Microenvironment and Immunology

Molecular Imaging of CXCR4 Receptor Expression in Human Cancer Xenografts with $^{[64\text{Cu}]\text{AMD3100}}$ Positron Emission Tomography

Sridhar Nimmagadda, Mrudula Pullambhatla, Kristie Stone, Gilbert Green, Zaver M. Bhujwalla, and Martin G. Pomper

Abstract

The chemokine receptor CXCR4 and its cognate ligand CXCL12 are pivotal for establishing metastases from many tumor types. Thus, CXCR4 may offer a cell surface target for molecular imaging of metastases, assisting diagnosis, staging, and therapeutic monitoring. Furthermore, noninvasive detection of CXCR4 status of a primary tumor may provide an index of the metastatic potential of the lesion. Here, we report the development and evaluation of $^{[64\text{Cu}]\text{AMD3100}}$, a positron-emitting analogue of the stem cell mobilizing agent plerixafor to image CXCR4 in human tumor xenografts presel ected for graded expression of this receptor. This imaging method was evaluated in lung metastases derived from human MDA-MB-231 breast cancer cells. Ex vivo biodistribution studies, performed to validate the in vivo imaging data, confirmed the ability of $^{[64\text{Cu}]\text{AMD3100}}$ to image CXCR4 expression. Our findings show the feasibility of imaging CXCR4 by positron emission tomography using a clinically approved agent as a molecular scaffold. Cancer Res; 70(10): 3935–44. ©2010 AACR.

Introduction

The ability of malignant cells to metastasize to distant sites is one of the most lethal aspects of cancer. CXCR4, a member of the surface G protein–coupled seven-span transmembrane receptor class, plays a critical role in the homing of cancer cells to distant sites (1, 2) by binding to its ligand CXCL12, which is highly expressed where metastatic lesions are commonly observed (1, 3).

Increased CXCR4 expression is associated with an aggressive phenotype. Metastases frequently exhibit increased CXCR4 receptor expression compared with the primary lesion (4–6). Overexpression of CXCR4 in primary tumors is directly related to the degree of lymph node metastasis (7, 8). Similarly, elevated CXCR4 expression in estrogen and progestin receptor–negative breast cancers and triple negative breast cancers is closely associated with lymph node metastasis (9, 10). Inhibition of CXCR4-CXCL12 signaling by antibodies, peptides, small molecules, or siRNA knockdown has been found to reduce metastatic burden in various orthotopic and metastatic models of breast cancer (2, 11–15).

The critical role of CXCR4 in metastasis makes it an important biomarker to identify primary tumors that are more likely to metastasize (7, 16–18). Preclinical and clinical studies have detected high concentrations of CXCR4 receptors in the primary brain tumors compared with normal brain parenchyma (19, 20). The invading regions of glioblastomas and satellite tumors, which are the primary foci of recurrence, have been observed to express high levels of CXCR4 (21). Tissue microarray analyses of patient biopsies have shown that nuclear staining for CXCR4 increases with tumor grade (5) and that elevated CXCR4 expression levels are associated with poor survival in patients with breast cancer (8, 22, 23). CXCR4 expression has therefore been proposed as a prognostic factor in several cancers including brain, breast, colon, prostate, melanoma, and osteosarcoma and considered a therapeutic target because of its role in tumor development, growth, and metastasis (24, 25).

