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^{89}Zr-bevacizumab PET: Potential Early Read Out for Efficacy of Everolimus in Metastatic Renal Cell Carcinoma Patients.

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Running title: Everolimage study

Abstract

Rationale: Currently, biomarkers that predict efficacy of everolimus in metastatic renal cell carcinoma (mRCC) patients are lacking. Everolimus inhibits vascular endothelial growth factor A (VEGF-A) expression. We performed PET scans in mRCC patients with ^{89}Zr-bevacizumab, a VEGF-A-binding antibody tracer. Aims were to determine change in tumor tracer uptake after start of everolimus and to explore if ^{89}Zr-bevacizumab PET can identify patients with early disease progression.

Methods: ^{89}Zr-bevacizumab PET was done before and 2 and 6 weeks after start of everolimus 10 mg/day in mRCC patients. Routine CT scans were performed at baseline and every 3 months thereafter. Tumor tracer uptake was quantified using maximum Standardized Uptake Value (SUVmax). Endpoints were change in tumor tracer uptake and treatment response on CT after 3 months.

Results: Thirteen patients participated. Median SUVmax of 94 tumor lesions was 7.3 (range 1.6-59.5). Between patients, median tumor SUVmax varied up to 8-fold. After 2 weeks, median SUVmax was 6.3 (1.7-62.3) corresponding to a mean decrease of 9.1% ($P < 0.0001$). Three patients discontinued everolimus early. At 6 weeks, a mean decrease in SUVmax of 23.4% compared to baseline was found in 70 evaluable lesions of 10 patients, with a median SUVmax of 5.4 (1.1-49.4, $P < 0.0001$). All 10 patients who continued treatment had stable disease at 3 months.

Conclusions: Everolimus decreases ^{89}Zr-bevacizumab tumor uptake. Further studies are warranted to evaluate predictive value of ^{89}Zr-bevacizumab PET for everolimus antitumor efficacy.

Key words: Molecular imaging, positron emission tomography, renal cell carcinoma, everolimus, biomarker

Introduction

Clear cell renal cell carcinoma (RCC) is characterized by Von Hippel-Lindau gene inactivation. This results in expression of pro-angiogenic growth factors such as vascular endothelial growth factor A (VEGF-A) and typical vascular tumors. Patients with RCC have higher circulating VEGF-A levels than healthy controls (*1*). A high baseline plasma VEGF-A level is associated with shorter progression free and overall survival (OS) in metastatic RCC (mRCC) and is an independent prognostic factor (*2*). VEGF-A pathway targeting agents such as the VEGF-A binding antibody bevacizumab with interferon alpha (IFN-α) or the tyrosine kinase inhibitors (TKIs) sunitinib, sorafenib, pazopanib and axitinib are established treatment options for mRCC (*3-7*). Inhibitors of mammalian target of rapamycin (mTOR) also have antitumor activity in mRCC (*8,9*). mTOR plays a key role in cell growth, protein translation, autophagy and metabolism. Blocking mTOR causes cell cycle arrest in a broad range of tumor types but in addition to a direct antitumor effect, mTOR inhibitors block VEGF-A expression, vascular permeability, endothelial cell proliferation and angiogenesis (*10*).

Everolimus is an orally administered mTOR inhibitor. A phase III study in mRCC patients with progressive disease during or after treatment with sunitinib, sorafenib or both demonstrated doubling of median progression free survival (PFS) for everolimus compared to placebo (4.9 months versus 1.9 months) (*11*). Recently nivolumab was proven to be superior to everolimus in pretreated mRCC patients, with median OS of 25.0 months compared to19.6 months, and less high grade treatment-related adverse events (*12*).

Only a subset of mRCC patients profits from treatment with mTOR inhibitors. Regretfully no predictive biomarkers that can identify patients who are likely to benefit of mTOR inhibitors are available. The novel treatment option with the immunotherapeutic drug nivolumab after first-line anti-angiogenic treatment even more reinforces the need for such predictive biomarker. ^{89}Zr-bevacizumab is a positron emission tomography (PET) tracer that binds VEGF-A. We demonstrated in a previous study that ^{89}Zr-bevacizumab PET scans can visualize RCC tumor lesions, and that tracer uptake in these lesions changed during treatment with sunitinib or bevacizumab/IFN-α. Furthermore, results suggested that high baseline tumor uptake was associated with longer time to progression (TTP) (*13*). Serial ^{89}Zr-bevacizumab PET scans might also be useful to assess down regulation of tumor VEGF-A expression after start of a mTOR inhibitor and serve as an early read out of efficacy. We hypothesize that successful down regulation of VEGF-A expression by everolimus results in a decrease of tumor tracer uptake after start of treatment.

We conducted a feasibility study in patients with mRCC who started treatment with everolimus. The primary objective of this study was to demonstrate change in tumor tracer uptake after start of treatment. Secondary objective was to explore if ^{89}Zr-bevacizumab PET could identify patients with disease progression on the first follow-up CT after 3 months of treatment.

Patients and methods

Patients

Adult mRCC patients with measurable disease, WHO performance score ≤2 who were candidate to start treatment with everolimus were eligible. The study was approved by the institutional review board, and all subjects gave written informed consent. The trial is registered on ClinicalTrials.gov (NCT01028638).

