

## Targeting CXCR4 (CXC Chemokine Receptor Type 4) for Molecular Imaging of Aldosterone-Producing Adenoma

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**Abstract**—Primary aldosteronism is the most frequent cause of secondary hypertension and is associated with increased morbidity and mortality compared with hypertensive controls. The central diagnostic challenge is the differentiation between bilateral and unilateral disease, which determines treatment options. Bilateral adrenal venous sampling, currently recommended for differential diagnosis, is an invasive procedure with several drawbacks, making it desirable to develop novel noninvasive diagnostic tools. When investigating the expression pattern of chemokine receptors by quantitative real-time polymerase chain reaction and immunohistochemistry, we observed high expression of CXCR4 (CXC chemokine receptor type 4) in aldosterone-producing tissue in normal adrenals, adjacent adrenal cortex from adrenocortical adenomas, and in aldosterone-producing adenomas (APA), correlating strongly with the expression of CYP11B2 (aldosterone synthase). In contrast, CXCR4 was not detected in the majority of nonfunctioning adenomas that are frequently found coincidentally. The specific CXCR4 ligand <sup>68</sup>Ga-pentixafor has recently been established as radiotracer for molecular imaging of CXCR4 expression and showed strong and specific binding to cryosections of APAs in our study. We further investigated 9 patients with primary aldosteronism because of APA by <sup>68</sup>Ga-pentixafor–positron emission tomography. The tracer uptake was significantly higher on the side of increased adrenocortical aldosterone secretion in patients with APAs compared with patients investigated by <sup>68</sup>Ga-pentixafor–positron emission tomography for other causes. Molecular imaging of aldosterone-producing tissue by a CXCR4-specific ligand may, therefore, be a highly promising tool for noninvasive characterization of patients with APAs. (*Hypertension*. 2018;71:317-325. DOI: 10.1161/HYPERTENSIONAHA.117.09975.) • [Online Data Supplement](#)

**Key Words:** adrenal cortex ■ aldosterone ■ Ga68-pentixafor ■ humans ■ molecular imaging

Primary aldosteronism (PA) is characterized by adrenocortical oversecretion of aldosterone, which causes increased cardiovascular and metabolic morbidity.<sup>1-4</sup> In most cases, PA is either caused by bilateral adrenal hyperplasia or unilateral aldosterone-producing adenoma (APA).<sup>5</sup> Swift and thorough diagnostic evaluation is of high importance for further therapeutic decision-making: although APA may be cured by surgery, therapy of bilateral adrenal hyperplasia consists of life-long treatment with mineralocorticoid receptor

antagonists.<sup>6</sup> Conventional imaging reveals adrenal lesions with an age-dependent increase and an overall prevalence of 2% to 10%, mainly representing nonfunctioning adenomas (NFAs).<sup>7</sup> Thus, NFA coincident with biochemical PA may be falsely interpreted as APA instead of bilateral adrenal hyperplasia, potentially leading to avoidable surgical interventions. Accordingly, conventional imaging methods still do not allow for an unequivocal differential diagnosis in patients with PA.<sup>8-10</sup> Adrenal vein sampling (AVS) has, therefore, remained

Received July 15, 2017; first decision July 25, 2017; revision accepted November 28, 2017.

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The online-only Data Supplement is available with this article at <http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.117.09975/-/DC1>.

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*Hypertension* is available at <http://hyper.ahajournals.org>

DOI: 10.1161/HYPERTENSIONAHA.117.09975

the standard procedure in these cases, despite its expense and availability only in major centers.<sup>6,11,12</sup> Moreover, AVS is an invasive technique that is not well standardized between centers and heavily depends on the expertise of the interventional radiologist.<sup>13,14</sup> AVS furthermore requires the use of contrast agents in a patient population that often experiences impaired renal function.<sup>15</sup> To date, proposed clinical prediction scores cannot replace AVS.<sup>16–20</sup> An interesting novel approach consists of the measurement of a selected steroid profile by mass spectrometry.<sup>21,22</sup> Nevertheless, additional diagnostic steps will be needed to localize potential APA.

In this context, functional imaging techniques are of great interest. As a potential target, CYP11B2 (aldosterone synthase) has been investigated, and a selective CYP11B2 inhibitor has recently shown promising results as radiotracer for positron emission tomographic (PET) imaging in first pre-clinical studies.<sup>23</sup>

Chemokines belong to a class of cytokines that exert important physiological effects on organ development, cell migration, and tissue ultrastructure by binding to their specific receptors. In recent years, the role of chemokine receptors in malignant behavior of human cancers has emerged as a particular research focus.<sup>24–26</sup> CXCR4 (CXC chemokine receptor type 4) binds its ligand—the CXC chemokine CXCL12 (stromal cell-derived factor-1). Recently, a second CXCL12 receptor—CXCR7 (the nonclassical/atypical CXC chemokine receptor 7)—has been identified.<sup>27,28</sup> To date, limited data are available on the expression of chemokine receptors in the adrenal cortex, and the presence and functional relevance of chemokine receptors in adrenocortical tumors have not yet been evaluated in detail.

