BRIEF CORRESPONDENCE

PET/CT with ^{89}Zr-girentuximab can aid in diagnostic dilemmas of clear cell renal cell carcinoma suspicion

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Abstract

Based on the high expression of Carbonic Anhydrase IX (CAIX) in 95% of clear cell renal cell carcinoma (ccRCC), the anti-CAIX monoclonal antibody girentuximab can be used for the detection of ccRCC. This clinical study explores the value of ^{89}Zr-labeled girentuximab PET/CT imaging in diagnostic challenges regarding ccRCC. PET/CT imaging was performed 4 or 5 days after injection of ^{89}Zr-girentuximab in patients with a primary renal mass (n=16) or a history of ccRCC (n=14). Scans were used for decision making (surgery/active surveillance) in case of indistinct renal masses. All resected PET-positive primary lesions proved to be ccRCC, while no lesion progression was seen in PET-negative masses. In patients suspected of recurrent/metastatic ccRCC, PET/CT with ^{89}Zr-girentuximab was useful to confirm or exclude ccRCC, to evaluate the extent of the disease and to differentiate from other cancers. In this group ^{89}Zr-girentuximab PET/CT resulted in a major change in clinical management in five patients (36%), while in three patients (21%) repeat biopsies could be avoided. We conclude that ^{89}Zr-girentuximab PET/CT is a valuable diagnostic tool that can guide clinical decision making in case of diagnostic dilemmas concerning ccRCC suspicion.

Patient summary: ^{89}Zr-girentuximab PET/CT imaging can be a valuable diagnostic tool to identify ccRCC.

Non-invasive confirmation of the presence or absence of clear cell renal cell carcinoma (ccRCC) can be useful to guide clinical decision making in patients with indistinct primary renal tumors or patients suspected of recurrent or metastatic ccRCC. The high expression of Carbonic Anhydrase IX (CAIX) in 95% of ccRCC allows detection with the anti-CAIX monoclonal antibody girentuximab[1]. Multiple studies have confirmed the high accuracy of radionuclide imaging using radiolabeled girentuximab[2-4]. A PET-tracer, like ^{89}Zr-girentuximab, allows better contrast and spatial resolution compared to SPECT-tracers. Furthermore, animal studies have demonstrated that ^{89}Zr-girentuximab outperforms ^{124}I-girentuximab in terms of tumor-to-normal tissue ratios[5, 6].

This phase I/II study aims to evaluate the value of ^{89}Zr-labeled girentuximab PET/CT imaging in 30 patients suspected of ccRCC in whom the clinician faced a diagnostic dilemma defined as doubt about the best medical treatment despite conventional diagnostics. Two subgroups were distinguished: 1. patients with an indistinct renal mass, and 2. patients suspected of recurrent/metastatic ccRCC. Whole body PET/CT images were obtained 4-5 days after injection of 5mg ^{89}Zr-girentuximab (37 MBq). Lesions of interest were considered PET-positive or PET-negative based on a visual scale taking into account the background signal in surrounding organs and blood. Patient management was discussed in a multidisciplinary meeting before and after PET/CT results. A major change in management was defined as a change in one of the next step strategies; surgery (including surgical strategy), surveillance, or systemic therapy. The study was approved by Regional Internal Review Board (clinicaltrials.gov; NCT02883153). All patients signed informed consent.

In group 1 (n=16), ^{89}Zr-girentuximab PET/CT imaging was used in the decision to either perform surgery or active surveillance. In all cases follow-up data supported the decision (Table S1). In six patients tumors were PET-positive and five of them underwent surgery confirming ccRCC (Fig. S1). In the sixth patient (Fig. S2), with Von Hippel Lindau (VHL) syndrome, PET/CT revealed additional PET-positive lesions compared to MRI and the largest lesion was treated by cryoablation for debulking purposes. In patient #12, active surveillance of a 0.9cm PET-positive (follow-up: stable disease) and a 4cm PET-negative lesion (follow-up: shrinking) was initiated due to extensive previous abdominal surgery. In nine patients tumors were PET-negative and they were followed by active surveillance. None of the PET-negative tumors progressed during follow-up (mean 13.0±4.9 months), and these lesions are considered most likely to be benign or indolent non-ccRCC. However, longer follow-up is needed since average growth of renal tumors is 3 mm/year[7].

