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Brief Communication

^{68}Ga-Pentixafor-PET/CT imaging represents a novel approach to detect chemokine receptor CXCR4 expression in myeloproliferative neoplasms

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ABSTRACT

C-X-C motif chemokine receptor 4 (CXCR4) is an attractive target for cancer diagnosis and treatment, as it is overexpressed in many solid and hematological malignancies. This study investigated the feasibility of CXCR4-directed imaging with positron emission tomography/computed tomography (PET/CT) using ^{68}Ga-Pentixafor to visualize and quantify disease involvement in myeloproliferative neoplasms (MPNs).

Methods: 12 patients with MPNs (*n*=4 primary myelofibrosis, *n*=6 essential thrombocythemia, *n*=2 polycythemia vera) and 5 controls underwent ^{68}Ga-Pentixafor-PET/CT. Imaging findings were compared with immunohistochemical stainings, laboratory data and splenic volume.

Results: ^{68}Ga-Pentixafor-PET/CT was visually positive in 12/12 patients and CXCR4 target specificity could be confirmed by immunohistochemical staining. A significantly higher tracer uptake could be detected in the bone marrow of MPN patients (SUV_{mean} 6.45±2.34 vs. 4.44±1.24). Dynamic changes of CXCR4 expression determined by ^{68}Ga-Pentixafor-PET/CT corresponded with treatment response.

Conclusion: ^{68}Ga-Pentixafor-PET/CT represents a novel diagnostic tool to non-invasively detect and quantify the extent of disease involvement in MPNs.

Keywords:

Myeloproliferative neoplasms; CXCR4; molecular imaging; PET; theranostics

NTRODUCTION

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of rare, potentially life-threatening, hematopoietic stem cell disorders characterized by aberrant proliferation of one or more myeloid lineages (*1*). Due to similarities in pathogenesis and symptoms, diagnosis is often challenging. However, no imaging technique is presently established for the assessment of bone marrow (BM) involvement in MPNs

C-X-C motif chemokine receptor 4 (CXCR4) is a widely studied transmembrane chemokine receptor involved in tumor growth, metastasis and hematopoietic stem cell/progenitor homing and retention in hematopoietic sites (*2*). In addition, previous studies have shown CXCR4 overexpression in more than 30 different tumor entities (*3-5*) including multiple myeloma (*6*), diffuse large B-cell lymphoma (*7*), and small cell lung cancer (*8*). Recently, the radiolabeled CXCR4-targeted ligand ^{68}Ga-Pentixafor has been developed for positron emission tomography (PET) imaging and has been shown to non-invasively visualize CXCR4 expression in multiple hematological malignancies as well as inflammatory disease conditions *in vivo* (*3,5,7,9,10*). However, there is limited knowledge regarding imaging features of MPNs.

The aim of this proof-of-principle study was to assess the feasibility of non-invasive CXCR4-directed imaging with PET/CT in patients with MPNs.

MATERIALS AND METHODS

Subjects and study design

Between April 2015 and May 2017, 12 patients (6 males, 6 females, age 37-73 years, mean 58.2±9.1 years) with MPNs underwent molecular imaging with ^{68}Ga-Pentixafor-PET/CT. All patients had a clinically, molecularly and/or histologically confirmed myeloproliferative disorder (PMF, n=4; PV, n=2; ET, n=6). Detailed characteristics of the patient cohort are shown in Table 1. Five non-oncologic patients (3 males, 2 females; mean age 59±8 years) were included as a control group (detailed in Supplementary Methods).

PET/CT imaging

^{68}Ga-Pentixafor was prepared as previously described (*11*) (detailed in Supplementary Methods). After injection of ^{68}Ga-Pentixafor (median, 130 MBq; range, 74-190 MBq) all PET/CT scans were performed on a dedicated PET/CT scanner (Siemens Biograph mCT 64; Siemens Medical Solutions, Germany) using standard acquisition and reconstruction protocols (detailed in Supplementary Methods).