We, therefore, investigated the expression of chemokine receptors in the normal adrenal (NA) and in benign adrenocortical tumors. Because we found high expression of CXCR4 particularly in the zona glomerulosa of the adrenal cortex, we were further interested in the potential of the recently developed CXCR4 ligand 68Ga-pentixafor<sup>29–31</sup> (68GaCPCR4.2) for molecular imaging of aldosterone-producing tissue.

## Materials and Methods

### Availability of Data, Analytical Methods, and Study Materials

The data that support the findings of this study are available from the corresponding author on reasonable request.

### Immunohistochemical Analysis and Quantitative Polymerase Chain Reaction

Immunohistochemistry was performed in formalin-fixed and paraffin-embedded specimens of 2 NAs and 220 patients with known adrenocortical adenomas (117 APAs, 49 NFAs, and 54 cortisol-producing adenomas [CPAs]). The adjacent adrenal cortex was evaluated in 191 of 220 tumor samples. Tissue specimens and corresponding clinical data were derived from 5 tertiary referral centers from the University Hospitals of Berlin, Essen, Munich, Turin, and Wuerzburg. Patients with unilateral APA had been diagnosed according to the 2008 Endocrine Society Practice Guidelines.<sup>32</sup> Collection of patient data and samples was approved by the ethical committees of the respective institutions (Wuerzburg 88/11, Essen 01-187-1787-Z, Charité Berlin EA1/169/08,<sup>33</sup> Munich 379/10, Turin CEI/349). All patients had provided written informed consent.

Chemokine receptor mRNA expression levels were investigated by quantitative real-time polymerase chain reaction. RNA was isolated from fresh frozen tissue of 12 APAs, 11 CPAs, and 3 NFAs using the RNeasy Lipid Tissue Minikit (Qiagen, Alameda, CA) and reversely transcribed using the QuantiTect Reverse Transcription Kit (Qiagen).

For details, please see the [online-only Data Supplement](#).

### Imaging With 68Ga-Pentixafor

Synthesis of 68Ga-pentixafor was performed in Munich and Wuerzburg in a fully automated, good manufacturing practice-compliant procedure using a GRP module (Scintomics GmbH, Fürstenfeldbruck, Germany) equipped with disposable single-use cassette kits (ABX, Radeberg, Germany), using the method and standardized labeling sequence described previously.<sup>30,31,34</sup> Before injection, the quality of 68Ga-pentixafor was assessed according to the standards described in the European Pharmacopoeia for 68Ga-Edotreotide (European Pharmacopoeia, monograph 01/2013:2482, available at [www.edqm.eu](http://www.edqm.eu)). The radiochemical purity of the ready-to-inject formulation as confirmed by radio-high-performance liquid chromatography and radio-thin-layer chromatography was >99%.

In autoradiographic experiments, binding of 68Ga-pentixafor to cryosections from 4 APAs, 4 NFAs, 2 adrenocortical carcinomas, and 3 NAs was analyzed. One slide was treated with 2 MBq 68Ga-pentixafor, and another slide was incubated with 2 MBq 68Ga-pentixafor plus 10  $\mu$ mol/L of nonradioactive compound to monitor specific binding.

To evaluate the suitability of 68Ga-pentixafor for in vivo imaging of APA, 9 patients with APA were investigated: 3 at the University Hospital Wuerzburg (all by PET/computed tomography [CT]) and 6 at the Technical University of Munich (1 patient by PET/CT and 5 patients by PET/magnetic resonance imaging). Reference values for 68Ga-pentixafor uptake in NA glands were established by retrospective analysis of 68Ga-pentixafor-PET imaging results from patients with multiple myeloma (n=20),<sup>29</sup> pleural mesothelioma (n=6), and gastrointestinal tumors (n=18) that had been investigated for individual assessment of eligibility for endoradiotherapy with 177Lu-Pentixafor. In these patients, no adrenal tumor lesion was documented on conventional CT. CT and magnetic resonance imaging scans were scored by a board-certified radiologist, and all PET scans were scored by a board-certified nuclear medicine physician. For details, please see the [online-only Data Supplement](#).

