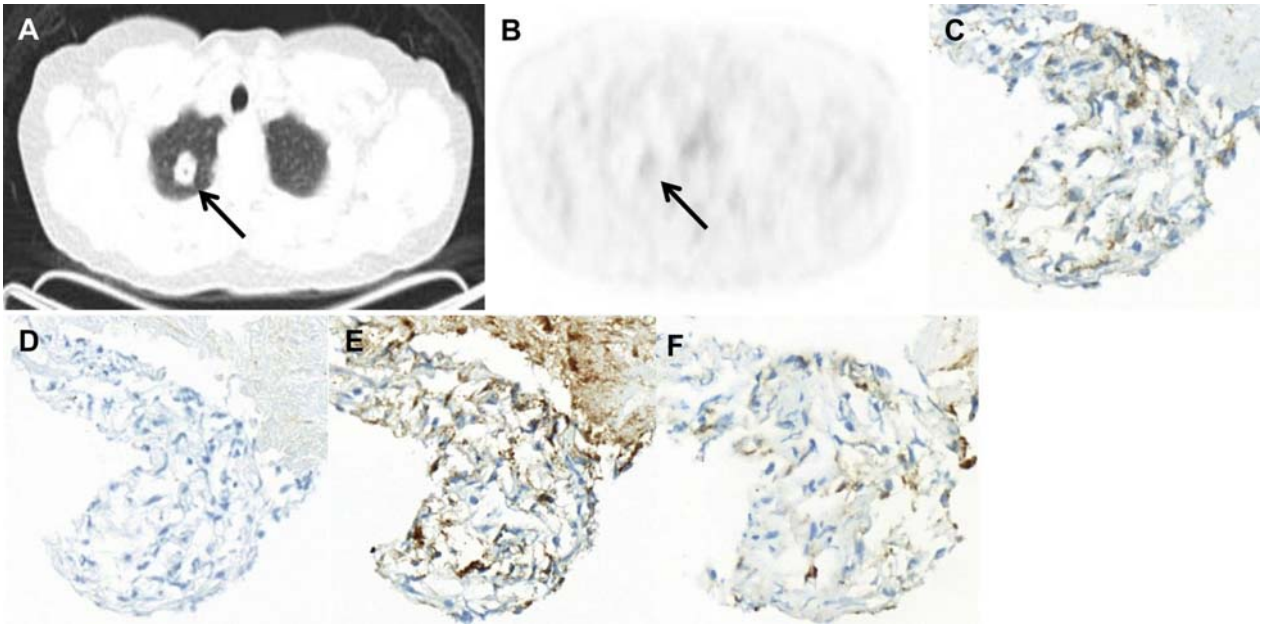
Immunohistochemical Staining of xCT, CD44s, and Inflammatory Cell Markers

Tissue samples from core-needle biopsies for routine diagnostic pathologic examinations were obtained before or after ^{18}F-FSPG PET/CT and used for immunohistochemical (IHC) analysis of xCT, CD44s, and inflammatory cell markers. A protocol employing an automatic IHC staining device (Benchmark XT, Ventana Medical Systems, Tucson, AZ, USA) with the OptiView DAB IHC Detection Kit (Ventana Medical Systems, Tucson, AZ, USA) was used on formalin-fixed, paraffin-embedded tissue sections. Briefly, 4-

