

### 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 <sup>68</sup>Ga-Pentixafor-PET/CT. Magnification: ×400.

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Supplemental Table 1: Patients`	characteristics and i	maging results

	Anatomic sites					
No.	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 <sup>3</sup> )	532	294	450	260	689	336
CXCR4 uptake						
SUV <sub>mean</sub> BM	4.13	3.86	3.97	2.86	5.48	3.87
SUV <sub>mean</sub> 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  $59\pm8$  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<sup>®</sup> module (SCINTOMICS GmbH, Germany) equipped with a disposable single-use cassette kit (ABX, Germany). The eluate ( $^{68}$ Ga<sup>3+</sup> 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 <sup>68</sup>Ga-Pentixafor-PET/CT examinations. To confirm specific binding of <sup>68</sup>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.