Image analysis

PET/CT scans were visually assessed by two board-certified nuclear medicine physicians (CL and MK). First, a visual inspection of scans for elevated intramedullary tracer uptake (higher than mediastinal blood pool) was performed. For semi-quantitative analysis, mean standardized uptake values (SUV_{mean}) were determined as follows: In an initial step, transaxial slices in the middle of the lumbar vertebral bodies of L2-L4 were selected. Next, tracer uptake in each vertebral body was determined by placing a region-of-interest (ROI) of 10 mm diameter in the center of each vertebra. The individual overall SUV_{mean} (L2-L4) was calculated as the mean of the respective SUV_{mean} of these three ROIs. For assessment of the spleen, a ROI with a diameter of 4 cm was used. Background activity was measured by placing a 15 mm ROI in the center of the right atrium ($SUV_{blood\ pool}$). Mean TBR (TBR_{mean}) were calculated by dividing the SUV_{mean} of the lumbar vertebral bodies L2-L4 and $SUV_{blood\ pool}$. Splenic volumes were assessed by means of CT.

Immunohistochemical stainings of patient biopsy material

Immunohistochemical (IHC) stainings were performed on 10% formalin-fixed, paraffin-embedded BM biopsies from 9/12 MPN patients (detailed in Supplementary Methods).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.0 software (GraphPad Software, Inc., San Diego, CA). Results are shown as mean ± standard deviation or median and range as indicated. All

statistical tests were performed two-sided and a *P*-value <0.05 was considered to indicate statistical significance.

RESULTS

CXCR4-directed PET-imaging with ^{68}Ga-Pentixafor was visually positive in all patients (n = 12/12 patients). ^{68}Ga-Pentixafor-PET/CT depicted significantly increased tracer uptake both in the BM as well as extramedullary hematopoietic sites as compared to controls with an SUV_{mean} of 4.44±1.3 (controls: 2.67±0.41; *P*=0.01) in the BM and an SUV_{mean} of 6.45±2.34 (controls: 4.44±1.24; *P*=0.09) in the spleen, respectively (example given in Figure 1). Individual imaging results can be found in Supplemental Table 1 and Supplemental Table 2, respectively.

No significant differences were observed between SUV_{mean} of the different subtypes of MPN. Patients with $JAK2V617F^+$ or $CALR^+$ mutations did not show higher BM uptake than $JAK2V617F^-$ patients (r=0.19, *P* =n.s.). Additional BM biopsies were available in 9/12 patients and confirmed moderate to strong CXCR4 expression in dysplastic cells of the megakaryocytic lineage in all samples (Supplemental Figure 1).

Three patients additionally underwent follow-up CXCR4-directed PET/CT after a median of six months (range, 4-7 months) after treatment initiation with ruxolitinib (patient #1, patient #6) and hydroxyurea (patient #5). In these patients, initially high tracer accumulation in the spleen and BM (with a SUV_{mean} of 6.12±2.15, 3.60±1.14 and 4.10±0.32, respectively) declined (SUV_{mean} BM 3.97 vs. 2.86; SUV_{mean} spleen 7.72 vs. 4.89) in response of treatment (Figure 2). Furthermore, the decrease of SUV_{mean} after treatment corresponded with spleen volume reduction and normalization of hemoglobin, peripheral leukocyte count, thrombocyte count as well as lactate dehydrogenase levels (Supplemental Table 2).

DISCUSSION

Although PET/CT imaging is widely used for the diagnosis, staging, and response assessment of various types of hematological malignancies (*12*), it is not routinely used in patients with MPNs.

Currently, with the diagnosis being based solely on the assessment of clinical, hematological, histopathological and genetic parameters (*1*), no imaging technique is established for the assessment of BM involvement. Here, we present the first proof-of-principle study investigating the *in vivo* application of ^{68}Ga-Pentixafor in patients with MPNs. We demonstrate the feasibility of ^{68}Ga-Pentixafor-PET/CT to non-invasively detect and quantify the extent of BM involvement with MPN patients demonstrating significantly higher tracer uptake in the BM and extramedullary hematopoietic sites than non-malignant controls. With SUV_{mean} ranging between 2.9 and 7.4, the intensity of ^{68}Ga-Pentixafor uptake in the BM of MPN patients was similar to that previously reported for BM involvement in patients with chronic lymphatic leukemia (*13*) and acute myeloid leukemia (*14*).

