Imaging of C-X-C Motif Chemokine Receptor 4 Expression in 690 Patients with Solid or Hematologic Neoplasms Using ⁶⁸Ga-Pentixafor PET

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In recent years, molecular imaging addressing the C-X-C motif chemokine receptor 4 (CXCR4) has increasingly been used in various clinical settings. Here, we aimed to assess radiopharmaceutical uptake and image contrast to determine the most relevant clinical applications for CXCR4-directed imaging. We also investigated the impact of specific activity on scan contrast. Methods: Patients (n = 690) with a variety of neoplasms underwent a total of 777 PET/CT scans with ⁶⁸Ga-Pentixafor, serving as the CXCR4-specific radioligand. A semiquantitative target lesion analysis was conducted (providing SUVmax and target-to-blood pool ratio [TBR], defining as SUVmax [target lesion] divided by SUVmax [blood pool]). The applied specific activity (in MBq/μg) was compared with semiquantitative assessments. Results: Of the 777 scans, 242 did not show discernible uptake in disease sites, leaving 535 PET scans (68.9%) for further analysis. Very high tracer uptake (SUVmax > 12) was found in multiple myeloma (n = 113), followed by adrenocortical carcinoma (n = 30), mantle cell lymphoma (n = 20), adrenocortical adenoma (n = 6), and small cell lung cancer (n = 12). Providing information on image contrast, comparable results for TBR were recorded, with TBR (>8) in multiple myeloma, mantle cell lymphoma, and acute lymphoblastic leukemia (n = 6). When comparing specific activity with semiquantitative parameters, no significant correlation was found for SUVmax or TBR (R² ≥ 0.612). Conclusion: In this large cohort, ⁶⁸Ga-Pentixafor demonstrated high image contrast in a variety of neoplasms, particularly for hematologic malignancies, small cell lung cancer, and adrenocortical neoplasms. The present analysis may provide a roadmap for detecting patients who may benefit from CXCR4-targeted therapies.

Key Words: CXCR4; C-X-C motif chemokine receptor 4; ⁶⁸Ga-Pentixafor; PET

Because of its pivotal role in cancer progression, the C-X-C motif chemokine receptor 4 (CXCR4) orchestrates organ-specific tumor spread through several mechanisms. These include promotion of angiogenesis, growth of malignant cells, or inhibition of antitumor immune response (1). Numerous CXCR4-directed molecular imaging agents have been developed recently to define precisely the utility of CXCR4 as an anticancer target (2–5). Among them, the ⁶⁸Ga-labeled radiotracer Pentixafor (cyclo(D-Tyr1-D-[NMe]Orn2(AMBS-68Ga-DOTA)-Arg3-Nal4-Gly5) demonstrated high selectivity for CXCR4 along with rapid renal excretion (6,7). Accordingly, ⁶⁸Ga-Pentixafor has been used in a wide variety of clinical scenarios in oncology. These include in patients with multiple myeloma (MM), marginal zone lymphoma or solid tumor entities, such as small-cell lung cancer (SCLC) and non–small-cell lung cancer, neuroendocrine neoplasms, and adrenocortical carcinoma (8–13). Of note, head-to-head comparison with established imaging modalities or other reference radioisotopes revealed improved lesion detection rates by ⁶⁸Ga-Pentixafor PET (8,14,15). This may promote wider adoption of this imaging agent in patients for whom existing modalities are lacking. Furthermore, ¹⁷⁷Lu⁶⁸Y-Pentixafor, a therapeutic counterpart to target CXCR4, has been applied for targeted radionuclide therapies in hematologic malignancies, such as MM or diffuse large B cell lymphoma (16,17). Such theranostic approaches have demonstrated not only a favorable outcome (16,17), but also tolerable adverse effects, although stem cell support is mandatory (18).

The beneficial use of ⁶⁸Ga-Pentixafor PET/CT, along with its potential to identify patients eligible for treatment with β-particle emitters, favors wider clinical use. However, before widespread adoption or clinical development programs leading to market authorization, comprehensive characterization of its performance should be undertaken.