CXCR4 is expressed on the surface of several cell types including those of the central nervous, gastrointestinal, and immune systems (26). In the immune system, it is expressed on peripheral blood lymphocytes, monocytes, neutrophils, pre-B cells, mast cells, and CD34+ hematopoietic progenitor cells (27) and mediates leukocyte homing (28) and bone marrow homeostasis (29). Because of these expression patterns, extended or chronic use of CXCR4 targeted therapeutics may result in unwanted toxicity. A CXCR4-based imaging agent can be used to identify patients likely to respond to CXCR4-based therapies, thereby reducing unnecessary toxicity. Attempts have been made to image CXCR4 expression in cancer models using $^{111}\text{In}$-labeled peptides and $^{125}\text{I}$-labeled monoclonal antibodies with single-photon emission computed tomography (SPECT)/computed tomography (CT) (30, 31). Synthesis and evaluation of the positron emission tomography (PET) imaging agent $^{[64\text{Cu}]\text{AMD3100}}$ were recently described in normal mice (32), but studies in tumor models have not been reported to date.
To image CXCR4 expression in tumors, we used the ability of the cyclam function of the prototype CXCR4 inhibitor AMD3100 to form strong complexes with Cu to develop $[^{64}\text{Cu}]$AMD3100 as an imaging agent for PET. AMD3100 is a high-affinity, specific CXCR4 antagonist that inhibits binding of the natural chemokine ligand CXCL12 (33–35). Previous studies have shown that Cu bound to the cyclam increases the affinity of AMD3100 to the CXCR4 receptor by 7-fold (36). Here we present a detailed in vivo evaluation of $[^{64}\text{Cu}]$AMD3100 in tumor models. In proof of principle studies, we evaluated CXCR4 expression in subcutaneous brain tumor models stably expressing CXCR4. We showed that CXCR4 expression imaging is feasible in orthotopic and metastatic models of breast cancer by PET. Ex vivo biodistribution analyses were performed to further validate the in vivo studies.

**Materials and Methods**

**Cell lines.** The human glioblastoma cell line U87 and the breast carcinoma epithelial cell line MDA-MB-231 were gifts from Drs. John Laterra and Zaver Bhujwalla, respectively. DU4475, a breast carcinoma epithelial cell line, was purchased from American Type Culture Collection (ATCC) and has been cultured for <6 months in our laboratory. Both U87 and MDA-MB-231 cell lines were authenticated and purchased from ATCC but have not been authenticated in our laboratory. U87 and breast cancer cell lines were maintained in MEM and RPMI, respectively, supplemented with 10% fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 mg/mL of streptomycin. A U87 cell line stably transfected with human CD4 and CXCR4 (U87-stb-CXCR4) was obtained from NIH AIDS Research and Reference Reagent Program (Dr. HongKui Deng and Dr. Dan R. Littman; ref. 37) and maintained in DMEM supplemented with 15% FBS, 1 μg/mL puromycin, 300 μg/mL G418, 100 units/mL of penicillin, and 100 mg/mL of streptomycin. All cell lines were maintained in a humidified incubator with 5% CO₂. All cell culture reagents were purchased from Invitrogen.

**Radiopharmaceutical preparation.** AMD3100 was purchased from Sigma-Aldrich, and $[^{64}\text{Cu}]$CuCl₂ was obtained from Nordion or The University of Wisconsin. $[^{64}\text{Cu}]$AMD3100 was prepared according to literature methods for the corresponding unlabeled material (36). Briefly, 200 μg of AMD3100 were added to 370 to 740 MBq (10–20 mCi) of $[^{64}\text{Cu}]$CuCl₂ buffered with 100 mmol/L sodium acetate (pH, 5.5) and heated at 60°C for 40 minutes. $[^{64}\text{Cu}]$AMD3100 (Fig. 1A) was purified on a reverse phase high-performance liquid chromatography (HPLC) system (Varian) using a C-18 column (Luna; 5 μm, 250 × 10 mm). The mobile phase consisting 89% water with 0.1% trifluoroacetic acid and 11% methanol with 0.1% trifluoroacetic acid was used at a flow rate of 5 mL/min. UV absorbance was monitored at 266 nm, and radioactivity was detected by a model 105S single-channel radiation detector (Bioscan). A radioactive peak from 20 to 22 minutes containing the major product was collected and concentrated. The final product was then formulated in PBS, sterile filtered, and used for in vitro and in vivo experiments.