Interestingly, in our small cohort, the highest tracer accumulation was detected in the BM of a patient with essential thrombocythemia at the time of initial diagnosis. However, further research to establish and validate cut-off values both detection of BM involvement and differentiation between various disease types is still needed.

Our findings are consistent with the concept of MPNs as chronic inflammatory diseases (*15*) in conjunction with an impaired microenvironment that favors malignant over normal hematopoiesis through profound changes in the BM stromal compartment and increased cytokine levels. Interestingly, megakaryocytes have been shown to contribute to MPN pathology and to be a major driver of BM fibrosis (*16*). In line with that observation, our IHC data show CXCR4 expression predominantly on the surface of dysplastic cells of the megakaryocytic lineage. In this context, the extent of CXCR4 uptake may, therefore, also serve as a prognostic factor to further stratify MPN patients.

We monitored three newly-diagnosed MPN patients (n=3/12) over a median of six months after treatment initiation, showing that CXCR4 uptake determined by ^{68}Ga-Pentixafor-PET/CT might correlate with treatment response. The extent of both BM and splenic uptake (SUV_{mean} BM 3.97 vs. 2.86; SUV_{mean} spleen 7.72 vs. 4.89) decreased with treatment initiation. Interestingly, tracer uptake in BM and spleen corresponded with hematological parameters and splenic volume. The reduction of SUV_{mean} after treatment correlated with normalization of hemoglobin, peripheral leukocyte count, thrombocyte count as

well as lactate dehydrogenase. Whereas recent data suggest additional utility of CXCR4-directed PET/CT imaging for response assessment in hematologic malignancies such as extranodal marginal zone lymphoma (*17*) or central nervous system B-cell lymphoma (*18*), the extent to which ^{68}Ga-Pentixafor-PET/CT can be used for response assessment in MPNs needs to be investigated in future prospective studies.

Our pilot observation suffers from several limitations. The radiation exposure associated with PET/CT prevented the use of a control group of healthy individuals. Instead, we included Conn's adenoma patients as a control group who received CXCR4-PET/CT imaging as part of their endocrinological investigation (*19*) and ^{68}Ga-Pentixafor uptake in other controls might be even lower than in our endocrinological control group. However, when compared with previously reported upper limits of physiologic BM uptake measurements in cancer patients without BM involvement, values are in broad agreement with our study (SUV_{mean} BM 1.7 pancreatic adenocarcinoma; SUV_{mean} BM 2.3 MALT lymphoma) (*13*). Furthermore, although immunohistochemical analysis could demonstrate CXCR4 expression in all BM biopsies, CXCR4 expression was relatively low and did not clearly correspond with the intensity of the PET signal. However, surface expression of CXCR4 is a dynamic process and can be influenced by therapeutic interventions (*20*).

CONLUSION

In summary, our data are the first to demonstrate that CXCR4-directed imaging with ^{68}Ga-Pentixafor-PET/CT is feasible to visualize and quantify disease involvement in MPN patients. Further evaluation in larger, prospective studies is warranted to determine the clinical impact in primary staging, response assessment and to evaluate the potential for a theranostic approach.

DECLARATIONS

Conflict of interest/Competing interests: Hans-Jürgen Wester is founder and shareholder of Scintomics GmbH. All other authors declare no conflicts of interest.

Ethical approval: The data in this study represent a retrospective analysis of imaging and therapies and not a prospective clinical trial. Studies and analyses were performed in accordance with the ethical standards of the institutional research committee.

Informed consent: All patients signed written informed consent to the diagnostic procedures as well as to the recording and analysis of their data and the anonymized publication of the results.

KEYPOINTS

QUESTION: Is CXCR4-directed PET imaging with ^{68}Ga-Pentixafor feasible to visualize and quantify disease involvement in myeloproliferative neoplasms?

PERTINENT FINDINGS: This retrospective analysis revealed that CXCR4-directed imaging is positive in all investigated cases and showed a significantly higher tracer uptake in the bone marrow of patients with myeloproliferative neoplasms.

IMPLICATIONS FOR PATIENT CARE: CXCR4-directed PET imaging with ^{68}Ga-Pentixafor is feasible to non-invasively detect and quantify the extent of bone marrow involvement in patients with myeloproliferative neoplasms.

