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RESEARCH REPORT

Exceptional response to everolimus in a novel tuberous sclerosis complex-2 mutation–associated metastatic renal-cell carcinoma

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Abstract Everolimus, an oral inhibitor of the mammalian target of rapamycin (mTOR) pathway, is currently approved for treatment of advanced renal-cell carcinoma (RCC) after failure of initial treatment with the tyrosine kinase inhibitors. Patients with tuberous sclerosis complex (TSC) syndrome can also develop RCC primarily mediated through mTOR signaling. However, the efficacy and duration of response of mTOR inhibition in patients with TSC-associated RCC is not well known. Herein, we describe a case of a patient with *TSC2*-associated metastatic RCC with mutations *H1620R* and *Y1650C* who has had an exceptional response to everolimus in the frontline setting and continues to derive benefit from mTOR inhibition 2 yr into therapy. Furthermore, the alteration *H1620R* in exon 37 resulting in a missense mutation is likely deleterious given our findings and previous analyses of the *TSC2* gene. Further studies of somatic mutations in extended responders to mTOR inhibitors will help personalize therapy for these patients. It also emphasizes the value of targeted therapies based on genomic analyses.

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder with multiorgan involvement affecting the brain, skin, heart, lungs, liver, and kidneys (Curatolo and Bombardieri 2007). Diagnosis is usually made based on clinical findings and family history. Genetic testing to confirm the diagnosis is not universally needed. TSC is caused by mutations in either the *TSC1* (hamartin) gene on Chromosome 9 or the *TSC2* (tuberin) gene on Chromosome 16 (Carbonara et al. 1994; Au et al. 1999; Kobayashi et al. 2001). This tumor-suppressing function of the *TSC* genes is mostly related to inhibition of cellular signaling and growth mediated by the mammalian target of rapamycin (mTOR) pathway (Tee et al. 2002). This is supported by the clinical phenotype in patients with TSC whereby they can develop numerous benign and malignant tumors in different organ systems. For example, patients with TSC can develop subependymal giant cell astrocytoma (SEGA) in the brain and angiomyolipomas in the kidneys. Additionally, patients with TSC syndrome are at increased risk of developing renal-cell carcinomas (RCCs) (Bjornsson et al. 1996; Henske 2004; Rakowski et al. 2006).

Everolimus, an oral inhibitor of the mTOR pathway, is currently approved for treatment of advanced RCC after failure of initial treatment with the tyrosine kinase inhibitors. It is not yet approved for treatment of RCC in the frontline setting. Additionally, the drug is approved for a number of indications in patients with TSC syndrome. In adults it is approved for patients with TSC syndrome and renal angiomyolipomas; and in both children and adults with TSC

syndrome who develop SEGA. In patients with TSC-associated RCC, the efficacy and duration of response are not known. Herein we describe a case of a patient with TSC-associated metastatic RCC who has had an exceptional response to everolimus in the frontline setting and continues to derive benefit from mTOR inhibition 2 yr into therapy.

CLINICAL PRESENTATION AND FAMILY HISTORY

A 47-yr-old Caucasian male with a past medical history only significant for infantile seizures before age 5 presented to the emergency department with gross hematuria. Physical exam was significant for multiple hypomelanotic cutaneous lesions mostly noted on the back and the lower extremities. Additionally, multiple facial cutaneous lesions most consistent with facial angiofibromas were noted. Of note, the patient's mother was clinically diagnosed with TSC. She was reported to have classic skin findings, angiofibromas, brain tubers and, bilateral clear cell RCC at age 48. The patient's brother was also diagnosed with TSC at age 35 based on classical skin findings and brain tubers. The patient's nephew, son of the affected brother, had seizures, autism, and skin abnormalities consistent with TSC.