Study Design and Treatment

Patients underwent ^{89}Zr-bevacizumab PET imaging before, and 2 and 6 weeks after start of everolimus 10 mg orally once daily. Treatment was continued until disease progression or unacceptable toxicity. The primary endpoint was change in ^{89}Zr-bevacizumab uptake in tumor lesions between the baseline PET scan and the scans after 2 and 6 weeks of treatment. The secondary endpoint was progressive disease according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST1.1) after 3 months of treatment.

Imaging Techniques

Four days prior to each PET scan, patients were injected intravenously with 37 MBq ^{89}Zr-bevacizumab, protein dose 5 mg. No therapeutic effects are to be expected from this dose as therapeutic bevacizumab doses vary from 7.5 to 15 mg per kg body weight. Conjugation and labeling were done as described before (*14*). Patients were scanned from head to upper thigh in up to 8 consecutive bed positions as described earlier (*13*), with a final reconstruction resolution of about 10 mm.

At baseline and every 3 months during treatment, patients underwent computed tomography (CT) imaging. CT was performed with oral and intravenous contrast with a maximal slice thickness of 5.0 mm. The mean interval between baseline CT and baseline PET scan was 15 days.

Imaging Data Analysis

The baseline PET scans were fused with the baseline CT scans and analyzed by visual and quantitative assessment of tumor lesions. All hotspots on PET that were concordant with tumor lesions on CT were documented as being metastases. For reliable quantification of ^{89}Zr-bevacizumab uptake, lesions had to be > 10 voxels and had to be delineable from normal organ background. Irradiated lesions were excluded from quantification. Quantification was performed with AMIDE Medical Image Data Examiner software (version 0.9.1, Stanford University) (*15*). Maximum and mean standardized uptake values (SUVmax and SUVmean) were calculated for lesions and not involved organs/tissues. SUVmax and SUVmean were strongly correlated, r = 0.99, $P < 0.0001$ (Suppl. Fig. 1). SUVmax is less operator dependent and was therefore used for calculations of tumor tracer uptake. SUVmean was used for measuring uptake in healthy organs. CT scans were evaluated by a radiologist (S.V.K.M.). All tumor lesions ≥10 mm on the baseline CT scan were measured for comparison with PET. Treatment response was assessed according to RECIST1.1.

Biomarker Analysis

Serum samples were collected before every tracer injection, at day -4, day 11 and day 39 for analysis of circulating VEGF-A levels, with day 0 being the day of baseline PET scan and start of everolimus treatment. The blood was centrifuged and platelet poor plasma samples were stored at -80 ˚C until analysis. VEGF-A was analyzed using an enzyme-linked immunosorbent assay (ELISA) (R&D systems, Minneapolis, MN). Plasma VEGF-A was compared to tumor tracer uptake.

Statistical Assessments

We estimated that 11 patients were required to predict with 80% power (two-sided $\alpha = 0.05$) that there is a true difference in SUV (≥ 1.25 SD) between the baseline scan and the scan after 2 and/or 6 weeks. Change in ^{89}Zr-bevacizumab tumor lesion uptake between the baseline scan and the scan after 2 and/or 6 weeks of treatment was calculated and presented as a percentage (mean with 95% confidence interval CI). Wilcoxon's paired rank test was used to assess significance of the change in tumor and healthy organ tracer uptake and plasma VEGF-A. Correlations were analyzed by using Spearman's rank test.

Analyses were performed with SPSS version 22 (IBM, New York, NY).

Results

Patients

Between December 2009 and October 2014, 13 patients were included. The median interval between stop TKI and start everolimus was 10.57 weeks, the mean interval was 16.8 weeks (range 2-44.3). Ten patients completed the study; two patients stopped everolimus between 2 and 6 weeks because of adverse events and one patient had clinically progressive disease before the third PET-scan. Median time to progression was 5.8 months (1.9-24.9). For patient characteristics, see Table 1.

^{89}Zr-bevacizumab PET

^{89}Zr-bevacizumab uptake in tumor lesions

^{89}Zr-bevacizumab PET images were of high quality and visualized tumor lesions clearly (Fig. 1). Out of 147 tumor lesions ≥ 10 mm on CT, 103 were detected as hot spots on the PET scan (71%). A total of 94 tumor lesions fulfilled the criteria for reliable quantification and were used for calculations (Fig. 2).

Median SUVmax at baseline was 7.3 (range 1.6-59.5). All 13 patients underwent the second PET scan after 2 weeks of treatment. Median SUVmax was 6.3 (range 1.7-62.3) corresponding to a mean change of -9.1% (95% CI -3.4% to -14.9%, $P < 0.0001$). On the third PET scan, performed in 10 patients, a total of 70 evaluable lesions were apparent with a median SUVmax of 5.4 (range 1.1-49.4). This corresponds to a mean change of -23.4% (95% CI -16.5% to -30.2%, $P < 0.0001$) compared to baseline (Fig. 3 and 4).

^{89}Zr-bevacizumab uptake in healthy organs

^{89}Zr-bevacizumab uptake in healthy organs at baseline was comparable to previous studies (*13,16*) (Suppl. Fig. 2).

^{89}Zr-bevacizumab PET and treatment outcome

All 10 patients still on everolimus after 3 months had stable disease according to RECIST1.1 at first response evaluation. The single patient with clinical progressive disease within 3 months had a baseline median tumor SUVmax of 8.7 (range 6.8-14.9) and a mean decrease of 12.2% of tumor SUVmax after 2 weeks of treatment which resembles the pattern seen in patients without early progression. Exploratory analysis showed a correlation between baseline mean tumor SUVmax and time on treatment (r = 0.63, *P* = 0.02, Suppl. Fig. 3). Mean tumor SUVmax at baseline of patients who were on treatment longer than the 12 months was higher than mean tumor SUVmax of patients who were on treatment <12 months (median mean tumor SUVmax 22.2 versus 7.2) (*P* = 0.09).