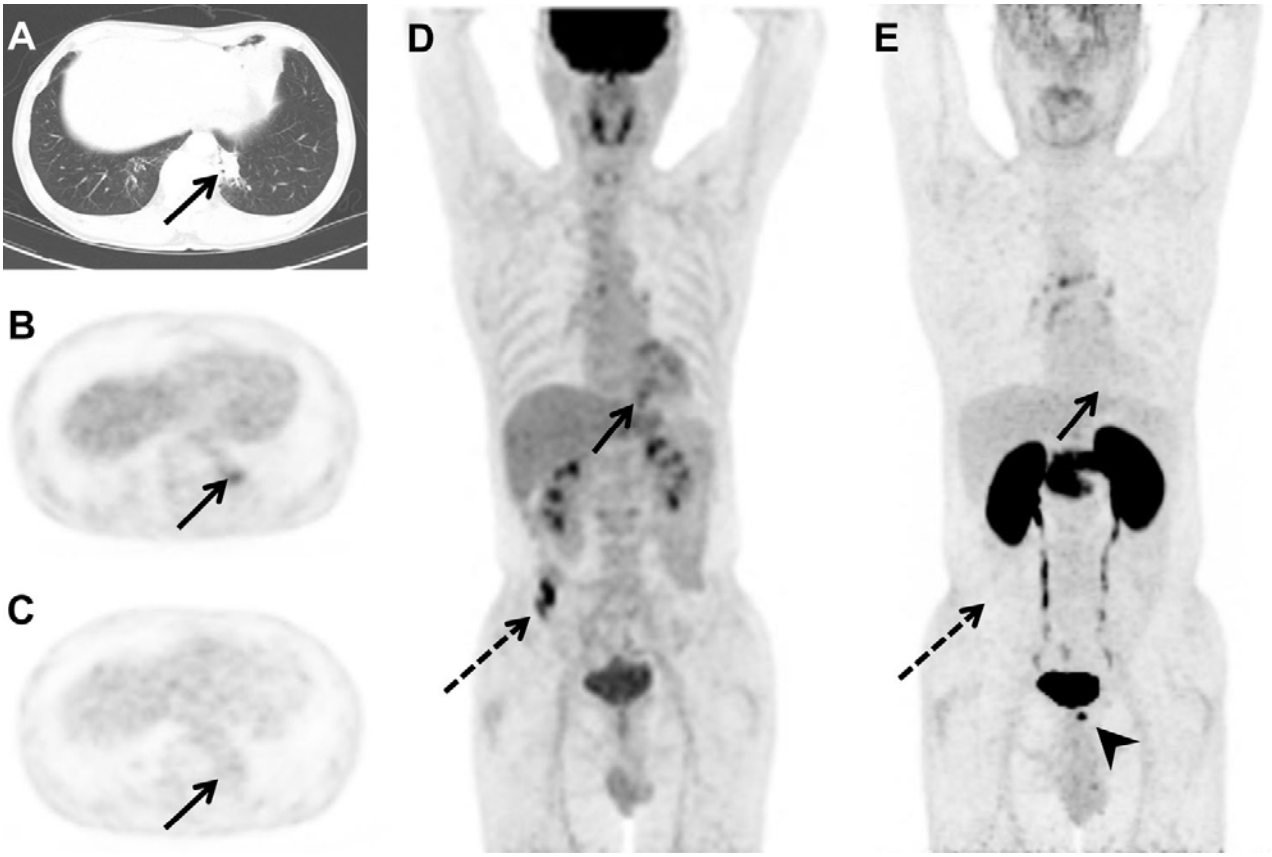
mm-thick whole-tissue sections were transferred onto silanized charged slides and allowed to dry for 10 min at room temperature, followed by 20 min in an incubator at 65°C. Sections were processed using the heat-induced epitope retrieval method with Cell Conditioning 1 (CC1) buffer for 32 min and were then incubated for 16 min with antibodies to xCT (1:250 dilution, NB300-318, clone poly, Novus, Colorado, USA), CD44 (1:200 dilution, M7082, clone DF1485, Dako, Glostrup, Denmark), CD68 (1: dilution, M0814, clone KP1, Dako, Glostrup, Denmark, for identification of macrophages and dendritic cells), CD11c (1:200 dilution, AB52632, clone mono, Abcam, Cambridge, UK, for identification of dendritic cells), CD163 (1:400 dilution, NCL-CD163, clone 10D6, Novo, Newcastle, UK, for identification of M2 macrophages), CD206 (1:500 dilution, MAB25341, clone 685645, R&D system, MN, USA, for identification of M2 macrophages), and CD138 (1:100 dilution, M7228, clone MI-15, Dako, Glostrup, Denmark, for identification of plasma cells) in the autoimmunostainer. Antigen-antibody reactions were visualized using the Ventana OptiView DAB IHC Detection Kit (Optiview HQ Linker 8 min, Optiview HRP Multimer 8 min, Optiview H2O2/DAB 8 min, Optiview Copper 4 min). Counterstaining was performed by using Ventana Hematoxylin II for 8 min and bluing reagent for 4 min. Finally, all slides are removed from the stainer, dehydrated, and coverslipped for microscopic examination. Incubation with the primary antibody was omitted in the negative controls. The levels of expression of xCT, CD44, and inflammatory cell markers in inflammatory and infectious lesions were examined by an experienced pathologist who was completely masked to the patient and imaging information. The IHC results were reported as the proportion of positively stained cells to inflammatory cells.