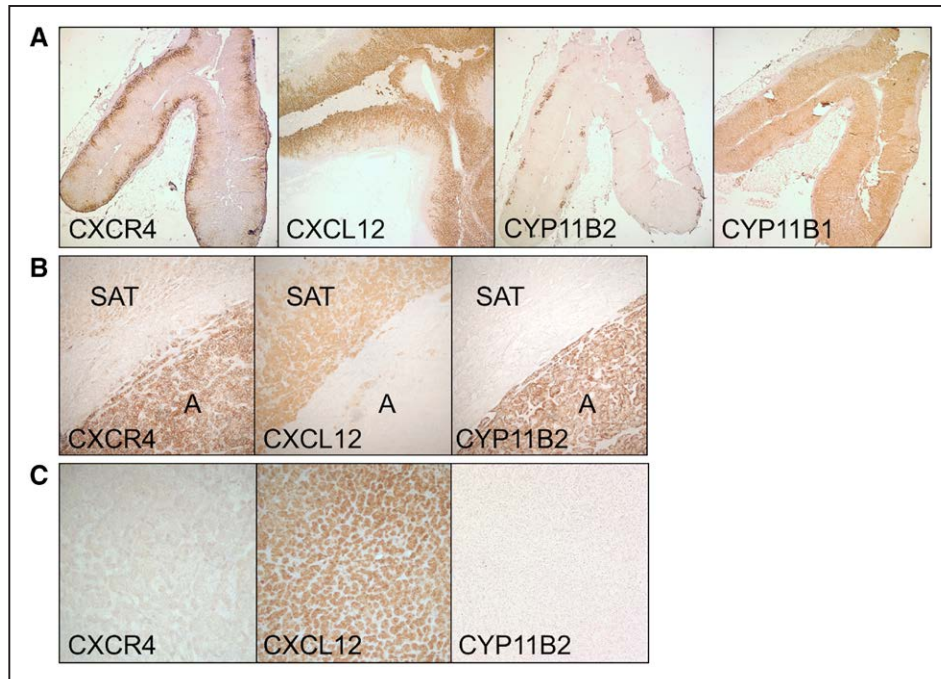
### Statistical Analysis

All statistical tests were performed using either SPSS23 or Graphpad PRISM6. *P* values <0.05 were considered statistically significant. Quantitative values were expressed as mean $\pm$ SEM or median and range as appropriate. Comparisons of related metric measurements were performed using Wilcoxon signed-rank test, and the Mann-Whitney *U* test was used to compare quantitative data between 2 independent samples. In addition, the  $\chi^2$  test was performed for comparison of APA, CPA, and NFA grouped for high or low expression of CXCR4. Correlation between CYP11B2 and CXCR4 staining scores was examined by calculation of Pearson correlation coefficient. Receiver operating characteristic analysis was performed for maximum standardized uptake values (SUV<sub>max</sub> values) of controls and APA with Graphpad PRISM6.

## Results

### Immunohistochemical Analysis of CXCR4, CYP11B1, CYP11B2, and CXCL12

In NA, CXCR4 staining was particularly strong in the subcapsular region. CYP11B2-positive areas exhibited particularly strong CXCR4 expression (Figure 1A). However, the CXCR4-positive area was broader compared with the area of CYP11B2-positive cells. CXCL12 showed a complementary distribution pattern with low expression in the subcapsular area of the adrenal cortex but strong staining in the inner adrenocortical zones (Figure 1A). As for NA, a similar staining



**Figure 1.** CXCR4 (CXC chemokine receptor type 4) staining is particularly high in aldosterone-producing tissue, whereas CXCL12 (stromal cell-derived factor-1) shows a complementary expression pattern. **A**, Immunohistochemical staining of CXCR4, CXCL12, CYP11B2 (aldosterone synthase), and CYP11B1 in the normal adrenal. Magnification  $\times 25$ . Strong CXCR4 staining was detected in the outer part of the normal adrenal cortex covering a broader and more continuous area compared with CYP11B2, which was predominantly found in subcapsular aldosterone-producing cell clusters. CXCL12 showed a complementary staining pattern to CXCR4 with missing expression in the outer cortical part but strong staining in the inner adrenocortical zones. **B**, Representative images for aldosterone-producing adenoma (APA). Strong CXCR4 staining was observed in the APA, which correlated with CYP11B2 staining, whereas CXCL12 staining was observed only in the surrounding adrenocortical tissue (SAT). Magnification  $\times 10$ . **C**, Representative images for nonfunctioning adenoma (NFA). Immunohistochemical staining for CXCR4 and CYP11B2 was negative; in contrast, strong CXCL12 staining was observed in the adenoma (A). Magnification  $\times 10$ .

pattern was found for CXCR4 and CXCL12 in APA ( $n=5$ ), CPA ( $n=5$ ), and NFA ( $n=5$ ; Figure 1B and 1C). CXCR7 was expressed equally in all adrenocortical zones (data not shown).

In adrenocortical adenomas, high CXCR4 membrane staining was found in 71% of APAs (Figure 1B; Figure S2 in the [online-only Data Supplement](#)), 26% of CPAs and 4% of NFAs. Eight NFAs were completely CXCR4 negative (Table 1).

Comparison of membrane staining intensity revealed highly significant differences between NFA and APA ( $P<0.001$ ). Likewise, a significant positive correlation was observed between CXCR4 membrane staining and CYP11B2 staining intensity in APA ( $r=0.42$ ;  $P<0.01$ ). CYP11B1 was coexpressed in 81% of APAs, 100% of CPAs and 98% of NFAs (Table 2).

CXCR4 immunostaining was observed in all adjacent adrenal cortices independent of the underlying adrenal tumor (Table 1).

No differences were observed on clinical parameters or mutational status among patients with APA when grouping them according to the CXCR4 staining intensity of their respective tumor (Table 2).