The confirmation of the presence or absence of ccRCC by ^{89}Zr-girentuxumab PET/CT imaging results provided valuable information for clinical decision making in challenging cases, such as patients with a relative contraindication for surgery. ^{89}Zr-girentuximab is not nephrotoxic and can be used in patients with renal insufficiency. Due to the highly specific CAIX-targeting , ^{89}Zr-girentuximab does not target other RCC subtypes. However, a negative ^{89}Zr-girentuximab PET/CT lowers the a priori chance of lesions with a high malignant potential, which also aids in clinical decision making.

Group 2 contained fourteen patients suspected of recurrent or metastatic ccRCC (Table S2). In case of oligometastatic disease, surgery with curative intent can be considered, whereas in case of extensive metastatic disease, there are only systemic treatment options with a palliative effect. Confirmation of oligometastases prior to metastasectomy is of utmost importance to avoid futile surgery. Eight patients (#17 to #24) were considered for surgery with curative intent. In three of them high uptake in the lesion of interest was seen and surgery was performed confirming ccRCC (Fig. 1). In patient #17 surgery was performed despite a negative PET/CT due to a high clinical suspicion (CAIX-expressing papillary growing ccRCC, false negative PET/CT). In patient #22 surgery could be limited to an adrenalectomy, since the enlarged contralateral lymph node was PET-negative (follow-up: stable disease). In three patients (#19-20, #24) curative surgery was cancelled since PET/CT imaging revealed additional metastases or did not confirm ccRCC recurrence (Fig. 2).

The remainder of the patients in group 2 had multiple suspicious lesions. Patients #25-#26 were previously diagnosed with metastatic ccRCC and ^{89}Zr-girentuximab PET/CT imaging was used to evaluate the extent of the disease. In patients #27–30 previous biopsies were inconclusive or considered to be too invasive to perform. The invasive nature can make a re-biopsy less desirable. In patients #27-29 multiple PET-positive lesions were visualized indicating metastatic ccRCC with follow-up indeed showing lesion progression (#27 lost to

follow-up). In patient #30 the pulmonary lesions were PET-negative. Under the suspicion of lung cancer, a lobectomy was performed that confirmed primary lung adenocarcinoma (Fig. S3). As ^{89}Zr-girentuximab PET/CT visualizes the underlying tumor biology (CAIX expression), it can be used to distinguish ccRCC from other cancers, which is important since treatment strategies can be essentially different. Hypoxia-driven CAIX expression has been described in other malignancies, but the mutational loss of VHL in ccRCC leads to a much higher expression[1].

The impact on clinical management in group 1 was difficult to express objectively, since treatment of renal masses did not follow strict guidelines but was subject to preferences of both the clinician and the patient. In group 2, a major change in clinical management occurred in five patients (36%). In three patients (21%) repeated biopsies were avoided, since the ^{89}Zr-girentuximab PET/CT was positive, highly suggestive for ccRCC. Clinical management was never changed solely on ^{89}Zr-girentuximab PET/CT imaging results, but with all clinical data taken into consideration. In the remainder of the patients PET/CT imaging confirmed clinical practice, but provided more certainty to decide on clinical management.

The aim of the current study, to evaluate the value of ^{89}Zr-girentuximab PET/CT imaging in diagnostic challenges, led to the inclusion of a heterogeneous study population, which can be considered a limitation of this study. Furthermore, the absence of histopathological confirmation in some patients precludes adequate calculation of diagnostic accuracy. A large phase III trial will be initiated.

In conclusion, this study shows the diagnostic value of ^{89}Zr-girentuximab PET/CT for the detection of primary, recurrent or metastatic ccRCC. It may guide clinical decision making in case of diagnostic dilemmas concerning ccRCC suspicion.