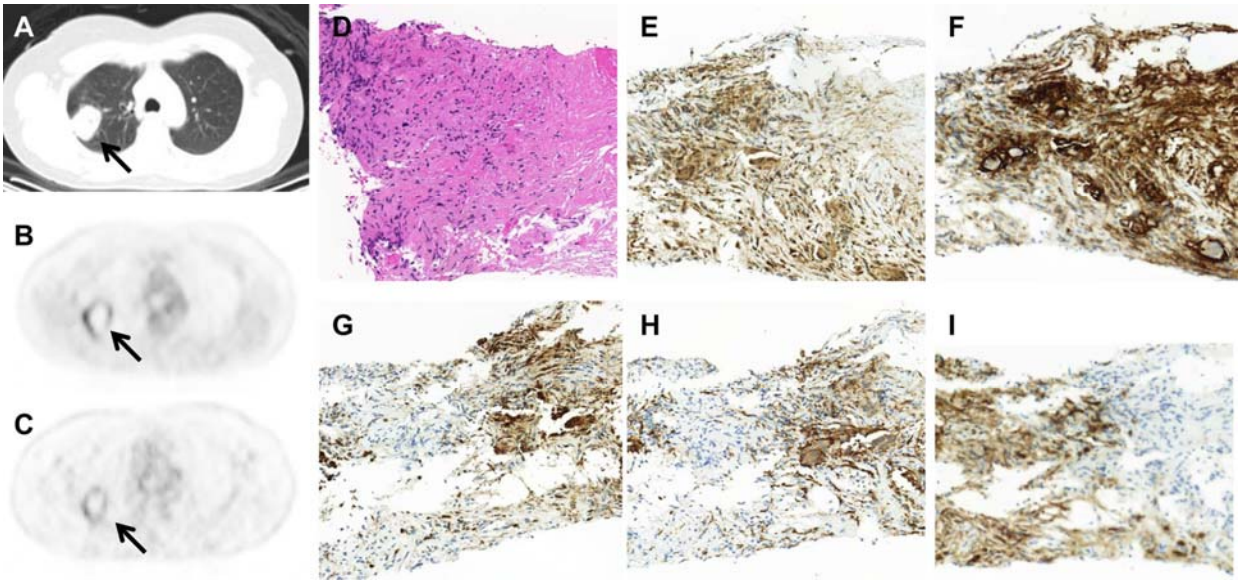
SUPPLEMENTAL FIGURE 1. Mean SUV_{mean} of ^{18}F-FSPG distribution in pancreas, kidney, liver, spleen, blood pool, and the largest reference inflammatory/infectious lesions (sarcoidosis and nonsarcoidosis) over time. The kidney and pancreas show high ^{18}F-FSPG uptake. The pancreas activity gradually increases, reaching a plateau at approximately 15–60 minutes. The activities in kidney and other remaining regions decrease over time. Spleen activity is barely distinguishable from blood-pool activity at 105 minutes after injection. On average, the tracer accumulates in inflammatory lesions, followed by a plateau phase (A). The mean SUV_{mean} of nonsarcoid lesions decreases continuously after injection over time from 2.9 ± 1.0 at 30 sec to 1.3 ± 0.5 at 105 min (P = 0.012). By contrast, sarcoidosis lesions reach a plateau at about 60 min (7.3 ± 2.8), and the mean SUV_{mean} is maintained at 105 min (6.2 ± 0.9, P = 0.655) (B).



SUPPLEMENTAL FIGURE 2. A 43-y-old woman with pulmonary tuberculosis (patient 3). A transverse CT image (A) shows a nodular lesion in the right upper lobe of the lung. Minor uptake of ^{18}F-FSPG is visible in the lesion (B, arrow). The proportions of inflammatory cells positive for xCT (C), CD44 (D), CD68 (E), and CD163 (F) on IHC were 100%, 0%, 100%, and 50% (x400), respectively.