Because of the patient's significant hematuria, computed tomography (CT) of the abdomen was performed and showed multifocal bilateral renal masses suspicious of a malignant process with associated para-aortic lymphadenopathy. CT of the chest demonstrated numerous subcentimeter pulmonary nodules with a pattern most consistent with metastatic disease. These findings and family history raised the suspicion for TSC, and MRI of the brain with and without gadolinium was also performed and showed numerous nonspecific FLAIR/T2 hyperintense lesions involving the cerebral hemispheres bilaterally with predominant involvement of the cortical gray matter without associated vasogenic edema, enhancement, or restricted diffusion most consistent with TSC. Figure 1 summarizes the spectrum of findings involving different organ systems noted in our patient.

The patient underwent a needle biopsy of one of the renal masses and pathology showed RCC with mixed papillary and clear cell features. The patient was evaluated by a medical geneticist who, based on clinical criteria and family history, diagnosed the patient with TSC. No confirmatory genetic testing was felt necessary. Tissue from the renal mass biopsy, however, was sent for genetic testing and that confirmed two *TSC2* gene mutations (*H1620R*, *Y1650C*) alongside other aberrations as noted in Tables 1 and 2. These aberrations were reported out as variants of unknown significance. We did not find any previous reports of these two mutations. There is one report similar to the *H1620R* as a missense alteration that has been previously reported (Au et al. 1998). However, in that particular report, the amino acid reported is asparagine and not histidine, as in our report. Analysis of the *TSC2* gene by Au and colleagues in 90 TSC patients did notice this alteration (DNA sequence change: 4859ArT; Codon change: Asn1620Ile; Exon 37). This H1620R is likely another deleterious mutation. We did not find any previous reports of the *TSC2 Y1650C* alteration and this likely represents a variant of unknown significance (VUS) unless other reports or detailed family germline analyses corroborate these findings.

CLINICAL COURSE AND MANAGEMENT

Given the unique pathogenesis of RCC in TSC and the important dominant role of mTOR signaling, the patient was started on personalized off-label therapy with an mTOR inhibitor, everolimus, at a dose of 10 mg oral on a daily basis in the frontline setting. The patient has tolerated the treatment well with no untoward side effects other than grade-1 stomatitis, hyperglycemia, and hypertriglyceridemia, which were managed conservatively without any

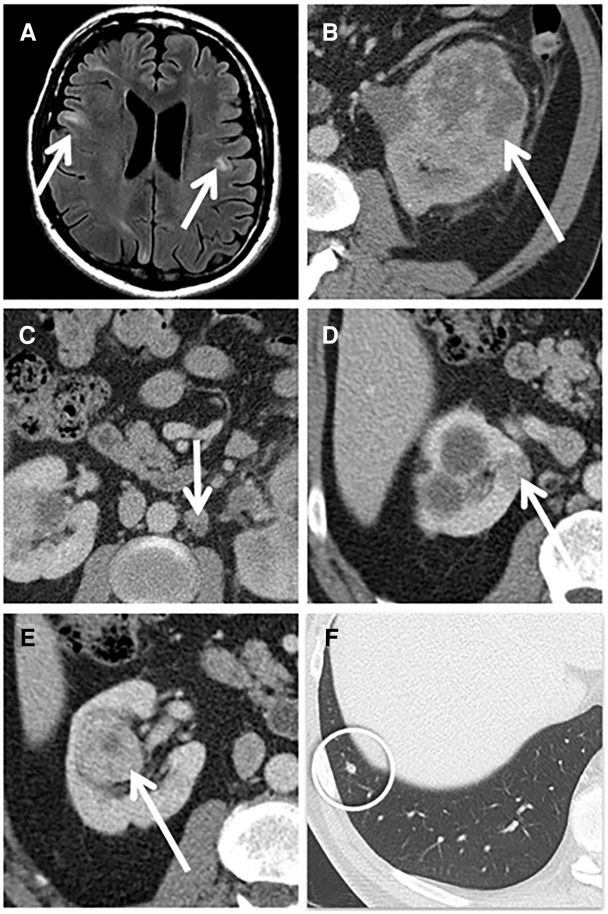

Figure 1. The spectrum of manifestations seen in our patient with tuberous sclerosis (TSC)-associated renal-cell cancer. (*A*) MRI of the brain with gadolinium contrast showing numerous nonspecific FLAIR/T2 hyperintense lesions involving the cerebral hemispheres bilaterally with predominant involvement of the cortical gray matter without associated vasogenic edema, enhancement, or restricted diffusion consistent with TSC; (*B*) CT scan of the abdomen showing RCC forming multiple masses in the left kidney; (*C*) metastatic para-aortic adenopathy alongside cysts seen in the right kidney; (*D*) angiomyolipomas seen in the right kidney alongside several cysts; (*E*) angiomyolipoma seen in the right kidney; and (*F*) subcentimeter nodules seen in the lung consistent with metastases from RCC.