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Figures

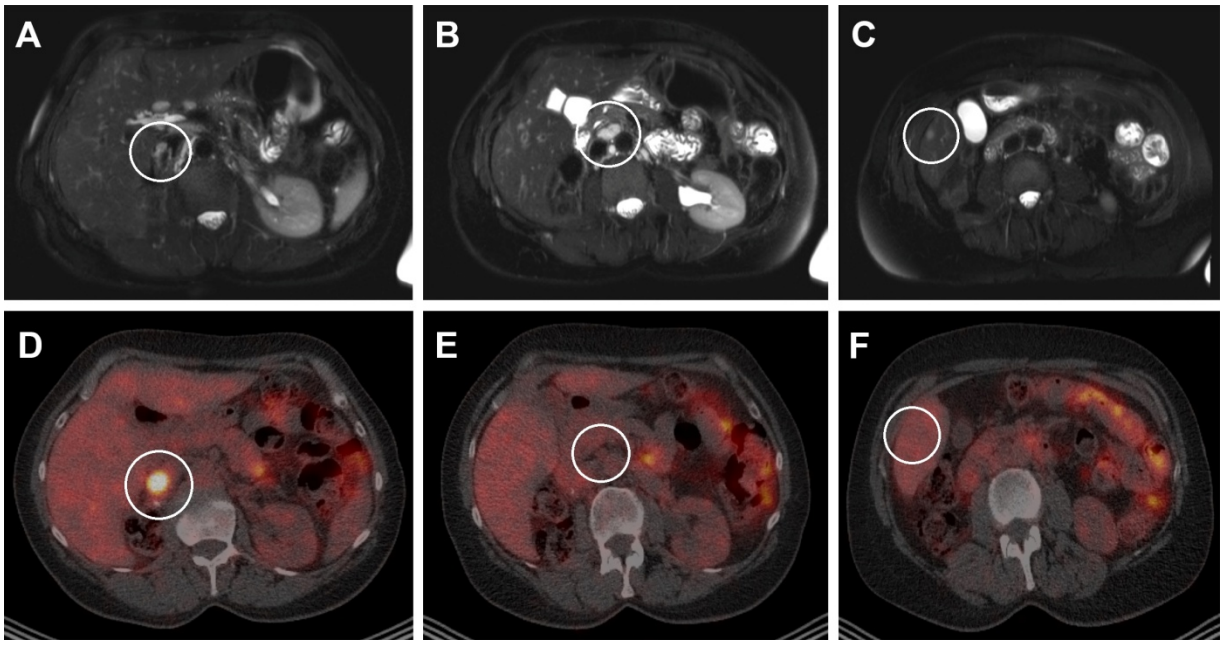

Figure 1: Patient #18 presented with a suspicious lesion in the inferior caval vein (VCI 10 mm) 6 months after nephrectomy (pT3a ccRCC with a positive surgical margin at the renal vein). CT and multiparametric MRI (A) could not distinguish with certainty between tumor thrombus and a blood clot, and furthermore mpMRI revealed several enlarged lymph nodes (B, short axis up to 12 mm) and a liver lesion (C, 7 mm). ^{89}Zr-girentuximab PET/CT imaging showed uptake of ^{89}Zr-girentuximab in the inferior caval vein (D) thereby non-invasively confirming the presence of recurrent ccRCC. No uptake of ^{89}Zr-girentuximab in the lymph nodes (E) nor the liver lesion (F) was seen, making ccRCC metastases less likely. Surgery was performed with resection of the lesion in the IVC (ccRCC) and several enlarged lymph nodes (benign). No change of the liver lesion was seen over 15 months. The value of the ^{89}Zr-girentuximab PET/CT was to confirm the local recurrence, and, prior to metastasectomy, provide more certainty about the enlarged lymph nodes and the liver lesion.