### Quantitative Analysis of Chemokine Receptor Expression

Quantitative real-time polymerase chain reaction revealed high mRNA levels for CXCR4 and CXCR7 in all adrenocortical

tumor tissues. However, CXCR4 and CXCR7 expression showed considerable variation and did not differ significantly between adenoma types at mRNA level. Furthermore, other chemokine receptors were found to be expressed at lower

**Table 1. CXCR4 Protein Expression in Samples From Standard Full Slides in 117 APAs, 54 CPAs, and 49 NFAs**

CXCR4	n	Low Membrane Staining, n (%)	High Membrane Staining, n (%)
		$\leq 1$	$> 1$
APA	117	34 (29%)	83 (71%)
CPA	54	40 (74%)	14 (26%)
NFA	49	47 (96%)	2 (4%)
Adjacent adrenal			
APA	108	66 (61%)	42 (39%)
CPA	47	31 (66%)	16 (34%)
NFA	36	26 (72%)	10 (28%)

Semiquantitative  $h$  score was calculated by multiplying the staining intensity grading score with the proportion score (0.1=1%–9%, 0.5=10%–49%, 1=50% or more cells with CXCR4 staining, respectively). Significant differences were observed for comparison of APA with NFA and also for comparison of APA with CPA,  $P<0.001$ , respectively. CPA versus NFA,  $P=0.002$ . No significant differences between tumor entities were observed for CXCR4 membrane staining intensity in adjacent adrenal cortex. APA indicates aldosterone-producing adenoma; CPA, cortisol-producing adenoma; CXCR4, CXC chemokine receptor type 4; and NFA, nonfunctioning adenoma.

**Table 2. Clinical Parameters of Patients With APA Included in the Immunohistochemical Analysis**

Patient Data	CXCR4	CXCR4	P Value
	<i>h</i> score ≤1	<i>h</i> score >1	
	n=34	n=83	
Age at diagnosis, y (median; range)	49 (26–67)	49 (18–75)	0.9
Sex (M/F), %	44/56	39/61	0.6
SBP, mm Hg	170±22	160±26	0.1
DBP, mm Hg	100±11.5	100±15	0.2
Serum potassium, mmol/L	3.0±0.6	3.0±0.7	0.9
Plasma aldosterone, ng/L	n=23	n=48	0.5
	520 (208–1950)	500 (57–1148)	
Mutation status of APA, n	25	58	0.1
WT	15	23	
KCNJ5	7	28	
ATPAS	1	5	
CACNA1D	2	2	
Additional CYP11B1 expression detected by IHC (percentage of tumors)	73	84	0.2

Group comparison according to CXCR4 membrane staining of the respective APA. No statistically significant differences were found between groups. APA indicates aldosterone-producing adenoma; ATPAS, ATPase (Na/K-ATPase); CACNA1D, calcium voltage-gated channel subunit alpha1 D; CXCR4, CXC chemokine receptor type 4; DBP, diastolic blood pressure; F, female; IHC, immunohistochemistry; KCNJ5, G protein-activated inward rectifier potassium channel 4; M, male; SBP, systolic blood pressure; and WT, wild type.

levels, especially CCR11, CCR7, and CCR3 (C-C chemokine receptor types 11, 7 and 3) in NA and CXCR2 (CXC chemokine receptor type 2), CCR2 (C-C chemokine receptor type 2), and CCR11 in APA, CPA, and NFA (Figure 2).

### Autoradiographic Experiments With 68Ga-Pentixafor

Autoradiographic visualization of 68Ga-pentixafor revealed strong specific binding to APA and adrenocortical carcinoma tissues and the outer cortical zones of the NA (Figure 3A). No binding was observed to NFA.

### 68Ga-Pentixafor PET Imaging In Vivo

Further information on patients with PA, results of imaging, AVS, and clinical outcome is given in Table 3. 68Ga-pentixafor uptake in the area of the adrenal nodule of patients with PA was observed with median  $SUV_{max}=8.6$  (4.7–18.3), in the contralateral adrenal values were significantly lower:  $SUV_{max}=4.0$  (2.3–6.5;  $P<0.001$ ). Only 1 patient with a 6-mm tumor showed lower 68Ga-pentixafor uptake at the side of surgery. In the control group,  $SUV_{max}$  values ranged from 1.0 to 5.8 (mean  $SUV_{max}$ : left adrenal gland, 3.4; right adrenal gland, 3.0; Figure 3D). Histopathology confirmed the tumors to be adrenocortical adenomas. CXCR4 immunoreactivity was investigated in 4 patients being strongly positive for CXCR4 (*h* score, 2 in 3 cases and *h* score, 3 in 1 case). All tumors also showed positive CYP11B2 staining.