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FIGURES

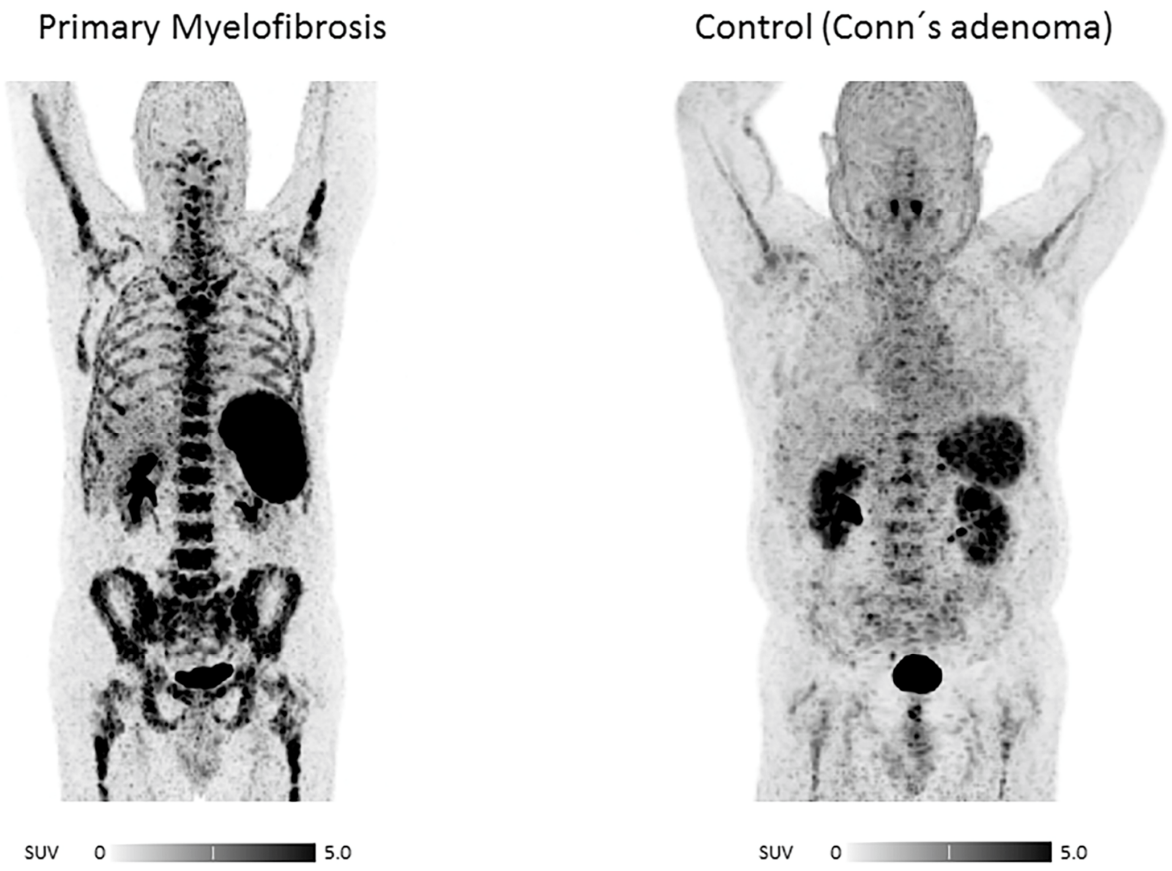


FIGURE 1: Display of a patient (patient #6) with primary myelofibrosis (PMF). ^{68}Ga-Pentixafor-PET/CT (maximum intensity projections) depicts significantly increased tracer uptake in the bone marrow as well as the spleen compared to the control group.