There was no correlation between change in SUVmax after 2 and 6 weeks of treatment and time on treatment.

Plasma VEGF-A

Median baseline plasma VEGF-A was 408 pg/mL (n = 13, range 144-2013 pg/mL). At day 11 median plasma VEGF-A was 97 pg/mL (n = 12, range < 38-644 pg/mL) representing a mean decrease of 68.7% (*P* = 0.001). After 6 weeks 10 patients were ongoing of whom 9 samples were available. Median VEGF-A was 83 pg/mL

(range < 38-384 pg/mL, decrease 74.6%, *P* = 0.004) (Fig. 6). Only one patient showed an increase in plasma VEGF-A level after 2 weeks of treatment; this patient had early clinical progression. At baseline there was no correlation between plasma VEGF-A and tumor SUVmax. There was also no correlation between change in tumor tracer uptake and change in plasma VEGF-A after 2 or 6 weeks.

Discussion

In this study in mRCC patients we found a decrease in ^{89}Zr-bevacizumab tumor uptake after 2 weeks, and a further decrease after 6 weeks of everolimus treatment. Because all patients had stable disease on CT at 3 months we could not assess the ability of ^{89}Zr-bevacizumab PET to early identify progressive disease. Substantial heterogeneity in tumor tracer uptake both within and between patients was seen at baseline, but also change in tumor tracer uptake during treatment varied considerably.

Predictive biomarkers that can differentiate patients likely to benefit from mTOR inhibitors from those who are unlikely to benefit, are needed to achieve patient tailored therapy. Several potential markers have been identified, such as functional mutations in the mTOR pathway genes, baseline serum LDH and off target effects resulting in specific side effects (*17-20*). However, these markers still have to be validated and their potential clinical usefulness have to be established.

^{18}F-fluorodeoxyglucose (FDG) PET has been investigated as a predictive biomarker for everolimus treatment in 50 mRCC patients. There was no correlation between baseline SUVmax and change in tumor size, but baseline SUVmax showed a significant negative correlation with OS and PFS. A modest correlation between

ΔSUVmax and change in tumor size was found, but ΔSUVmax did not correlate with OS or PFS. Altogether, the investigators did not recommend any additional studies (*21*).

We developed ^{89}Zr-bevacizumab PET imaging to non-invasively study tumor VEGF-A concentration. Previously we performed serial ^{89}Zr-bevacizumab PET scans (at baseline, after 2 and 12 weeks) in patients who started everolimus treatment for neuroendocrine tumors (NETs) (*16*). In that study, tumor lesions were visualized in 10 out of 14 patients with a median SUVmax of 5.8 (range 1.7-15.1). Tumor SUVmax decreased by 7% after 2 weeks and by 35% after 12 weeks of treatment, which mirrors the results of the current study in mRCC patients. The highest tumor SUVmax in NET patients however was 15.1, while in the current study we measured tumor SUVmax values up to 59.5 in RCC patients. Furthermore, only 19% of all lesions ≥ 10 mm was visualized in the 10 NET patients with a positive PET scan, while in the present study we visualized 71% of mRCC lesions ≥ 10 mm. These differences are likely explained by tumor biology. Sporadic VHL mutations are a unique characteristic of RCC and result in high VEGF-A production by tumor cells.

In the current study we did not perform biopsies. However a good correlation between tumor uptake of radioactive labeled bevacizumab and VEGF-A in tumor tissue was shown earlier (*14, 22, 23*). We therefore assume that the decrease in tumor tracer uptake represents reduction in VEGF-A production by the tumor cells as a result of everolimus treatment. Another potential explanation may be antiangiogenic effects of everolimus, such as reduced tumor perfusion or reduced vascular permeability (*24*). However, dynamic contrast-enhanced magnetic resonance imaging using K(trans) measurement in patients with advanced cancer showed no decrease in tumor perfusion

on everolimus treatment (*25*). Whether mainly tumor VEGF-A concentration or also antiangiogenic effects of everolimus are visualized with serial ^{89}Zr-bevacizumab PET remains to be determined.

Patients who participated in the current study received prior treatment with a TKI. In a previous studies we demonstrated that sunitinib results in a heterogeneous change in tumor ^{89}Zr-bevacizumab uptake with an overall slight decrease and a rebound phenomenon after 2 stop weeks (*13*). The median interval between stop TKI and start of everolimus was 10.57 weeks (range 2 – 44.3). For the two patients who had an interval of 2 weeks between TKI and tracer injection we cannot exclude influence of prior treatment on tumor tracer uptake. For the overall study results however, influence of previous treatment is likely limited.

Median plasma VEGF-A decreased after 2 weeks of everolimus treatment. Overall a small further decrease was measured after 6 weeks, but in half of the patients plasma VEGF-A increased between week 2 and week 6.

The current study was conducted before publication of the CheckMate 025 and METEOR trials (*12, 26*). Nivolumab, a programmed death 1 (PD1) targeting antibody that acts as immune checkpoint inhibitor prolonged OS compared to everolimus in previously treated patients with advanced RCC. Cabozantinib, a tyrosine kinase inhibitor of VEGF receptors (VEGFR), MET and AXL was also shown to be superior to everolimus in previously treated mRCC patients. These trials strongly enforce the need for predictive biomarkers that can identify patients who will likely benefit from everolimus to allow individualized treatment choices. In an exploratory analysis we found that the baseline ^{89}Zr-bevacizumab PET results correlated with time on treatment. Tumor tracer

uptake was higher in patients that were treated longer. Therefore, ^{89}Zr-bevacizumab PET might be useful for predicting treatment success.