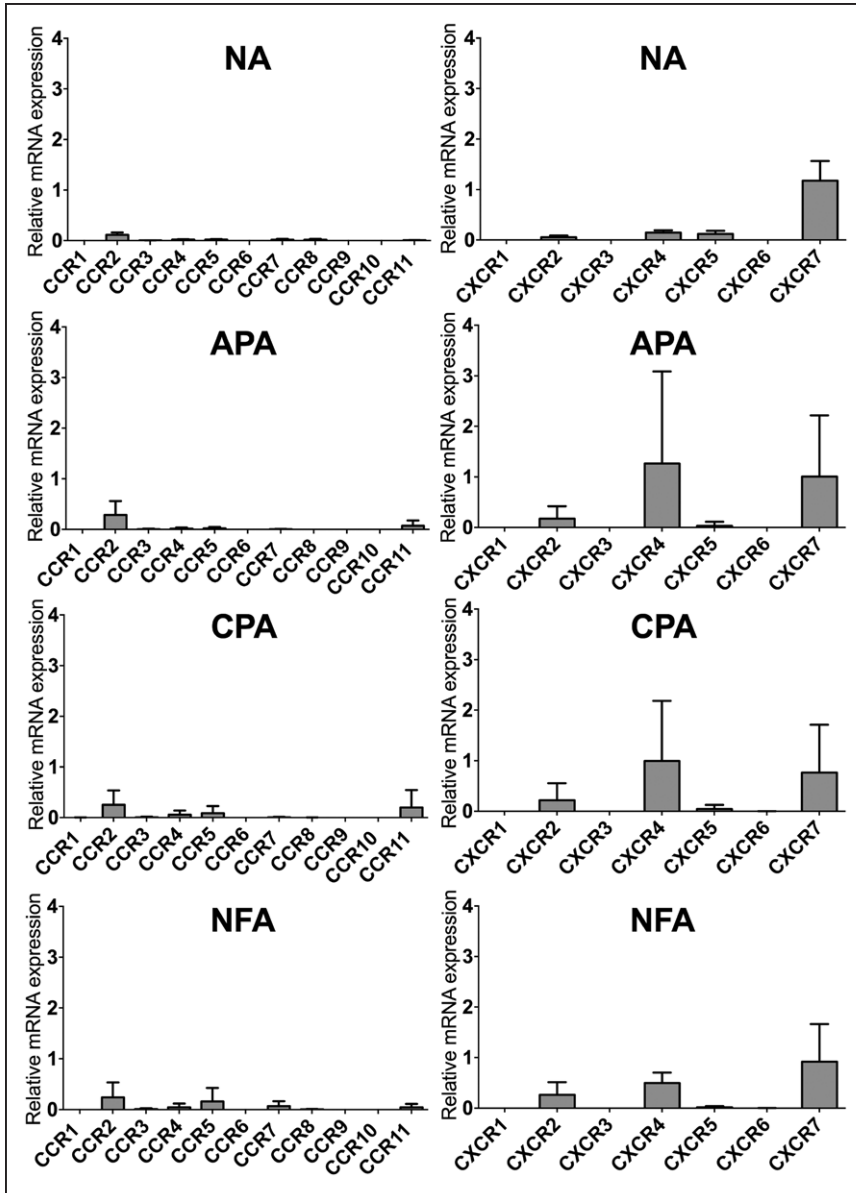
Additionally, ratios were calculated for  $SUV_{max}$  values by dividing the higher SUV through the lower level of the respective contralateral side in controls and by dividing the  $SUV_{max}$  value of the side of APA through the contralateral side in patients with PA. Ratios from patients with APA significantly differed from ratios of controls (APA: mean,  $2.8\pm0.5$ ; control: mean,  $1.5\pm0.08$ ;  $P<0.001$ ). In receiver operating characteristic analysis, a cutoff value for  $SUV_{max}$  of 4.9 revealed a sensitivity of 88.9% (95% confidence interval, 51.8–99.7) and a specificity of 87.2% (95% confidence interval, 78.3–93.4) for diagnosing APA. Setting specificity to 100% a cutoff value of 7.3 was calculated (sensitivity, 77.8%), whereas a sensitivity of 100% was reached at a cutoff value of 4.7 (specificity, 83.7%). Area under the curve was 0.964 (95% confidence interval, 0.92–1.01; Figure 3E).

### Discussion

The key in vitro finding of our investigation is the high expression of the chemokine receptors CXCR4 and CXCR7 in aldosterone-producing tissue. In contrast, expression of CXCR4 was negative or only weak in almost all nonfunctioning adrenocortical adenomas. Based on this differential expression pattern, we investigated the available PET tracer 68Ga-pentixafor, which selectively binds to human CXCR4,<sup>29,35</sup> thus representing a potentially suitable imaging biomarker for noninvasive differential diagnosis in PA.

Quantitative real-time polymerase chain reaction revealed the expression of several chemokine receptors in both NA and adrenocortical adenomas. The most highly expressed receptors were CXCR7 and CXCR4: the high expression levels found in NAs suggest an important role in adrenal physiology. Interestingly, CXCR4 protein expression in the adrenal cortex was particularly high in the outer part of the adrenal cortex. In contrast, immunohistochemical staining of its ligand CXCL12 was highest in the inner adrenocortical zones. Because migration of CXCR4-expressing cells along a CXCL12 gradient has been demonstrated for different cells,<sup>26,36</sup> it is possible to speculate that the expression pattern of CXCR4 and CXCL12 is involved in the proposed centripetal migration and transdifferentiation of adrenocortical cells. In this model, it is hypothesized that undifferentiated progenitor cells in the subcapsular region give rise to differentiated mineralocorticoid-producing zona glomerulosa cells, which migrate centripetally and undergo lineage conversion into glucocorticoid-producing zona fasciculata and reticularis cells.<sup>37,38</sup> In this context, the CXCL12 gradient might play a major role for the migration direction of adrenocortical cells. An alternative hypothesis is that the observed expression pattern is relevant for the ultrastructural organization of the adrenal cortex and local cell–cell interaction as has been shown for other tissues.<sup>39,40</sup> CXCR7 also binds CXCL12. It has been shown to exert a scavenger function and modulate CXCL12–CXCR4 signaling.<sup>41</sup> However, the precise physiological role of the CXCL12/CXCR4/CXCR7 system in the NA remains to be elucidated.