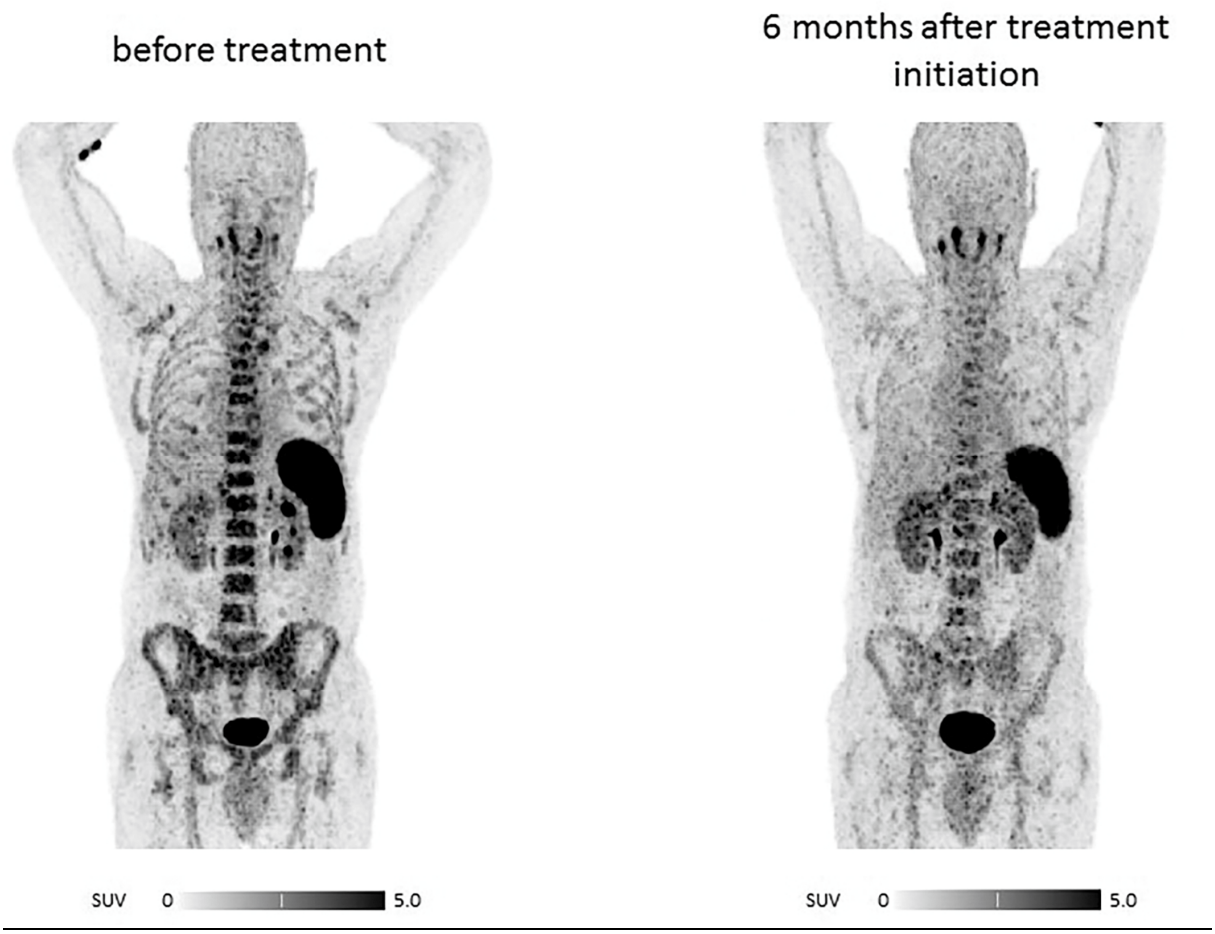


FIGURE 2: Response assessment with CXCR4-directed PET/CT imaging (maximum intensity projections). Example of therapy-induced CXCR4 downregulation in a patient with essential thrombocythemia (patient #5).