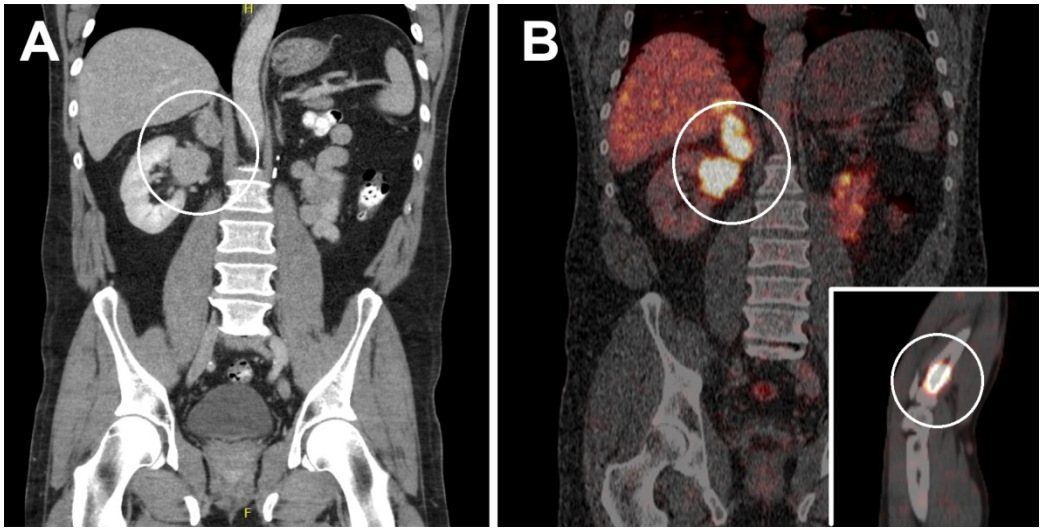

Figure 2: Patient #20 underwent a radical nephrectomy for ccRCC six years earlier and now presented with a new renal tumor and a solitary adrenal metastasis on CT-thorax/abdomen(A). Prior to metastasectomy (radical nephrectomy + adrenalectomy), ^{89}Zr-girentuximab PET/CT imaging was performed to confirm the diagnosis and to exclude other metastatic sites. Uptake of ^{89}Zr-girentuximab in the primary renal mass and the adrenal lesion was seen (B), but also in the proximal radius (insert). The proximal radius was not in the field of view of the initial conventional imaging (CT-thorax/abdomen). Biopsy of the radius confirmed ccRCC metastasis which was treated with radiotherapy. If the bone lesion had not been detected, the patient would have undergone a radical nephrectomy and as a result would have been dependent on dialysis. This would have been futile surgery and a major decrease in quality of life.

Supplemental tables and figures

Table S1: ^{89}Zr-girentuximab PET/CT imaging in patients with renal masses

#	Age	Dilemma/contra-indication to surgery	Lesion of interest	Size (cm)	PET	Action after PET/CT	Follow-up/ histology
1	76	Mono-kidney	Bosniak 3	2.6	+	PN	ccRCC
2	63	Mono-kidney	Bosniak 3	4.9	-	FU	SD
3	76	Multifocal disease, comorbidity	8 solid tumors, bilateral	1.5-9.5	-	Biopsy/FU	Oncocytoma
4	49	Multifocal disease, VHL syndrome (Fig S2)	Bosniak 3 Bosniak 3** Myelum (L5) Epididymis Cerebellum	2 1.32 1.2 < 1.0 <1.0	+ + + + +	Cryoablation FU	Biopsy inconclusive SD Hemangioblastoma Cystadenoma Hemangioblastoma
5	29	Multifocal disease, biopsy inconclusive DD angiomyolipoma.	Solid Solid	3.5 5.3	- -	FU FU	SD SD
6	66	Multifocal disease, DD with metastases of other malignancies*	Solid Solid	1.2 2.2	- -	FU FU	SD SD
7	64	DD with metastasis of other malignancies (Synchronous lung carcinoma)	Bosniak 3-4	3	+	PN	ccRCC
8	50	Central lesion, PNx not feasible	Bosniak 3	5.5	-	FU	SD
9	47	Central lesion, PNx not feasible (Fig S4)	Bosniak 3-4	2.8	-	FU	SD
10	59	Central lesion, PNx not feasible	Bosniak 3	1.1	-	FU	SD
11	66	Extensive previous abdominal surgery (Fig S1)	Bosniak 3	4.2	+	RN	ccRCC
12	49	Extensive previous abdominal surgery	Bosniak 3-4 Solid (Contralateral kidney)	4 0.9	- +	FU	Shrinking SD
13	70	Comorbidity	Bosniak 3	2.7	+	PN	ccRCC
14	63	Comorbidity	Bosniak 3	9	-	FU	SD
15	65	Other; metastases?	Bosniak 3 Adrenal	3.7 3.7	+ -	PN LA	ccRCC Adrenal adenoma
16	45	Other; Changing cystic lesion after marsupialisation; cystic ccRCC?	Bosniak 3	10	-	Marsupialisation because of symptomatic cyst	No malignancy

* History of colorectal carcinoma, prostate cancer and urothelial cell carcinoma. Metastases unlikely due to low tumor markers. Patient refused biopsy. ** PET/CT imaging revealed accumulation of ^{89}Zr-girentuximab in multiple cystic lesions. Based on the PET/CT a bilateral radical nephrectomy was advised. PN: partial nephrectomy; RN: radical nephrectomy; LA: laparoscopic adrenalectomy; FU: follow-up; SD: stable disease.