Corresponding to the high expression of CXCR4 in the outer adrenal cortex, high expression levels were also found in APA, whereas expression in NFA was almost negligible. CXCR4 expression and CYP11B2 expression levels



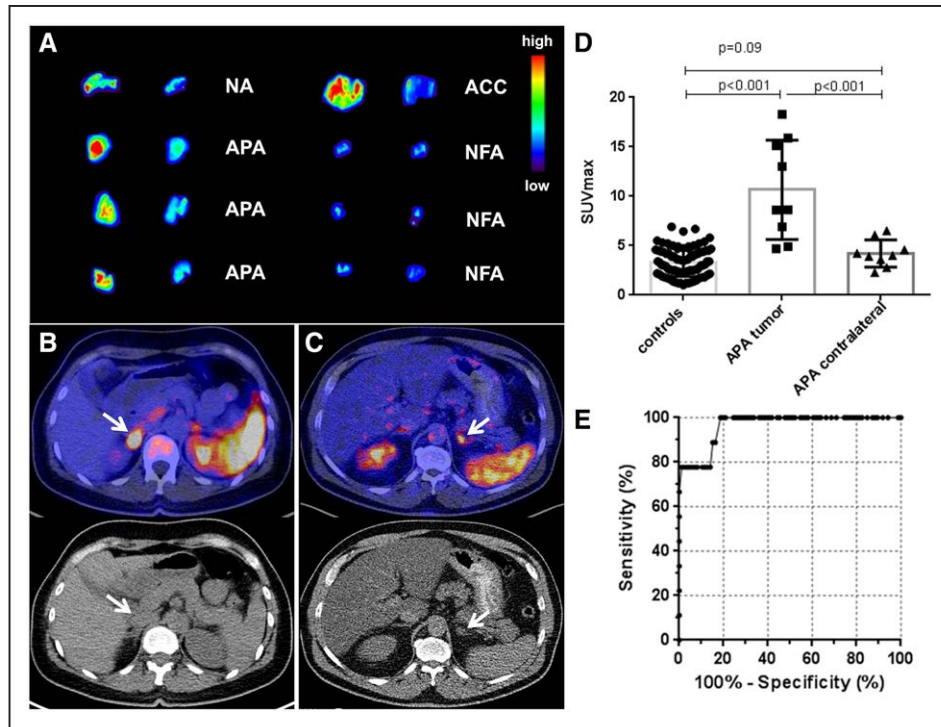
**Figure 2.** Chemokine receptor mRNA expression in adrenocortical adenomas. Relative chemokine receptor mRNA expression of CC chemokine receptors (left) and CXC chemokine receptors (right) in normal adrenal (NA, n=4), aldosterone-producing adenoma (APA, n=12), cortisol-producing adenoma (CPA, n=11), and nonfunctioning adenoma (NFA, n=3).

correlated significantly, further emphasizing the particularly high expression of CXCR4 in aldosterone-producing tissue, which was independent of sex, clinical parameters, or mutational status. On the background of these findings, <sup>68</sup>Ga-pentixafor PET imaging might be a useful tool for non-invasive characterization of adrenal lesions in PA. The results from the autoradiographic studies fit well with the findings from our immunohistochemical analyses. Strong binding of <sup>68</sup>Ga-pentixafor could be documented for APA and parts of the NA cortex in contrast to NFA that did not show relevant tracer binding.

<sup>11</sup>C-metomidate PET imaging has recently been used in patients with primary PA and was able to identify aldosterone-producing tissue in these patients with high imaging quality. Metomidate binds to both enzymes CYP11B1 and CYP11B2 with high avidity.<sup>42</sup> Our observation of expression of both CYP11B1 and CYP11B2 in most APAs matches with the observed high <sup>11</sup>C-metomidate uptake in the aldosterone-producing tissue.<sup>43,44</sup> However, 98% of the NFAs in our series

also exhibited CYP11B1 staining, and both <sup>11</sup>C-metomidate uptake and <sup>123</sup>I-iodometomidate uptake in NFA have been demonstrated in clinical studies.<sup>45,46</sup> CYP11B1 downregulation may be achieved in normal surrounding adrenocortical tissue by dexamethasone pretreatment. However, it is unclear whether this also holds true for adrenocortical adenoma cells because ACTH (adrenocorticotrophic hormone) receptor expression is low in NFAs.<sup>47</sup> Nevertheless, the data obtained by the <sup>11</sup>C-metomidate studies represent an important proof of principle demonstrating that the PET imaging technique per se may be of value in PA.<sup>44</sup>