Previously, we published results of serial ^{89}Zr-bevacizumab PET imaging in mRCC patients who were treated with bevacizumab/IFN-α or sunitinib. A similar heterogeneous tumor tracer uptake at baseline was found with a slightly lower detection rate of tumor lesions ≥ 10 mm (56.7%). A correlation between baseline tumor tracer uptake and time to progression (TTP) was shown in an exploratory analysis, with a high uptake corresponding to longer TTP (*13*). This observation, in combination with the current study, supports the option that ^{89}Zr-bevacizumab PET has prognostic rather than predictive value. Interestingly, in nephrectomy samples of 137 patients with locally advanced or metastatic clear cell RCC, high VEGF expression determined by immunohistochemistry was associated with worse overall survival. Median overall survival was 206 months in patients with low and 65 months in patients with high VEGF expression ($P < 0.001$) (*27*). This observation raises the possibility that high tumor VEGF-A is an unfavorable prognostic factor but in the metastatic setting, by providing a target for treatment, identifies patients that show prolonged benefit from direct and indirect VEGF pathway inhibition with angiogenesis and mTOR inhibitors.

Larger trials are needed to evaluate whether this molecular imaging tool can serve as a predictive biomarker to select patients who derive clinically relevant benefit from everolimus.

Conclusion

^{89}Zr-bevacizumab tumor uptake decreases significantly during everolimus

treatment in mRCC patients. We could not demonstrate whether ^{89}Zr-bevacizumab PET can early identify progressive disease. Larger trials are warranted to determine whether ^{89}Zr-bevacizumab PET can be used to select patients for everolimus treatment.

Financial Disclosures

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Figure 1A **Figure 1B**

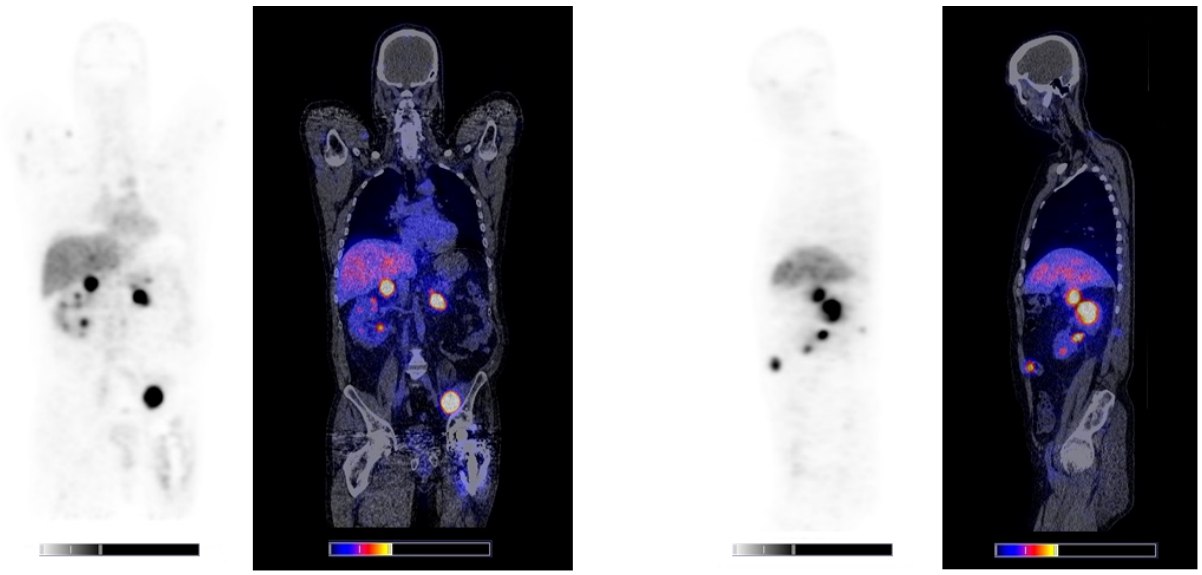


Figure 1. Baseline ^{89}Zr-bevacizumab PET images of a mRCC patient (A) Coronal 2D image through the right adrenal gland metastasis, showing additionally one local recurrence in the left kidney lodge, 1 kidney metastasis and a soft tissue metastasis near left iliac bone. (B) Sagittal 2D image in the coronal image through the right adrenal gland metastasis, showing additionally three kidney metastases and one soft tissue metastasis in the abdominal wall.