Table S2: The value of ^{89}Zr-girentuximab PET/CT in patients with a history of ccRCC

#	Age	Dilemma	Hypothetical step	Action after PET/CT	Lesions	Size (cm)	PET	Histology/FU	Comment
17	47	Screening prior to metastasectomy	Metastasectomy	Metastasectomy	Lymph node	1.4	-	Papillary ccRCC	False negative PET/CT
18	67	Screening prior to metastasectomy (Fig 1)	Metastasectomy	Metastasectomy	Thrombus ICV Lymph node Liver	1.0 1.2 0.7	+ - -	ccRCC Benign SD	Kidney recurrence 15 months after PET.
19	68	Screening prior to metastasectomy	**Metastasectomy**	**Consider metastatic ccRCC**	Lymp node (para-aortal) *Lymph node (Retroaortal) Muscle (iliac spine)*	1.8-2.6 <1.0 1.3	+ + +	PD PD PD	
20	52	Screening prior to metastasectomy (Fig 2)	**RN + LA**	**Radiotherapy bone**	Kidney Adrenal *Bone (Proximal radius)* *Myelum (L4)*	4.1 4.1 2.9 0.9	+ + + +/-	ccRCC ccRCC ccRCC PD	After a stable interval, surgery of the kidney and adrenal were performed.
21	48	Screening prior to metastasectomy	Metastasectomy	Metastasectomy	Pancreas tail Pancreas head *Liver*	1.7 0.5 ns	+ - +/-	ccRCC ccRCC SD	No liver lesion visualized with intraoperative ultrasound. Follow-up suggested an adrenal metastasis.
22	52	Screening prior to metastasectomy	**Metastasectomy (adrenal + contralateral LN)**	**Metastasectomy (adrenal)**	Adrenal LN	3.3 1.5	+ -	ccRCC SD	
23	66	Screening prior to RN after PN	RN + LA	RN + LA	Kidney Adrenal	1.2 1.2	+ +/-	ccRCC Adenoma	
24	57	Screening prior to RN after PN	**RN**	**Follow-up**	Kidney	2.3	-	SD	
25	60	Evaluate extent of the disease	Consider metastatic ccRCC	Maximize treatment Th7 Gastroscopy	Bone (Th7) Muscle *Stomach*	1.3 ns ns	+ - +	ccRCC SD Benign	
26	58	Evaluate extent of the disease	Consider metastatic ccRCC	Cryoablation kidney for local control MRI vertebra.	Kidney Thrombus ICV Lung *Bone (C4)*	3.0 1.9 1.2 ns	+ - - +	ccRCC Shrinking Shrinking MRI: inflammation	Multiple new pulmonary lesions six months after PET
27	69	Biopsy inconclusive	Biopsy	Consider metastatic ccRCC	Liver Lung Pancreas Adrenal Kidney *Subcutis* *Liver*	9.0 < 2.9 1.6-2.4 1.0 1.4-3.1 ns 0.9-1.5	+ + + + + + +	-	Lost to follow-up
28	73	Biopsy inconclusive	Biopsy	Consider metastatic ccRCC	Liver Pancreas *Bone (Th6)* *Thyroïd*	1.8 – 10 3.0 ns 2.7	+ + + +	Overall PD	
29	70	Biopsy challenging due to tumor location	Biopsy	Consider metastatic ccRCC	Lung *Bone (Clavicula)*	0.6-2.3 ns	5.0 15.6	PD SD	
30	72	Biopsy challenging due to tumor location:	**Biopsy/Consider metastatic ccRCC**	**Lobectomy**	Lung (multiple)	0.8-1.9	-	Primary lung carcinoma and granulomas	

	DD m+ ccRCC or primary lung carcinoma (Fig S3)

In **Bold** are patients in whom ^{89}Zr-girentuximab PET/CT imaging was considered to have a major impact on clinical management. In *italic* are additional lesions visualized due to ^{89}Zr-girentuximab PET/CT imaging.
+/- Inconclusive lesion on ^{89}Zr-girentuximab PET/CT imaging due to small lesion size, slightly enhanced uptake and/or absence of a substrate on conventional imaging.
PN: partial nephrectomy, RN: radical nephrectomy. LA: laparoscopic adrenalectomy. DD: differential diagnosis. SD: stable disease. PD: progressive disease. LN: lymph node. I.C.V.: inferior caval vein. Ns: no substrate on anatomical imaging. Consider metastatic ccRCC: in general this meant follow-up until progression of disease and then start systemic therapy.