Targeting CXCR4 in PA may be a promising alternative approach. This is highlighted by our 9 patients with APA who were investigated by <sup>68</sup>Ga-pentixafor PET/CT. All patients showed focal <sup>68</sup>Ga-pentixafor uptake in the adrenal nodule with SUV<sub>max</sub> values ranging between 4.7 and 18.3 (mean, 10.7), whereas the mean SUV<sub>max</sub> value measured in non-PA patients was 3.3. Uptake values in APA significantly differed both from values obtained in controls and in the contralateral



**Figure 3.** The CXCR4 (CXC chemokine receptor type 4) ligand  $^{68}\text{Ga}$ -pentixafor specifically accumulates in aldosterone-producing tissue. Ex vivo experiments and clinical positron emission tomographic (PET) imaging. **A**, Ex vivo autoradiographic visualization of  $^{68}\text{Ga}$ -pentixafor binding to cryosections of the normal adrenal gland (NA), aldosterone-producing adenoma (APA), adrenocortical carcinoma (ACC), and nonfunctioning adenoma (NFA). Red colors indicate highest binding of radio ligand, blue/dark blue colors indicate no binding or full competitive inhibition. **A**, 2MBq  $^{68}\text{Ga}$ -pentixafor; **B**, 2MBq  $^{68}\text{Ga}$ -pentixafor plus 10  $\mu\text{M}$  of the nonradioactive ligand. Strong ligand binding that could be blocked by coincubation with the unlabeled tracer compound could be documented in NA, APA, and ACC. **B** and **C**, Representative images of  $^{68}\text{Ga}$ -pentixafor PET/computed tomographic (CT) imaging in 2 patients with APA:  $^{68}\text{Ga}$ -pentixafor imaging in a 47-year-old female patient with primary aldosteronism after injection of 152 MBq  $^{68}\text{Ga}$ -pentixafor (**B**).  $^{68}\text{Ga}$ -pentixafor-PET/CT of a 66-year-old male patient with primary aldosteronism after injection of 126 MBq  $^{68}\text{Ga}$ -pentixafor. **C**, White arrows indicate the tumor lesion. **D**, Maximum standardized uptake values ( $\text{SUV}_{\text{max}}$ ) for  $^{68}\text{Ga}$ -pentixafor uptake in the adrenal of control subjects compared with patients with APA both at the tumor (APA tumor) and the contralateral (APA contralateral) side. Adrenal  $\text{SUV}_{\text{max}}$  values from controls are pooled data derived from both sides. **E**, Receiver operating characteristic analysis of  $\text{SUV}_{\text{max}}$  values. Data were obtained from controls ( $n=44$ ; 86 values were obtained from measurement of the left and right adrenal; in 2 patients, only the left adrenal was available) and 9 APAs. A cutoff value for  $\text{SUV}_{\text{max}}$  of 4.8 revealed a sensitivity of 89% (95% confidence interval [CI], 51.8–99.7) and a specificity of 85% (95% CI, 75.5–91.7). Setting specificity to 100% a cutoff value of 7.8 was calculated (sensitivity, 67%), whereas a sensitivity of 100% was reached at a cutoff value of 4.7 (specificity, 81%). Area under the curve, 0.964 (95% CI, 0.92–1.01).

adrenals. In 1 patient, APA measured only 6 mm and was not detected by  $^{68}\text{Ga}$ -pentixafor indicating the detection limit for small lesions because of reduced spatial resolution. In 2 patients,  $^{68}\text{Ga}$ -pentixafor identified the tumor side, which was unable to be localized by AVS.

As a future approach, the combination of steroid profiling by mass spectrometry as a first differential diagnostic step with subsequent functional imaging using  $^{68}\text{Ga}$ -pentixafor PET/CT or  $^{68}\text{Ga}$ -pentixafor PET/magnetic resonance imaging could enable noninvasive diagnosis in most patients with PA. As furthermore 96% of NFA exhibited either low or no staining for CXCR4, no tracer uptake would be expected in these tumors. Because immunohistochemistry revealed low CXCR4 staining in 29% of APAs, only this subgroup would remain for further investigation by AVS. A small group of patients with NFA is expected to be false-positive in  $^{68}\text{Ga}$ -pentixafor PET imaging because 2 of these adenomas (4%) in our series showed high CXCR4 expression. On the contrary, we recently found high CXCR4 expression in adrenocortical carcinoma.<sup>48</sup> Therefore, a high CXCR4 uptake in PET

imaging generally might be associated with a lower threshold for the decision for surgery. However, this would need to be corroborated by further studies. In both NFA patients with strong CXCR4 staining in our cohort, tumor growth was documented leading to the decision for adrenalectomy.

There are several limitations on the clinical aspects of our article: within this translational project, we did not perform a standardized clinical trial investigating healthy individuals, and the data used as controls were obtained from patient cohorts with malignant disease that had already undergone  $^{68}\text{Ga}$ -pentixafor PET imaging for other clinical reasons. It is to date unclear whether concurrent chemotherapy might influence CXCR4 expression in vivo. Many malignant tumors exhibit high CXCR4 expression, and although no adrenal metastases were detected by conventional imaging, CXCR4-positive micrometastases could not be excluded. Subgroup analysis of the 3 groups of control patients who received different treatments did, however, not reveal any group differences. Uptake values in the contralateral adrenals of patients with PA were not significantly higher compared with the controls without PA.