**Receptor binding assays.** U87, U87-stb-CXCR4, DU4475, and MDA-MB-231 cells seeded in six-well plates at 60% to 80% confluence were used for receptor binding assays. Cells

![Figure 1](image-url)
were incubated with 37 kBq (1 μCi)/mL of [64Cu]AMD3100 at 4°C for 30 minutes in a binding buffer [PBS containing 5 mmol/L MgCl2, 1 mmol/L CaCl2, 0.25% bovine serum albumin (pH 7.4)]. After incubation, cells were washed quickly four times with 4°C binding buffer and detached using non-enzymatic buffer, and cell-associated radioactivity was determined in a gamma spectrometer (Pharmacia/LKB Nuclear, Inc.). Radioactivity values were converted into percentage of injected dose per million cells. Experiments were performed in triplicate and repeated thrice.

**Animal models.** Procedures were conducted according to protocols approved by Johns Hopkins Animal Care and Use Committee. Female non-obese diabetic (NOD)/severe combined immunodeficient (SCID) mice, 6 to 8 weeks old, weighing between 20 and 25 g were purchased from The Johns Hopkins Immune Compromised Animal Core. Mice were implanted s.c. with U87 and U87-stb-CXCR4 brain tumor cells (3 × 10^6/100 μL) in the left and right upper flanks, respectively. Brain tumor models were used when the tumor size was 400 to 500 mm3. For breast tumor imaging, CXCR4low MDA-MB-231 (3 × 10^6/100 μL) and CXCR4high DU4475 (2 × 10^6/100 μL) cells were inoculated in the left and right upper thoracic mammary fat pads, respectively. Breast tumor models were used when the tumor size was 50 to 100 mm3.

**PET/CT imaging and analysis.** An eXplore Vista small animal PET (GE) and X-SPECT small animal SPECT/CT system (Gamma Medica Ideas) were used for image acquisition. Before the injection of radiotracer, mice were induced with 3% and maintained under 1.5% isoflurane. Dynamic imaging studies were performed in three mice harboring U87 and U87-stb-CXCR4 xenografts. After an i.v. infusion of [64Cu]AMD3100 (range, 8.2–13 MBq; mean, 10.5 MBq), a 60-minute dynamic imaging sequence (14 frames: 2 × 30 s, 3 × 60 s, 3 × 120 s, 2 × 300 s, 4 × 600 s) was acquired over the tumors in the left and right upper thoracic mammary fat pads. The tumor size was measured by caliper. The percentage of injected dose per gram (%ID/g) values were calculated based on a predetermined calibration factor using a known quantity of radioactivity. PET data were acquired using AMIDE software (SourceForge), and time-activity values were averaged over three animals. PET data were acquired using AMIDE software (SourceForge), and time-activity values were averaged over three animals.

**Ex vivo biodistribution.** NOD/SCID mice bearing either brain or breast tumors were injected i.v. with 740 kBq (20 μCi) of [64Cu]AMD3100 in 200 μL of saline. At 2, 10, 30, 60, 90, 120, and 240 minutes after injection, blood, lungs, liver, muscle, spleen, stomach, small intestine, kidney, and tumor samples were collected and weighed. Tissue sections were frozen and stored at −80°C for posterior analysis. Tumors of 100 to 200 mm3 size were used for experiments to minimize necrosis. An experimental lung metastasis model was established by tail vein injection of 2 × 10^6 MDA-MB-231 cells in 200 μL of HBSS. NOD/SCID mice injected with HBSS alone were used as controls. Lung metastasis animals were used for experiments at 35 days after i.v. injection of tumor cells.

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recognize amino acids 328 to 338 of human CXCR4 (38) with minimum cross-reactivity to mouse CXCR4 (Imgenex) at a 1:100 dilution. Specific staining was visualized using biotin-SP–conjugated AffiniPure goat anti-rabbit antibody (Jackson Immunoresearch) followed by peroxidase-labeled streptavidin (LSAB+ System-HRP, DakoCytomation) and incubation with substrate-chromogen solution according to the manufacturer’s instructions.