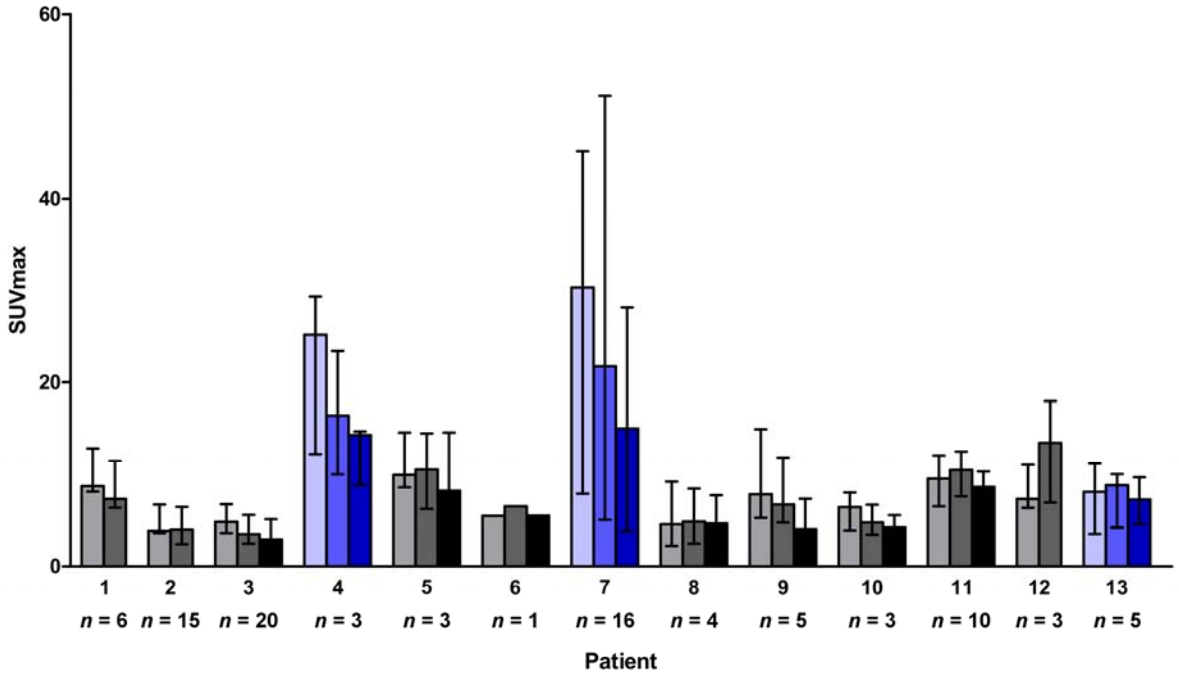


Figure 2. Median tumor tracer uptake with interquartile range per patient at baseline (light grey) after 2 weeks of everolimus (dark grey) and after 6 weeks of treatment (black). Patients in blue stayed on treatment > 12 months. n = number of quantified tumor lesions per patient.