**Table 3. Clinical Parameters of 9 Patients With Aldosterone-Producing Adenoma Investigated by 68Ga-Pentixafor**

Sex	Age, y	TU Side in CI	TU Size, mm	AVS, Result	SUV <sub>max</sub> <sup>1</sup> Right	SUV <sub>max</sub> <sup>1</sup> Left	Surgery	Outcome
F	47	R	23	Unsuccessful sampling R, suppression L	13.0	3.6	R	CBR
F	57	R	17	Lateralization R, no suppression L	15.1	2.8	R	CBR
F	55	R	37	No lateralization	8.6	4.0	R	CBR
M	50	R	6	Lateralization R	4.9	6.1	R	CBR
F	47	R	14	No AVS	18.3	4.2	R	CBR
M	63	L	18	Lateralization L	2.3	4.7	L	CBR
M	57	L	28	No lateralization	3.9	15.9	L	CBR
M	44	...	No TU	Lateralization R	6.9	6.5	No surgery	...
M	65	L	12	Lateralization L	4.6	8.6	No surgery	...

AVS indicates adrenal vein sampling; CBR, clinical improvement and biochemical remission; CI, conventional imaging; F, female; L, left; M, male; R, right; SUV<sub>max</sub>, maximum standardized uptake value; and TU, tumor.

In our patient series, most tumor lesions were >1 cm. Many APAs are <1 cm, which generally limits the sensitivity of PET imaging because of the reduced spatial resolution of this imaging approach.<sup>49</sup> Thus, 68Ga-pentixafor PET imaging may not fully replace but at least significantly reduce the number of AVS to a more selected patient subgroup.

### Perspectives

Taken together, we have demonstrated that CXCR4 is highly abundant in the zona glomerulosa and in APAs suggesting a significant role in adrenocortical physiology and further representing a potential target for molecular imaging of aldosterone-producing tissue. Considering the need for universal diagnostic alternatives to AVS in PA, 68Ga-pentixafor PET represents a novel and promising tool for differential diagnosis that warrants further evaluation in a prospective clinical trial.

### Acknowledgments

We thank all colleagues of Nuclear Medicine and the Endocrine Unit, Department of Medicine I, University of Wuerzburg, for excellent cooperation. B. Heinze, C.T. Fuss, and S. Hahner designed and performed in vitro experiments, collected and analyzed preclinical and clinical data, and wrote the article. K. Lang contributed to preclinical experiments; A. Schirbel performed radiosyntheses; P. Mulatero, F. Beuschlein, M. Reincke, T.A. Williams, M. Quinkler, N. Rayes, V. Wild, A.-C. Reis, S. Petersenn, T. Deutschbein, S. Monticone, Y. Rhayem, and M. Fassnacht provided biomaterials and clinical patient data; C.E. Gomez-Sanchez provided selective antibodies detecting human CYP11B1 and CYP11B2; M. Mustafa, C. Bluemel, K. Herrmann, and A.K. Buck performed patient imaging and analysis of imaging data; H.-J. Wester and S. Kropf provided the precursor for 68Ga-pentixafor. The article was edited and approved by all authors.

### Sources of Funding

This work was supported by the Deutsche Forschungsgemeinschaft (AL 203/I-1 to A.K. Buck, A. Schirbel, and S. Hahner; SFB-TR-B13 to S. Hahner and M. Reincke; and RE 752/20-1 to M. Reincke) and the Else Kröner-Fresenius-Stiftung (No. 2013\_A49 to S. Hahner and

A. Schirbel, and 2015\_A171 and 2013\_A182 to M. Reincke). M. Reincke is supported by the European Research Council under the European Union Horizon 2020 program (No. 694913).

### Disclosures

H.-J. Wester is the Chief Executive Officer (CEO) and shareholder of Scintomics. S. Kropf is the CEO of Scintomics. The other authors report no conflicts.

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## Novelty and Significance

### What Is New?

- CXCR4 (CXC chemokine receptor type 4) is highly expressed in the outer adrenocortical zones, as well as in aldosterone-producing adenomas, especially compared with nonfunctioning adenomas.
- The CXCR4-specific positron emission tomographic tracer <sup>68</sup>Ga-pentixafor exhibits strong binding to cryosections of aldosterone-producing adenomas, adrenocortical carcinomas, and the outer adrenal cortex.
- First in vivo imaging studies show the potential suitability of CXCR4-specific positron emission tomographic imaging for noninvasive subtype differentiation in patients with primary aldosteronism.

### What Is Relevant?

- Alternative tools for subtype differentiation in primary aldosteronism are urgently needed because of several drawbacks of the current diagnostic

standard adrenal vein sampling. Given the high expression of CXCR4, especially in aldosterone-producing adenomas, as well as strong tracer binding of <sup>68</sup>Ga-pentixafor to aldosterone-producing adenomas, CXCR4 seems to be a suitable target for noninvasive characterization of adrenal lesions in primary aldosteronism.

### Summary

CXCR4-targeted positron emission tomographic imaging represents a novel promising alternative to adrenal vein sampling for differential diagnosis in primary aldosteronism.