Data analysis. Statistical analysis was performed using an unpaired two-tailed \( t \) test. \( P \) values of < 0.05 for the comparison between CXCR4 high and CXCR4 low tumor uptake were considered to be statistically significant.

### Table 1. In vitro analysis of CXCR4 receptor expression and \(^{64}\text{Cu}\)AMD3100 binding

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Receptors/cell</th>
<th>Percentage of incubated dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>U87-stb-CXCR4</td>
<td>134,999 ± 20,341</td>
<td>13.1 ± 1.6</td>
</tr>
<tr>
<td>U87</td>
<td>3,664 ± 802</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>DU4475</td>
<td>16,640 ± 5,128</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>6,833 ± 1,570</td>
<td>0.1 ± 1.0</td>
</tr>
</tbody>
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Figure 2. PET/CT imaging of CXCR4 expression in subcutaneous brain tumor xenografts with \(^{64}\text{Cu}\)AMD3100. NOD/SCID mice bearing U87 and U87-stb-CXCR4 glioblastoma xenografts on the left and right flanks, respectively, were given ~11.1 MBq (300 μCi) of \(^{64}\text{Cu}\)-labeled radiotracers via tail vein injection, and PET/CT images were acquired. A, representative transaxial PET, CT, and fused sections of both the tumors from a \(^{64}\text{Cu}\)AMD3100-injected mouse at 90 min postinjection. B, volume-rendered whole body images of \(^{64}\text{Cu}\)AMD3100 (left), 50 mg/kg of AMD3100 blocking dose followed by \(^{64}\text{Cu}\)AMD3100 (middle), and \(^{64}\text{Cu}\)CuCl₂ alone (right). All images were scaled to the same maximum threshold value. C, dynamic time-activity curves acquired over 60 min in mice injected with \(^{64}\text{Cu}\)AMD3100. Points, means of four animals; bars, SEM. Specific accumulation of radioactivity in U87-stb-CXCR4 (red line) over U87 (blue line) is apparent. D, PET image of bone marrow uptake of \(^{64}\text{Cu}\)AMD3100 at 90 min postinjection. Scale was adjusted for clear visualization of bone marrow. Solid arrow, U87 tumor; unfilled arrow, U87-stb-CXCR4 tumor; arrow head, bone marrow; L, liver; K, kidney; B, bladder.
Results

Radiolabeling. The specific radioactivity of the $^{64}$Cu AMD3100 after purification was typically $134 \pm 61$ GBq/μmol (3.64 ± 1.6 Ci/μmol), with radiochemical purity of >98% as determined by radio-HPLC (Supplementary Fig. S1).

Receptor expression and in vitro radioligand binding. Flow cytometry analysis using 12G5 antibody revealed U87-stb-CXCR4, U87, MDA-MB-231, and DU4475 cells to be >95, 1 to 2, 6 to 18, and >95% positive for CXCR4 expression, respectively (Fig. 1B and C). In accordance with the percentage of CXCR4-positive cells observed, $^{64}$Cu AMD3100 showed specific binding in the order U87-stb-CXCR4 > DU4475 > MDA-MB-231 > U87. Further receptor quantification analysis using quantibrite beads revealed that DU4475 and U87-stb-CXCR4 express 16,640 ± 5,128 and 134,999 ± 20,341 receptors per cell, respectively, accounting for the differences in $^{64}$Cu AMD3100 binding observed. A detailed analysis of receptor expression status and radioligand binding is presented in Table 1.

PET imaging. Delineation of CXCR4-specific tumor uptake and retention of radioactivity is evident in the subcutaneous, orthotopic, and lung metastases models. The dynamic PET images acquired on the subcutaneous brain tumor models over 60 minutes showed a continuous accumulation of radioactivity in the U87-stb-CXCR4 tumor with %ID/g reaching 35 (Fig. 2A–C). Other than the U87-stb-CXCR4 tumor, whole body images at 90 minutes showed significant uptake in the liver, kidneys, and bladder. Bone marrow uptake of $^{64}$Cu AMD3100 was also evident (Fig. 2D). Blocking studies showed >90% reduction of radioactivity signal in both tumors. Relatively, less radioactivity was also observed in liver and kidneys (Fig. 2B). To show the specificity of $^{64}$Cu AMD3100, we also injected one mouse with $^{64}$Cu CuCl$_2$. The mouse receiving $^{64}$Cu CuCl$_2$ showed uniform distribution of radiotracer (Fig. 2B).