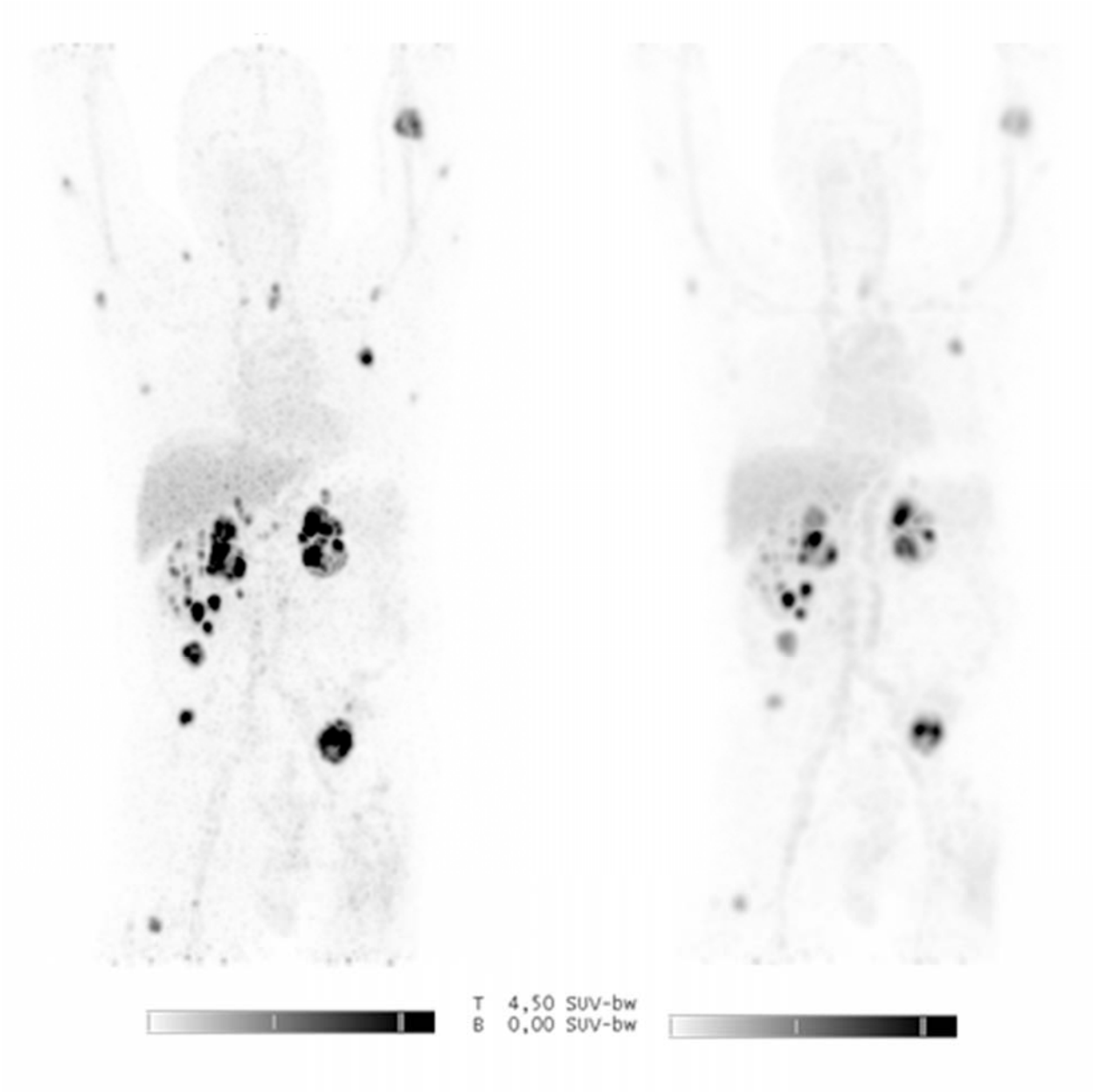


Figure 3. Maximum intensity projections prior to treatment showing multiple local recurrences in the left kidney lodge, metastases in the right kidney, right adrenal gland and soft tissue at baseline (left) and after 6 weeks of treatment, showing a heterogeneous decrease in uptake (right).

Figure 4A

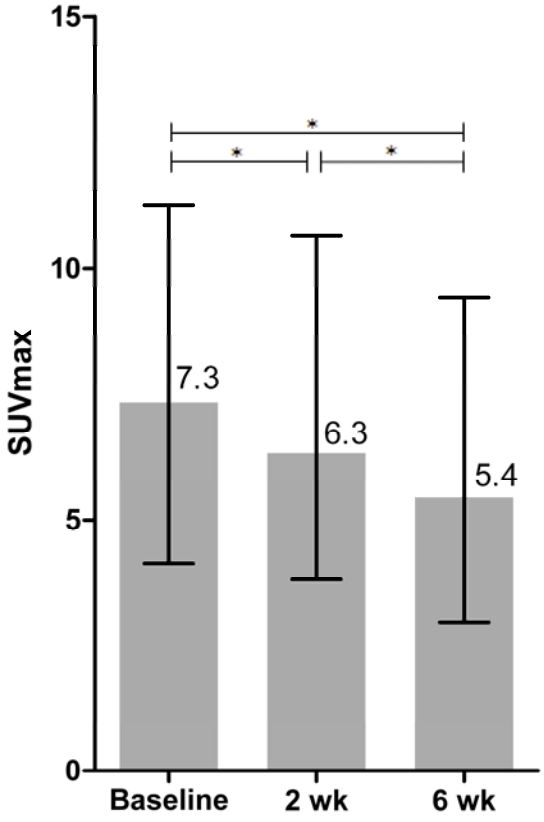


Figure 4B

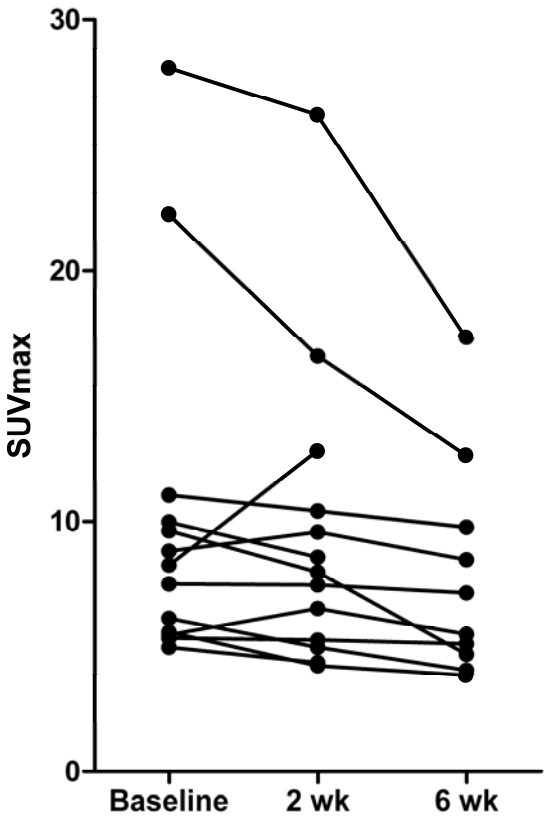


Figure 4. Tumor tracer uptake before and during treatment. (A) Median uptake of all lesions with interquartile range. (B) Mean tumor uptake per patient. *P < 0.0001.