SUPPLEMENTAL FIGURE 3. A 66-y-old man with pneumonia (patient 10). A transverse CT image shows an irregular mass in the left lower lobe of the lung (A). Higher ^{18}F-FDG accumulation (B) than that of ^{18}F-FSPG (C) is observed in the lesion. Maximum-intensity projection of ^{18}F-FDG (D) shows physiologic bowel uptake of ^{18}F-FDG in the cecum but no uptake of ^{18}F-FSPG (E) (dotted arrow). No physiological accumulation of ^{18}F-FSPG in the myocardium is observed (E). Interestingly, a focal uptake is observed in the prostate only on ^{18}F-FSPG PET (arrowhead). The nature of the focal prostate uptake has not yet been determined.



SUPPLEMENTAL FIGURE 4. Transverse CT (A), ^{18}F-FDG (B), and ^{18}F-FSPG PET (C) of a 42-y-old woman with necrotizing granulomatous inflammation (patient 6). The lesion in the right upper lobe of the lung shows major uptake of ^{18}F-FDG and ^{18}F-FSPG (B and C, arrows). Hematoxylin and eosin staining (D, x200) shows an abundance of plasma cells. The proportions of inflammatory cells positive for xCT (E), CD44s (F), CD68 (G), CD163 (H), and CD138 (I) on IHC were 95%, 90%, 35%, 20%, and 65% (x200), respectively.

SUPPLEMENTAL TABLE 1 Characteristics of patients and ^{18}F-FSPG PET/CT data

Patient No.	Age (y)	Sex	Final diagnosis	Clinical presentation	Visual ^{18}F-FSPG analysis	SUV_{max} at 60 min		Location
						^{18}F-FDG	^{18}F-FSPG	
1	50	F	NTM infection	^{18}F-FDG–positive lesions	M	16.0	3.6	Right lung*
					m	4.7	1.1	Right lung
					m	6.4	1.3	Right lung
					m	7.3	0.7	Right lung
					m	4.1	0.8	Right lung
2	50	F	Sarcoidosis	Cough, elevated serum ACE, and lymphadenopathy on CT	M	11.2	6.5	LN*
					M	9.5	7.1	LN
					M	11.9	5.7	LN
					M	10.2	4.3	Right lung
					M	6.5	6.7	LN
3	43	F	Pulmonary TB	Cavitary nodule on CT	m	NA	1.4	Right lung*
4	45	F	NTM infection	Lung nodule on CT	m	NA	1.4	Right lung*
5	53	M	Sarcoidosis	Cough, weight loss, and elevated serum ACE	M	18.4	9.2	LN*
					M	22.8	8.7	LN
					M	14.2	9.7	LN
					M	17.6	5.4	Right lung
					M	16.4	5.6	Right lung
6	42	F	Necrotizing granulomatous inflammation	Cavitary mass on CT	M	6.7	2.6	Right lung*
7	62	M	Pulmonary TB	Right hilar mass on chest radiograph	M	7.4	2.9	Right lung*
					m	5.4	1.3	Right lung
					m	3.0	1.4	Left lung
8	56	M	Radiation pneumonitis	Consolidation with ground-glass opacity on CT	M	7.6	2.5	Right lung*
9	44	M	Pulmonary TB	Lung nodule on CT	M	10.4	2.3	Right lung*
10	66	M	Pneumonia	Lung mass on CT	m	4.4	1.5	Left lung*

NTM = nontuberculous mycobacteria; TB = tuberculosis; ACE = angiotensin-converting enzyme; m = minor uptake; M = major uptake; LN = lymph node; NA = not assessed.
*The largest representative lesion.

SUPPLEMENTAL TABLE 2 ^{18}F-FSPG accumulation and IHC results of xCT, CD44, and surface markers of inflammatory cells in patients with available tissue specimens

Patient No.	^{18}F-FSPG at 60 min	Immunohistochemistry (% positive cells)						
	SUV_{max}	xCT	CD44	CD11c	CD206	CD68	CD163	CD138
3	1.4	100	0	0	50	100	50	0
5	9.2	80	80	80	50	80	1	1
6	2.6	95	90	40	20	35	20	65
7	2.9	50	1	NA	NA	5	1	0
10	1.5	75	30	NA	NA	10	10	70

NA = not assessed.