The 90-minute whole body images in the breast tumor model showed selective accumulation of activity in CXCR4$^{\text{high}}$ DU4475 tumors compared with CXCR4$^{\text{low}}$ MDA-MB-231...
tumors (Fig. 3A, B). Accumulation of radioactivity was similarly observed in the lung metastasis model (Fig. 4A, B).

**Ex vivo biodistribution, immunohistochemistry, and flow cytometry.** To quantify the degree of radiotracer uptake on a per organ basis, tissue and tumors were collected for up to 4 hours after injection of \[^{64}\text{Cu}\]AMD3100 from female NOD/SCID mice bearing subcutaneous brain tumors (Fig. 5A). The U87-stb-CXCR4 tumor showed consistently higher uptake relative to U87 tumors, except for the 2-minute time point, in agreement with the imaging data (Fig. 5A). Both the imaging and biodistribution studies clearly show that 90 minutes after radiotracer injection is the optimal time point for imaging these tumor models. The U87-stb-CXCR4-to-U87 tumor ratios reached a maximum of 6.16 ± 1.37 at 90 minutes. Also, the tumor-to-muscle ratios reached a maximum of 47.36 ± 6.93 and 13.39 ± 4.20 for U87-stb-CXCR4 and U87, respectively. Similarly, the tumor-to-blood ratios were 16.93 ± 3.40 and 2.62 ± 0.52 for U87-stb-CXCR4 and U87, respectively. Liver was noted to have the highest accumulation of radioactivity at all times. Specificity was shown in mice receiving either a 50 mg/kg blocking dose of AMD3100 followed by \[^{64}\text{Cu}\]AMD3100 or \[^{64}\text{Cu}\]CuCl\(_2\) alone. The administration of blocking dose resulted in >90% reduction in radioactivity accumulation in U87-stb-CXCR4 tumors. There was also reduction of radioactivity accumulation in U87 tumors, blood, and other tissues. In mice receiving blocking dose, the highest level of radioactivity was noted in kidneys, perhaps due to rapid clearance (Fig. 5B). Also, saturation of the plasma protein binding by unlabeled AMD3100 could have resulted in faster excretion. To investigate the role of Cu transchelation, if any, we also injected another group of mice with \[^{64}\text{Cu}\]CuCl\(_2\). Whereas blocking dose studies clearly showed the specificity of \[^{64}\text{Cu}\]AMD3100, the radioactivity distribution in mice receiving \[^{64}\text{Cu}\]CuCl\(_2\) was uniform, indicating that uptake observed could not be due to transchelated copper from \[^{64}\text{Cu}\]AMD3100.

Based on the above observations, biodistribution studies were carried out at 90 minutes postinjection in NOD/SCID mice harboring orthotopic MDA-MB-231 and DU4475 tumors and in MDA-MB-231-derived lung metastasis model. The tumor-to-muscle ratios were 26.24 ± 3.50 and 14.41 ± 0.73 for DU4475 and MDA-MB-231 tumors, respectively. Similarly, the tumor-to-blood ratios were 3.00 ± 0.39 and 1.93 ± 0.37 for the DU4475 and MDA-MB-231 tumors, respectively. There was a significant difference in %ID/g, tumor-to-muscle ratio, and tumor-to-blood ratio between the tumor types (Fig. 3C). No significant differences were observed in other tissues compared with the brain tumor models. Representative H&E-stained and