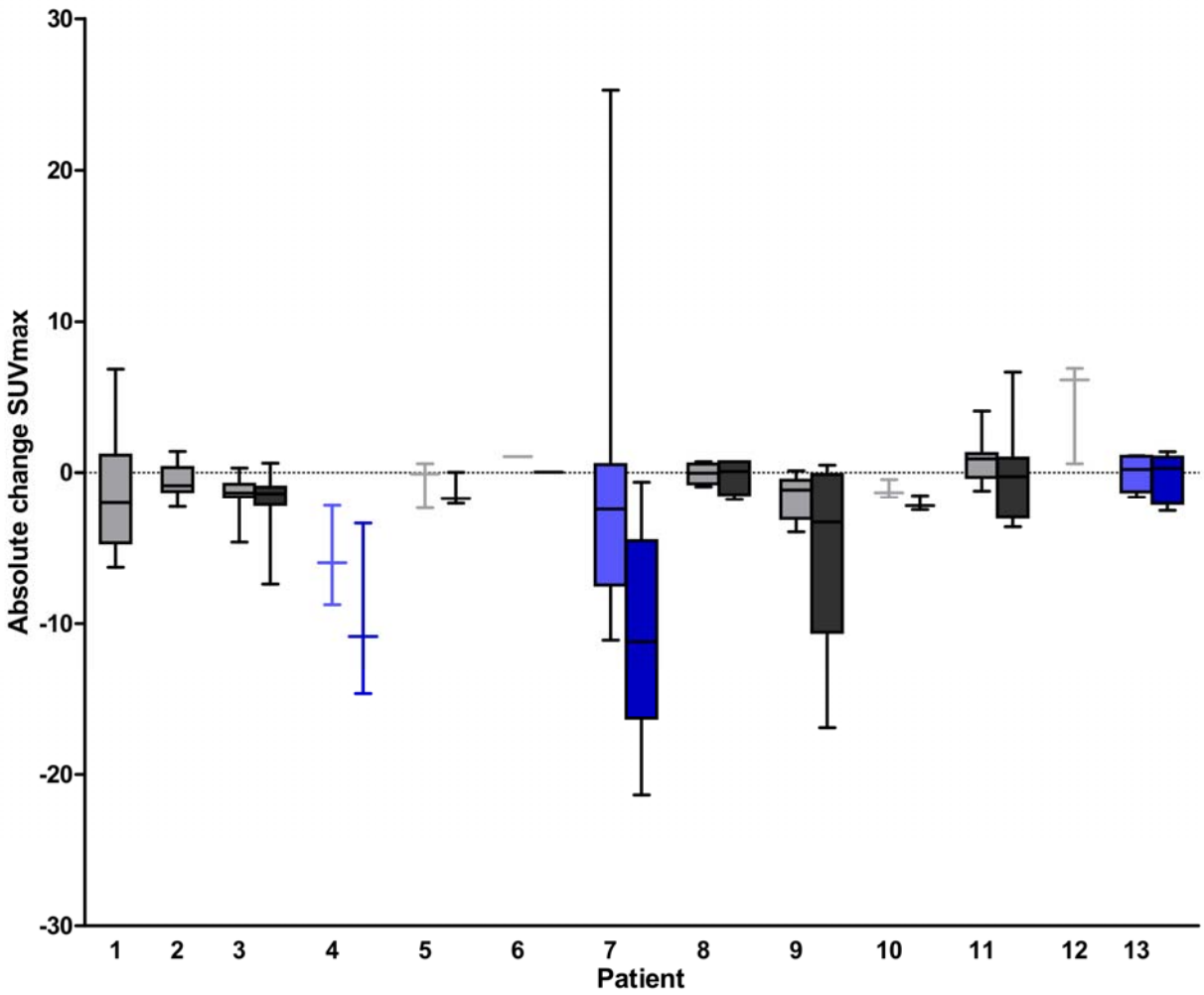


Figure 5. Absolute change in SUVmax for all lesions per patient after 2 weeks (grey) and 6 weeks (black) of everolimus treatment with median. Patients in blue stayed on treatment > 12 months.

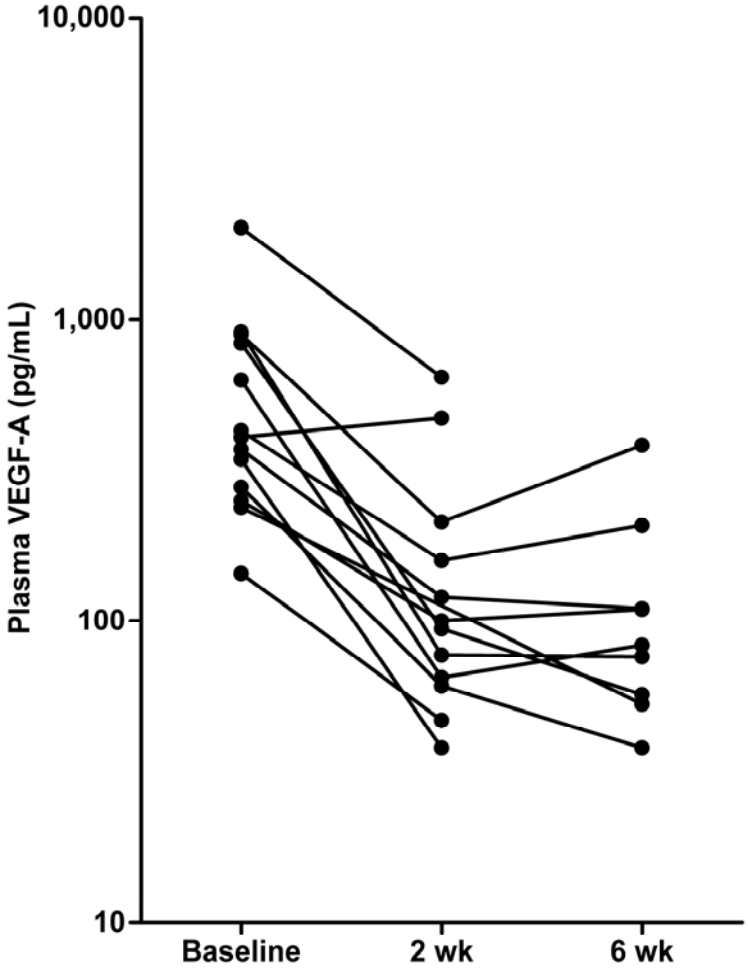
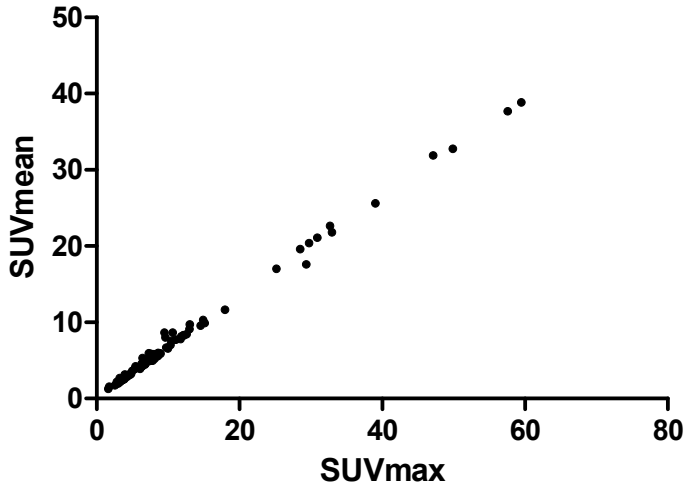