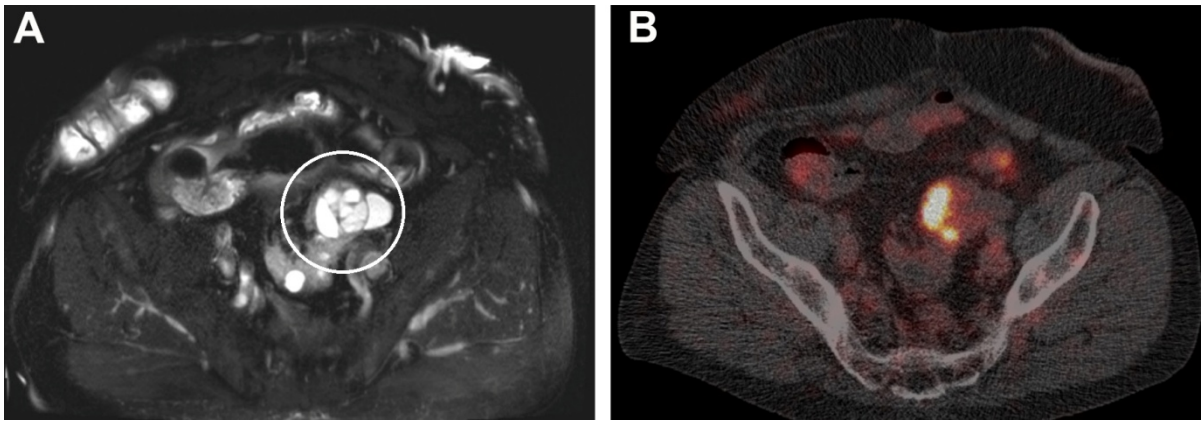

Figure S1: A. Patient #11 was referred with a complex cystic renal mass in a pelvic kidney on MRI. Because of a glomerular filtration rate of 25 ml/min and extensive previous abdominal operations, the clinician preferred to obtain certainty about the presence of ccRCC. B. ^{89}Zr-girentuximab PET/CT imaging visualized uptake of ^{89}Zr-girentuximab in the walls of the lesion and subsequent surgery confirmed the presence of ccRCC.