![Figure 4](image-url)
CXCR4-stained sections showing the difference in CXCR4 expression are shown in Fig. 3D. To evaluate if CXCR4 expression in metastases could be used for diagnostic purposes, we tested the feasibility in a MDA-MB-231–derived experimental lung metastasis model. The %ID/g values clearly show a significant difference in radioactivity uptake between the lung metastasis and saline-injected controls (Fig. 4C). The lung-to-muscle ratios at 90 minutes postinjection were 22.99 ± 2.50 and 14.32 ± 2.67 for lung metastasis and control animals, respectively. Representative CXCR4-stained section is shown in Fig. 4C. To validate the imaging and biodistribution results, we extracted lungs from a different group of mice and evaluated the CXCR4 expression levels in lung metastases. Flow cytometry analysis showed a 15% to 30% increase in the CXCR4 expression in the lung metastases–derived cell lines (Fig. 1B; Supplementary Fig. S2).

**Discussion**

Preclinical evaluation of [64Cu]AMD3100, a CXCR4 inhibitor known in unchelated form as Plerixafor, showed the feasibility of PET imaging of CXCR4 expression in a glioblastoma cell line stably expressing CXCR4 and in breast orthotopic and lung metastasis models. PET imaging of CXCR4 expression using [64Cu]AMD3100 may provide a novel strategy for detecting tumors and metastases.

We initially characterized the kinetics and biodistribution of [64Cu]AMD3100 in subcutaneous brain tumor models stably expressing CXCR4. In a preclinical study, AMD3100 used at pharmacologic doses caused growth inhibition of intracranial primary brain tumors and resulted in a synergistic effect, when combined with cytotoxic chemotherapy, in a CXCR4 expression–dependent manner (39). Here, by comparing a glioblastoma cell line with high or low CXCR4 expression, we showed that [64Cu]AMD3100 bound specifically to U87-stb-CXCR4 glioblastoma cells but not to the parental U87 cell line. The specificity in cells was validated by the in vivo uptake and retention in subcutaneous tumors derived from these cell lines. Dynamic PET imaging and biodistribution studies with [64Cu]AMD3100 exhibited a 6-fold increase in tumor uptake in U87-stb-CXCR4 tumors compared with U87 tumors. This uptake was blocked by unlabeled AMD3100, showing that the uptake was specific for the presence of CXCR4. Measurements of logP for [64Cu]AMD3100 were found to be 0.52 ± 0.02 (Supplementary Results), suggesting that [64Cu]AMD3100 may not be suitable for imaging CXCR4 beyond an intact blood-brain barrier.

We further validated our findings using established human breast cancer models and a model of experimental metastasis. Most breast tumor tissue has some levels of CXCR4 expression, and >40% of breast tumors show elevated expression (5, 40). We imaged CXCR4 expression in two human breast cancer cell lines preselected for high (DU4475) and relatively low (MDA-MB-231) expression. Significantly higher [64Cu]AMD3100 binding was observed in DU4475 breast cancer cells compared with MDA-MB-231 cells. Both the imaging and ex vivo biodistribution data showed CXCR4-specific uptake in the tumors. However, the uptake values in the DU4475 tumors were not as high as anticipated based on the receptor expression levels observed by flow cytometry. Histologic analysis revealed considerable necrosis in those tumors even when small, accounting for the low uptake and retention observed. Bone marrow uptake was evident in the images, possibly due to CXCR4 expression on hematopoietic cells, although the collection of bone but not bone marrow alone may have reduced the total %ID/g values. Also, the enlarged lymph nodes exhibited increased accumulation of radioactivity, indicating a potential role for [64Cu] AMD3100 PET imaging to identify lymph node metastasis. CXCR4 expression on immune cells and under inflammatory conditions is of particular interest in the context of immune cell trafficking and tumor evasion.
conditions may result in noncancer-specific uptake in lymph nodes, spleen, and thymus. Similar noncancer-specific uptake can also be observed in tissues that have a basal level of CXCR4 expression, such as brain and pancreas (26), although elevated CXCR4 expression in tumors would result in increased uptake.