Figure 6. Plasma VEGF-A concentrations, each line represents one patient.

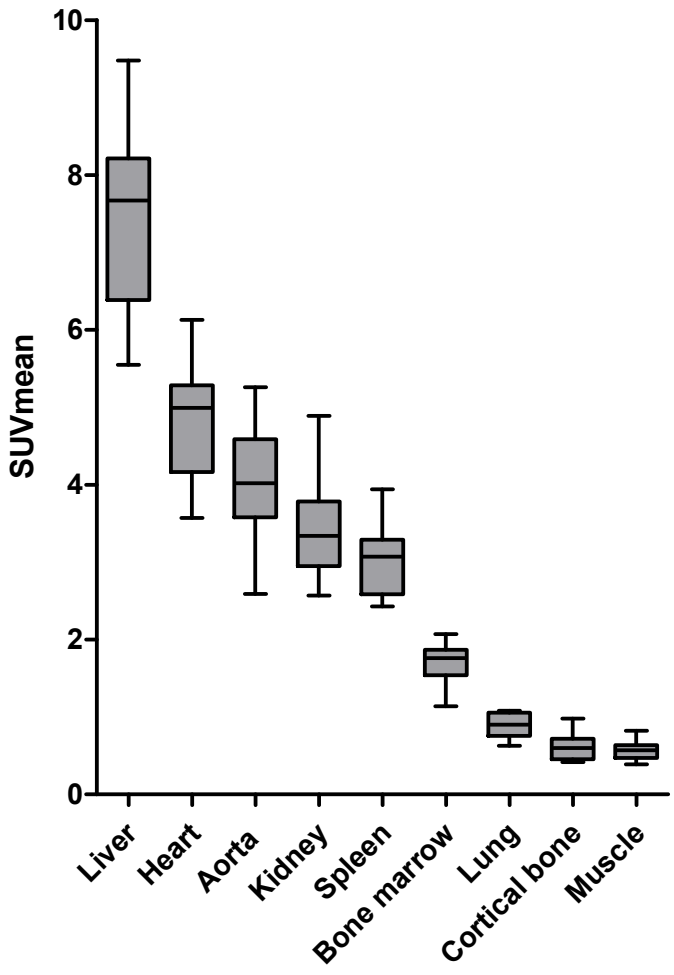
Table 1. Patient demographics and clinical characteristics.

Variable	*N*	*% (range)*
Sex		
Male	9	69.2
Female	4	30.8
Age (years)		
Median	63	(51-71)
Nephrectomy		
Yes	11	84.6
No	2	15.4
Histology		
Pure clear cell	12	92.3
Mixed	1	7.7
WHO performance		
0	4	30.8
1	2	15.4
2	7	53.8
Number of tumor sites		
Median	4	(2-7)
Tumor sites		
Kidney	6	46.2
- **primary**	2	
- **local recurrence**	2	
- **contralateral**	2	
Lung	9	69.2
Lymph node	7	53.8
Bone	6	46.2

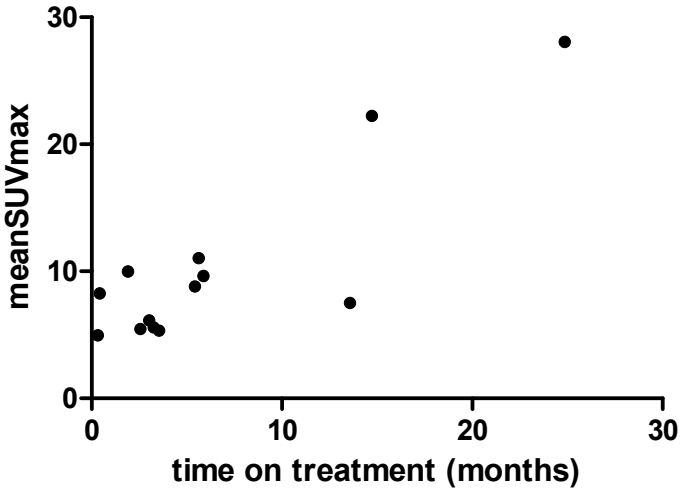
Liver	5	38.5
Adrenal	4	30.8
Muscle	4	30.8
Other	10	76.9
Previous treatment		
TKI	8	
TKI & IFN-α ± bevacizumab	2	
other	3	



Suppl. Figure 1. Correlation between SUVmean and SUVmax of all evaluable tumor lesions at baseline ($r = 0.99$, $P < 0.0001$).



Suppl. Figure 2. ^{89}Zr-bevacizumab uptake in healthy organs at baseline.



Suppl. Figure 3. Correlation between time on treatment and baseline tumor tracer uptake (r = 0.63, *P* = 0.02).