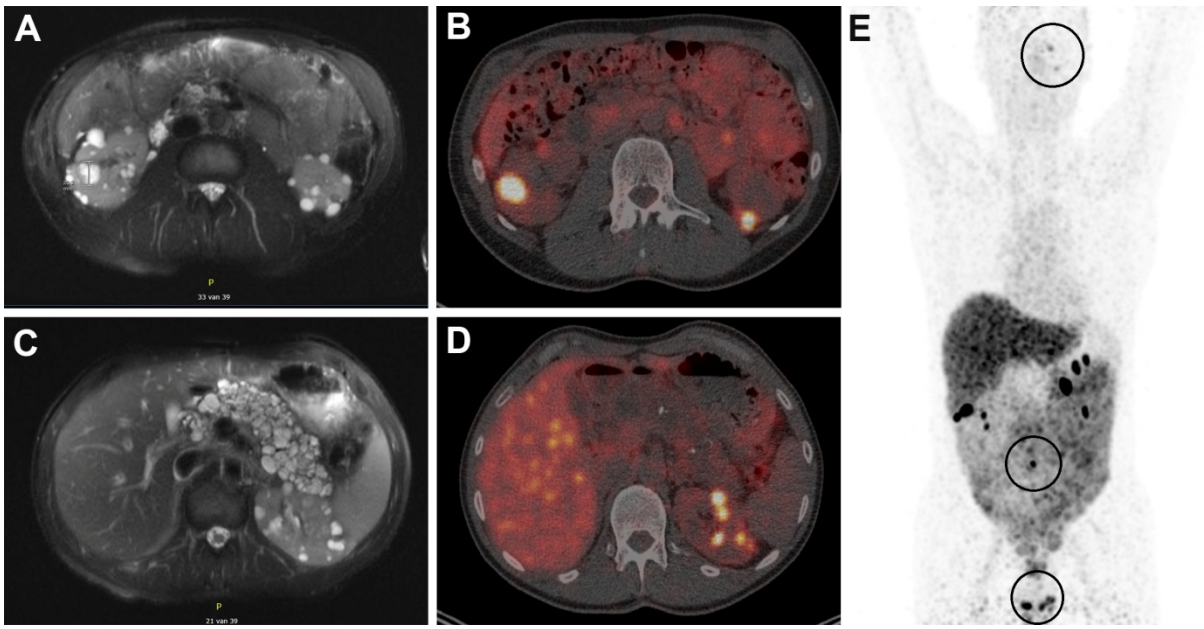

Figure S2: Patient #4 was known to have Von Hippel Lindau syndrome and had several primary ccRCC in the past. On MRI (A, C) two suspicious growing lesions were visualized, but PET/CT imaging revealed multiple lesions with high accumulation of ^{89}Zr-girentuximab (B, D). The largest renal lesion was treated by cryoablation, but unfortunately the biopsy that was retrieved during of the procedure was not representative. Focal uptake in the myelum on the level of the fifth lumbar vertebra,

the cerebellum and the epididymis was seen (E, circles), on conventional imaging matching with hemangioblastomas and cystadenomas respectively.

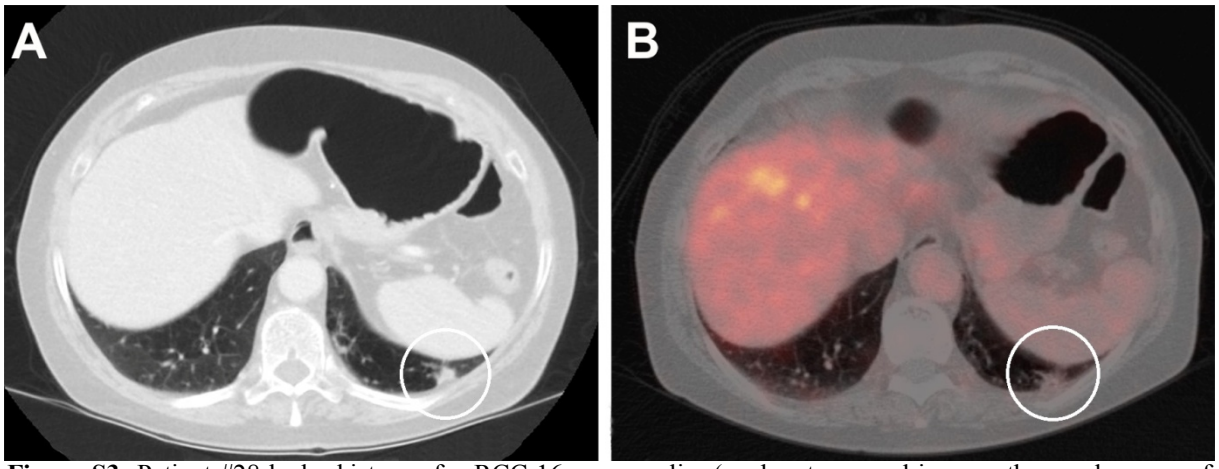

Figure S3: Patient #28 had a history of ccRCC 16 years earlier (nephrectomy and immunotherapy because of pulmonary metastases, followed by a partial response). Small residual lesions remained stable over the past years, except for slowly growing pulmonary lesions in the left lower lobe of the long, which had a differential diagnosis of a primary lung carcinoma (A). PET/CT imaging demonstrated no uptake of ^{89}Zr-girentuximab in the growing lung lesion, nor in the small residual lesions (B). Under the suspicion of a secondary primary lung cancer, a lobectomy was performed, confirming the presence of a primary lung carcinoma and several granulomas.