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FEATURED ARTICLE OF THE MONTH

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including assessment of radiopharmaceutical uptake and image contrast among a broad spectrum of neoplasms. In our bicentric study, which, to our knowledge, enrolled the largest cohort of patients imaged with $^{68}$Ga-Pentixafor PET/CT or PET/MR to date, we aimed to assess radiopharmaceutical accumulation and image contrast in several cancers to determine the most relevant clinical applications. In addition, lower specific activity characterized by higher amounts of cold mass could hamper image interpretation (1,9), for example, by an increasing occupation of the (sub)cellular target by nonradiolabeled components. Thus, we also investigated the impact of specific activity on quantification.

MATERIALS AND METHODS

Patient Population

Patients from 2 study sites were included (University of Würzburg and Medical University of Vienna). Parts of this cohort have been described before to determine the diagnostic usefulness of $^{68}$Ga-Pentixafor PET/CT (7–9,11–14,20–26), without evaluation of image contrast (including impact of specific activity) or comparing uptake among all included diagnoses. Patients signed written informed consent forms before the examination. Given the retrospective character of this study, the local ethics committee waived the need for further approval (no. 20210726 02).

Radiotracer Synthesis

Following good manufacturing practice, $^{68}$Ga-Pentixafor was provided using a synthesis module (Scintomics) and disposable single-use cassette kits (ABX, Radeberg, Germany), as described previously (27). Peptide mass (in μg), activity (in MBq), and specific activity (in MBq/μg) of injected $^{68}$Ga-Pentixafor were recorded for each patient.

Imaging

$^{68}$Ga-Pentixafor PET was performed either on a Siemens Biograph mCT (64 and 128, Siemens Medical Solutions) or on a Siemens Biograph mMR (Siemens Healthcare GmbH). Whole-body scans (covering the vertex of the skull to the proximal thighs) were conducted 60 min after injection of $^{68}$Ga-Pentixafor. We also performed low-dose CT scanning for attenuation correction and anatomic coregistration (120 keV, 512 × 512 matrix, 5-mm slices, increment: 30 mm/s, pitch index: 0.8, and rotation time: 0.5 s). PET images were reconstructed including corrections for CT-based attenuation, random events, and scatter. For MRI, we applied an integrated radiofrequency coil including a multistation protocol (slice thickness, 2 mm), as previously described (12,28).

Image Interpretation

All scans were obtained for clinical or research purposes. As part of this study, all images were reanalyzed by readers who were masked to respective clinical information. At Würzburg, image interpretation was performed as a single-reader analysis, verified by an expert reader. At Vienna, an expert reader performed the assessment.

Semiquantitative Assessment. A target lesion (TL) assessment was performed by investigating the visually most intense TL on PET. Three-dimensional volumes of interest applying an isocontour threshold of 40% were placed on the TL, providing $\text{SUV}_{\text{max}}$, $\text{SUV}_{\text{mean}}$, and $\text{SUV}_{\text{peak}}$. A target-to-blood-pool ratio (TBR) was derived by placing a volume of interest over the aortic arch. TBR was then provided by dividing $\text{SUV}_{\text{max}}$ (of the TL) by $\text{SUV}_{\text{mean}}$ (of the blood pool) (12).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (version 9.2.0, GraphPad Prism Software). Descriptive results are displayed as mean ± SD. Nonparametric Spearman correlation coefficients were calculated to investigate associations between semiquantitative parameters with specific activity (including an outlier correction using the ROUT-Method). A $P$ value of less than 0.05 was considered statistically significant.