TABLES

TABLE 1: Patients` characteristics

Patient #	Sex	Age (y)	Diagnosis	Disease stage	Mutation	Splenomegaly / Splenic volume	Treatment	Follow up PET/CT	CXCR4 IHC staining
#1	M	73	PV	Initial diagnosis	JAK2 V617F	yes (532 cm^3)	Ruxolitinib	yes	yes
#2	F	66	ET	Initial diagnosis	JAK2 V617F	no (226 cm^3)	none	no	yes
#3	M	37	ET	Initial diagnosis	CALR	yes (260 cm^3)	none	no	yes
#4	F	51	ET	Stable disease	JAK2 V617F	no (228 cm^3)	Anagrelide	no	yes
#5	M	60	ET	Initial diagnosis	CALR	yes (450 cm^3)	Hydroxyurea	yes	yes
#6	M	64	PMF	Initial diagnosis	JAK2 V617F	yes (689 cm^3)	Ruxolitinib	yes	yes
#7	M	58	PV	Stable disease	JAK2 V617F	no (280 cm^3)	Hydroxyurea	no	yes
#8	F	62	PMF	Progressive disease	JAK2 V617F	yes (508 cm^3)	Ruxolitinib	no	yes
#9	F	42	PMF	Initial diagnosis	none detected	no (250 cm^3)	None	no	no
#10	F	63	ET	Stable disease	JAK2 V617F	yes (625 cm^3)	Hydroxyurea	no	no
#11	M	58	PMF	Initial diagnosis	JAK2 V617F	no (214 cm^3)	None	no	yes
#12	F	52	ET	Stable disease	none detected	no (180 cm^3)	Anagrelide	no	no

PV: polycythemia vera; ET: essential thrombocythemia; PMF: primary myelofibrosis; CALR: Calreticulin; IHC: Immunohistochemistry

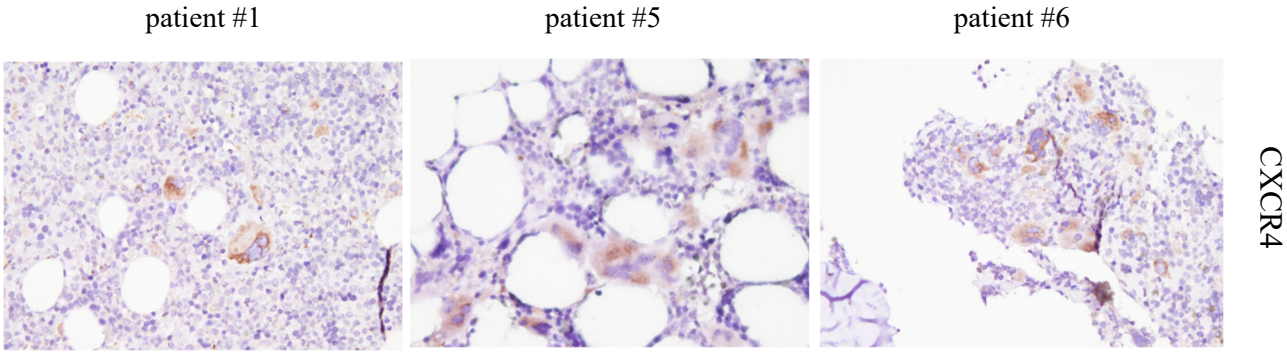
TABLE 2: Bone marrow CXCR4-uptakte (SUV_{mean}) and splenic uptake (SUV_{mean}) in MPNs

	All patients (n=12)		Primary myelofibrosis (n=4)		Essential thrombocythemia (n=6)		Polycythemia vera (n=2)		Controls (n=5)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Bone marrow										
SUV_{mean}	4.44 ± 1.3	2.9-7.4	5.48 ± 0.80	4.2-6.1	4.01 ± 1.40	2.9-7.4	3.92 ± 0.22	3.7-4.1	2.67 ± 0.41	2.3-3.3
Spleen										
SUV_{mean}	6.45 ± 2.34	3.5-12.5	6.12 ± 0.76	4.6-6.4	7.37 ± 2.53	4.1-12.5	4.72 ± 1.25	3.5-6.0	4.44 ± 1.24	2.9-5.4

SUV: Standardized uptake value

SUPPLEMENTARY MATERIALS

Supplemental Figure 1: Individual immunohistochemistry results for CXCR4



Exemplarily depicted is the expression of CXCR4 in bone marrow biopsies from three MPN patients. CXCR4 shows moderate to strong expression in dysplastic cells of the megakaryocytic lineage. Display of the individual results of immunohistochemistry (patient #1: polycythemia vera; patient #5: essential thrombocythemia; patient #6: primary myelofibrosis) undergoing ^{68}Ga-Pentixafor-PET/CT. Magnification: ×400.