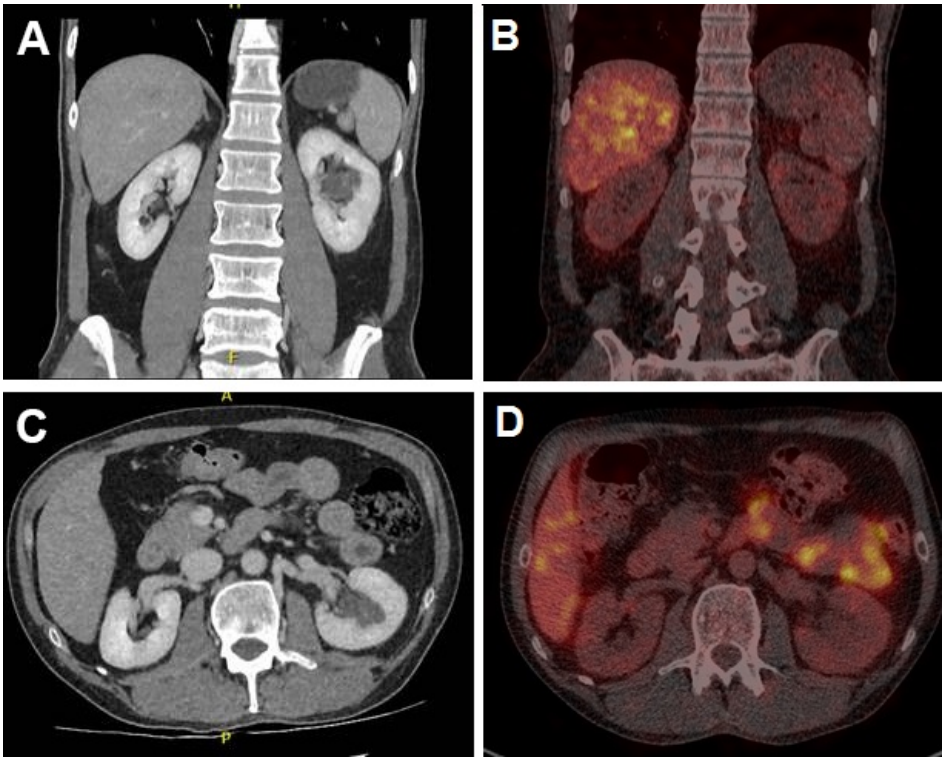

Fig S4: Patient #9 was diagnosed with a centrally located cystic lesion in the left kidney with thickened walls and enhancement of septa, classified as Bosniak 3-4 (CT; A coronal, C, transverse plane). Due to the central location of the tumor, a partial nephrectomy was considered not feasible. More certainty about the nature of the lesion prior to radical nephrectomy was desirable. Since the chance of malignancy was lowered due to the negative ^{89}Zr-girentuximab PET/CT (B coronal, D transverse plane), active surveillance was advised. Neither growth nor progression of the lesion was seen during 12 months of follow-up.

Supplemental methods

Patient population

Two subgroups of patients were distinguished; 1. patients with a renal mass of unknown origin, and 2. patients with a history of ccRCC and a suspicion of recurrent or metastatic disease. All patients received at least one of the following conventional diagnostic modalities: contrast-enhanced CT, MRI and/or biopsy. Patients were over 18 years old and signed informed consent. Exclusion criteria were pregnancy or lactation and a history of a CAIX-negative RCC. Lastly, patients that were on tyrosine kinase inhibitor (TKI) treatment within the last month were excluded, as treatment with TKI has demonstrated to decrease uptake of ^{111}In-girentuximab [8].

Preparation of ^{89}Zr-girentuximab

Conjugation of N-suc-desferal ester (VU Medical Center, Amsterdam, the Netherlands) to girentuximab (Wilex AG, Munich, Germany and Telix Pharmaceuticals, Melbourne, Australia) was performed as described previously [6]. At the day of injection 2 mg desferal-girentuximab was radiolabeled with 37 MBq of Zirconium-89 (Perkin Elmer, The Netherlands). The radiolabeling process was performed at a pH of 7.2. To achieve the desired pH value, oxalic acid, sodium carbonate and HEPES buffer (adjusted to pH 7.3 by use of sodium hydroxide solution) were added. After addition of 2 mg desferal-girentuximab and HEPES buffer, radiolabeling was allowed to take place during 60 minutes at room temperature. Next, unbound ^{89}Zr was complexed by the chelator EDTA by incubation for 15 minutes at room temperature. Then the product was purified using gelfiltration on disposable PD10 columns. To obtain a total protein dose of 5 mg, unlabeled girentuximab was added to lower undesired hepatic uptake of ^{89}Zr-desferal-girentuximab. Radiochemical purity was

determined by high-performance liquid chromatography and exceeded 90%. The end product was diluted to a total volume of 10 ml with NaCl 0.9% and administered intravenously in ten minutes within 4 hours after radiolabeling.