RESULTS

Highest Uptake in Hematologic Malignancies, SCLC, and Adrenocortical Neoplasms

No adverse events were recorded after injection of $^{68}$Ga-Pentixafor. Two hundred forty-two of 777 (31.1%) of the scans did not show discernible uptake, leaving 535 of 777 (68.9%) cases for further analysis (Table 1). As such, an overall number of 535 TLs were investigated. Among all TLs, $\text{SUV}_{\text{max}}$ was 13.01 ± 10.01 and the corresponding TBR was 8.59 ± 15.98. The highest average $\text{SUV}_{\text{max}}$ (>12) was found in MM ($n = 113$), followed by adrenocortical carcinoma ($n = 30$), mantle cell lymphoma (MCL; $n = 20$), adrenocortical adenoma ($n = 6$), and SCLC ($n = 12$; Figs. 1 and 2). The lowest average $\text{SUV}_{\text{max}}$ (<6) was recorded in osteosarcoma ($n = 1$), followed by bladder cancer ($n = 1$).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of scans</th>
</tr>
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<tbody>
<tr>
<td>Marginal zone lymphoma</td>
<td>187 (35)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>113 (21.1)</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>50 (9.3)</td>
</tr>
<tr>
<td>Adrenocortical carcinoma</td>
<td>30 (5.6)</td>
</tr>
<tr>
<td>Neuroendocrine neoplasm</td>
<td>30 (5.6)</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>20 (3.7)</td>
</tr>
<tr>
<td>Desmoplastic small round cell tumor</td>
<td>14 (2.6)</td>
</tr>
<tr>
<td>Myeloid disorders</td>
<td>13 (2.4)</td>
</tr>
<tr>
<td>Small cell lung carcinoma</td>
<td>12 (2.2)</td>
</tr>
<tr>
<td>B-cell lymphoma</td>
<td>10 (1.9)</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>9 (1.7)</td>
</tr>
<tr>
<td>Pancreas carcinoma</td>
<td>8 (1.5)</td>
</tr>
<tr>
<td>Non–small cell lung carcinoma</td>
<td>7 (1.3)</td>
</tr>
<tr>
<td>Adrenocortical adenoma</td>
<td>6 (1.1)</td>
</tr>
<tr>
<td>Acute lymphoblastoid leukemia</td>
<td>6 (1.1)</td>
</tr>
<tr>
<td>Liver carcinoma</td>
<td>4 (0.7)</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Pleural mesothelioma</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Mediastinal tumor*</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

*Not otherwise specified.

Data in parentheses are percentages.
mediastinal tumor (not otherwise specified, \(n = 1\)), head and neck cancer (\(n = 2\)), and Ewing sarcoma (\(n = 1\); Fig. 3). For SUV\(_{\text{peak}}\), comparable results were achieved (Supplemental Fig. 1). Moreover, high average TBR (>8) was recorded in MM, MCL, and acute lymphoblastoid leukemia (\(n = 6\)). Low average TBR (<4) was observed in head and neck cancer, colorectal cancer (\(n = 1\)), osteosarcoma, Ewing sarcoma, bladder cancer, renal cell carcinoma (\(n = 1\)), and mediastinal tumor (Fig. 4).

**FIGURE 1.** Maximum-intensity projections of patients with hematologic malignancies imaged with CXCR4-directed \(^{68}\)Ga-Pentixafor. Target lesion is also displayed on transaxial PET, CT, and PET/CT. Patient diagnosed with MM (A; SUV\(_{\text{max}}\) in target lesion, 74.3) and MCL (B; SUV\(_{\text{max}}\) in target lesion, 17.2). Substantially low background activity allowed for precise determination of disease sites.

**FIGURE 2.** Maximum-intensity projections of patients with solid tumor entities imaged with CXCR4-directed \(^{68}\)Ga-Pentixafor. Target lesion is also displayed on transaxial PET, CT, and PET/CT. Patient diagnosed with adrenocortical carcinoma (A; SUV\(_{\text{max}}\) in target lesion, 13.2) and small cell lung carcinoma (B; SUV\(_{\text{max}}\) in target lesion, 19.4). Background activity was substantially low, providing a precise read-out of disease sites.
Our study showed that the median injected peptide mass was 8.5 ± 2.56–35.61 μg, injected activity was 143 ± 38–239 MBq, and specific activity was 15.39 ± 2.19–43.70 MBq/μg. Comparing specific activity with semiquantitative parameters, only SUVmean (ρ = 0.138, P = 0.002), but none of the other correlative indices, reached significance (SUVmax: ρ = 0.01, P = 0.832; TBR: ρ = 0.023, P = 0.612; SUVpeak: ρ = −0.087, P = 0.053; Fig. 5).

**DISCUSSION**

In the present bicentric study investigating a large cohort imaged with 68Ga-Pentixafor, discernible uptake in putative sites of disease was noted in more than 68% of the scans. Among neoplasms studied, we determined MM had the highest uptake (SUV), with adrenocortical carcinoma and MCL closely after. Comparable results were recorded for image contrast (TBR). Specific activity had no impact on a semiquantitative level, supporting the notion that an excellent read-out can be achieved, even after administration at low specific activities.