Supplemental Table 1: Patients` characteristics and imaging results

No.	Anatomic sites			
	SUVmean BM	SUVmean spleen	TBRmean BM	TBRmean spleen
#1	4.13	3.47	3.50	2.94
#2	4.04	6.42	3.37	5.35
#3	7.38	12.51	4.47	7.58
#4	4.48	7.02	492	7.71
#5	3.97	7.72	3.36	6.54
#6	5.48	6.12	4.57	5.10
#7	3.70	5.96	2.53	4.08
#8	6.11	4.64	5.87	4.46
#9	2.98	5.19	2.54	4.44
#10	2.88	4.08	3.35	4.74
#11	4.19	6.36	3.46	5.26
#12	3.92	7.86	3.26	6.55

SUV: Standardized uptake value; BM: bone marrow; TBR: tumor-to-blood pool ratios

Supplemental Table 2:

Follow-up CXCR4 PET/CT after a median of 6 months after treatment initiation in patients with myeloproliferative neoplasms - Hematological parameters and splenic volume

	Polycythemia vera patient #1		Essential thrombocythemia patient #5		Primary myelofibrosis patient #6	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Hematological parameters						
Leucocyte count	15.900	6.800	29.600	12.200	9.100	5.100
Thrombocyte count	588.000	308.000	206.000	340.000	1.433.000	333.000
Hemoglobin (g/dl)	18.9	12.2	13.9	14.5	14.9	13.9
Lactate dehydrogenase (U/I)	272	222	378	298	180	176
Spleen						
Volume (cm^3)	532	294	450	260	689	336
CXCR4 uptake						
SUV_{mean} BM	4.13	3.86	3.97	2.86	5.48	3.87
SUV_{mean} spleen	3.47	4.59	7.72	4.89	6.12	4.91

SUV: Standardized uptake value; BM: bone marrow

SUPPLEMENTARY MATERIALS AND METHODS

Subjects and study design

Five patients (3 males, 2 females; mean age 59±8 years) who underwent CXCR4-directed PET/CT due to suspicion of benign Conn's adenoma were included as a control group. As previously described, these patients represent the most suitable control group because the exposure of healthy volunteers to unnecessary radiation cannot be justified (*19*).

PET/CT scans of seven patients were obtained as part of the initial staging prior to initiation of any chemotherapy or immunomodulatory agents. Three patients received a follow-up PET/CT scan after a median of 6 months (range 4-7 months) after treatment initiation. Taken together, eight patients had been treated with different agents including hydroxyurea (n=3), ruxolitinib (n=3) or anagrelide (n=2). At the time point of PET/CT scanning, patients' history, splenic volume and hematological parameters were recorded. Blood counts and serum chemistry including lactate dehydrogenase (LDH) were obtained for each patient.

PET/CT imaging

All syntheses were performed in a fully automated, GMP-compliant procedure using a GRP® module (SCINTOMICS GmbH, Germany) equipped with a disposable single-use cassette kit (ABX, Germany). The eluate ($^{68}Ga^{3+}$ in 0.1 M HCl) of a $^{68}Ge/^{68}Ga$-generator (Eckert & Ziegler Radiopharma GmbH, Berlin, Germany) was transferred to a cation exchange cartridge, eluted with 5 N NaCl, added to a solution of 20 µg Pentixafor (Scintomics, Fürstenfeldbruck, Germany) in HEPES-buffer and heated for 6 minutes at 125°C. The product was immobilized on a SepPak C18 cartridge, washed with water und eluted with ethanol/water 50/50. The eluate was passed through a sterile filter (0.22 µm) into a sterile vial und diluted with phosphate buffer solution to a total volume of 15 mL. Radiochemical purity was determined by gradient high performance liquid chromatography and thin-layer chromatography. Additionally, the product was also tested for ethanol content, pH, radionuclide purity, sterility, and endotoxins.

Immunohistochemical stainings of patient biopsy material

Biopsies were obtained within 4 weeks of the ^{68}Ga-Pentixafor-PET/CT examinations. To confirm specific binding of ^{68}Ga-Pentixafor, IHC staining for CXCR4 was conducted using an anti-CXCR4 rabbit polyclonal antibody (ab2074; Abcam, Cambridge, United Kingdom) followed by detection and visualization using the Dako EnVision-HRP rabbit labeled polymer/DAB. Counterstaining was performed with hematoxylin.