CXCR4 has been shown to play an important role in several steps of the metastatic cascade, such as cancer cell migration, invasion, and adhesion. Using gene expression profiling, Minn and colleagues showed that, in a subset of MDA-MB-231 cells with a propensity to metastasize to the lung, CXCR4 was one of the genes responsible for the metastatic phenotype (41). These data suggest that high levels of CXCR4 expression in cancer cells result in these cells homing to sites that are known to express high levels of the CXCR4 ligand CXCL12.

One goal of our studies was to evaluate the use of [64Cu] AMD3100 to detect metastases. Both in vivo and ex vivo data clearly showed detection of metastatic nodules in the lung, which were further confirmed by flow cytometry of single-cell extracts from these metastases as well as immunohistochemical analysis. The increase in CXCR4 expression in metastases due to clonal selection of cancer cells that metastasize to the lungs has been previously reported (42) and was confirmed by our findings.

Although we showed the ability to image CXCR4 expression in tumors and metastases using [64Cu]AMD3100, the liver and background tissue uptake observed should be discussed. Three major factors contribute to the accumulation of CXCR4 in normal tissue. One is the basal level of CXCR4 expression that produces a small background signal. The second is the moderate plasma protein binding observed with AMD3100. Nearly 58% of AMD3100 is protein bound. Although that increases the background signal, the advantage is that it also enhances the probability of delivering the radiotracer to the tumor. Additionally, as observed in our results, the background slowly deceased with time while the tumor uptake increased until 90 minutes postinjection. This suggests that, despite binding to plasma protein, [64Cu] AMD3100 was CXCR4-specific. The third is the instability of copper bound to the cyclam moiety and metabolism of [64Cu]AMD3100. Although [64Cu]-cyclam complexes are thermodynamically stable in vitro, several studies suggest that [64Cu] may dissociate and subsequently transchelate to superoxide dismutase, resulting in increased accumulation in the blood and liver (43–45). At 90 minutes, radioactivity in the blood of [64Cu]AMD3100-injected mice was 1.5-fold less compared with mice receiving [64Cu]CuCl2. In the case of transchelation, we would have detected similar blood radioactivity to that of [64Cu]CuCl2-injected mice, which was not observed. In addition, our metabolite studies using size exclusion columns showed that 30% to 40% of radioactivity was protein bound at 90 minutes after injection (Supplementary Fig. S3). Because of the plasma protein binding associated with AMD3100, it was difficult to further delineate the percentage of radioactivity bound to the cyclam moiety. However, the structurally similar bifunctional chelate CPTA [4-[(1,4,8,11-tetraazacyclotetradec-1-yl)methyl] benzoic acid] is routinely used for [64/67Cu] chelation, and blood analysis of radiolabeled antibodies injected animals suggests that >74% of radioactivity is still associated with the antibody at 24 hours postinjection (46, 47). The increased liver uptake observed could also be attributed to the positive charge associated with the cyclam moiety. The positive charge on cyclams has been identified as a cause of liver accumulation (48). In humans, AMD3100 elimination was primarily through kidneys, and nearly 70% of the drug was eliminated within 24 hours as parent, indicating metabolic stability (49).

Possible cyclam metabolites have been shown to have low affinity to CXCR4 (34, 36), suggesting that the uptake observed in our studies is due to intact [64Cu]AMD3100. Insights obtained from our studies and recent developments in bridged cyclam-based inhibitors may lead to the development of stable Cu-based CXCR4 imaging agents with optimized pharmacokinetics (50).

In summary, we have synthesized and evaluated a positron-emitting version of the prototype CXCR4 inhibitor AMD3100 in brain tumor xenografts stably expressing CXCR4. Our data with orthotopic breast xenografts and experimental metastases show that [64Cu]AMD3100 can be used to image graded levels of CXCR4 expression. The work presented here is a first step toward imaging a receptor directly involved in the metastatic process and provides a valuable strategy for noninvasive molecular characterization of tumors and metastases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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PET Imaging of CXCR4 Expression

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