A growing body of evidence supports the clinical utility of CXCR4-targeted 68Ga-Pentixafor PET/CT in a variety of disease entities, including hematologic malignancies (7,22) and solid tumors (29). Some of these studies also revealed that 68Ga-Pentixafor provided an increased detection rate at sites of disease when compared with conventional imaging or other PET agents such as 18F-FDG or somatostatin receptor–directed radiopharmaceuticals, thereby indicating that this agent can image malignancies that lack a more suitable modality (15,23,26). Here we aimed to provide a precise cohort of neoplasms that exhibit high tracer avidity.

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Increased CXCR4 expression on the tumor cells has been tightly linked to poor outcome in hematologic malignancies and
solid tumors (32,33), suggesting it as a viable therapeutic target. For example, the stromal cell–derived factor 1 neutralizing agent Olaptesed pegol (NOX-A12) or the CXCR4 antagonist Plerixafor have each been used in patients with refractory MM. In clinical phase I/II studies, such drugs achieved an overall response rate in almost half of the patients (Plerixafor) or partial response in 68% (NOX-A12) (34,35). Theranostic approaches based on 68Ga-Pentixafor scan results have also been conducted using the therapeutic analogs 177Lu/90Y-Pentixather (16,18). Although there is no study reporting an association between PET-based SUV_max and absorbed doses in lesions on CXCR4-directed imaging and radionuclide therapies to date, the markedly increased SUV_max observed in certain cases suggests that a substantial fraction of patients may also be eligible for CXCR4-directed radioligand therapies. However, CXCR4-targeted endoradiotherapy causes bone marrow ablation and, thus, subsequent stem cell support is needed (18), further emphasizing the importance of well-established algorithms for adequate patient selection.

Future studies should also evaluate the ability of CXCR4-directed molecular imaging to assess the retention capacities of nonradiolabeled CXCR4 neutralizing agents in vivo, preferably before treatment onset. For instance, a phase I study evaluating the CXCR4 inhibitor LY2510924 in patients with advanced solid cancers revealed favorable antitumor activity (36). Of note, a substantial patient fraction treated with this “cold” CXCR4 inhibitor had clinical diagnoses identical to those in the present study, for example, ovarian, lung, or pancreatic cancer (all demonstrating SUV_max > 6). As such, a baseline 68Ga-Pentixafor PET/CT revealing increased CXCR4 expression at disease sites may allow identification of patients who would most likely be suitable for nonradiolabeled CXCR4-directed drugs, including LY2510924 (36). This would then further expand the theranostic concept beyond identifying patients for treatment with β-emitters, but also to selecting individuals for nonradiolabeled CXCR4-targeted therapeutic options.

Our study has several limitations. We included both CT- and MR-based hybrid imaging, which may provide an additional variable that could be controlled better in future studies. Despite investigating the largest cohort to date, prospective trials should also be undertaken. In addition, the numbers of investigated patients per tumor entity substantially varied. Thus, resulting low numbers of cases and inter- and intrapatient heterogeneity of in vivo CXCR4 expression may have biased the results presented herein. Future studies should therefore consider more balanced subgroups enrolling comparable numbers of patients diagnosed with the identical tumor entity.

FIGURE 5. Correlation plots between specific activity in MBq/µg and SUV_max (A) and TBR (B). Significant Spearman ρ and P are displayed. No significance was reached.

CONCLUSION
We found high uptake and image contrast for a variety of neoplasms imaged with 68Ga-Pentixafor PET, such as MM and MCL, but also for adrenal neoplasms and SCLC. These results suggest clinical scenarios in which 68Ga-Pentixafor PET may prove beneficial for directing CXCR4-targeted therapies.

DISCLOSURE
Hans-Jürgen Wester is founder and shareholder of Scintomics. Anja Zehndner is an employee of Pentixapharm, Würzburg. No potential conflict of interest relevant to this article was reported.

KEY POINTS

QUESTION: What is the tracer avidity of malignant lesions, and what are the most avid tumors for CXCR4-targeted 68Ga-Pentixafor PET?

PERTINENT FINDINGS: We observed high uptake and image contrast of the radiopharmaceutical, in particular for hematologic malignancies as well as adrenal neoplasms and small cell lung cancer. Specific activity had no effect on 68Ga-Pentixafor uptake.

IMPLICATIONS FOR PATIENT CARE: Among a broad spectrum of neoplasms, the present bicentric study suggests entities eligible for CXCR4-directed molecular imaging and therapy.

REFERENCES