Table 1. Commercial next-generation sequencing (NGS)-based assay identifying multiple genomic alterations alongside a brief description of potential personalized targeted therapies

Genomic alterations detected[a]	Potential targeted therapy
TSC2 H1620R,Y1650C	Mammalian target of rapamycin (mTOR) inhibitors.
CDKN2A/B loss	Cyclin-dependent kinase (CDK) 4/6 inhibitors; mouse double minute 2 homolog (MDM2) inhibitors.
ARID2 L202	–

[a]Other variants of unknown significance that were also detected on NGS assay (FoundationONE) included *ARID1B* Q129_Q130insQQ; *BRCA2* D820G; *DICER1* T1214P; *FLT3* V579I; *MAP3K1* S939C; *RANBP2* K1479E; *RICTOR* rearrangement.

Table 2. Details on TSC variants identified on NGS assay

Gene	Chromosome position (GRCh37)	HGVS cDNA	HGVS protein	Type	Effect	dbSNP ID	Genotype	ClinVar (number of variants)	Allele Freq. ExAC/ gnomAD (all)
TSC2 (H1620R)	NC_000016.10: g.2086741A >G	NM_000548.4: c.4859A>G	NP_000539.2: p.His1620Arg	Single-nucleotide variant	Missense	397515177	Not known	Uncertain significance	NR[a]
TSC2 (Y1650C)	NC_000016.10: g.2086831A >G (GRCh38)	NM_000548.4: c.4949A>G	NP_000539.2: p.Tyr1650Cys	Single-nucleotide variant	Missense	45501091	Not known	Uncertain significance	NR[a]

TSC, tuberous sclerosis complex; NGS, next-generation sequencing; HGVS, Human Genome Variation Society; dbSNP, Database for Short Genetic Variations; NR, not reported.

[a]Allele frequencies (Allele freq.) were taken from Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org).

dose reductions or interruptions. Repeat CT scans approximately every 3 mo have shown excellent response in most disease sites and stable disease in the rest. Figure 2 shows the dramatic response in one of the main renal lesions alongside shrinkage noted in the associated para-aortic adenopathy 3- and 6-mo into therapy with everolimus.

DISCUSSION

Renal involvement in TSC is very common and is seen in >80% of patients and this includes angiomyolipomas, cysts, and RCC (Fig. 1; Kwiatkowski et al. 2011). Complications from renal disease are in fact the leading cause of death in TSC (Shepherd et al. 1991). The incidence of RCC in TSC is not very well documented in the literature but is estimated to be 2%–4% in some reports (Lipworth et al. 2006). TSC-associated RCC are thought to be a distinct pathological entity. To further classify the pathology of RCC in TSC, Yang et al. (2014) studied 46 renal tumors in a series of 19 patients and classified them into three groups based on histopathology. The most common subtype had a distinct prominent papillary architecture similar to findings in our patient.

RCC in the general population is usually diagnosed in the sixth to eighth decade of life with a median age at diagnosis of 64 yr (Siegel et al. 2015). Bilateral RCC and RCC diagnosed at a younger age should always raise the suspicion for hereditary kidney cancer syndromes. A careful personal and family history is of utmost importance. Although our patient had a strong family history of TSC, it is important to note that 80% of TSC cases are caused by de novo mutations (mostly in the *TSC2* gene) (Au et al. 2007).

Robust evidence exists to suggest abnormal activation of the mTOR pathway and its downstream mediators in benign and malignant tumors in TSC (El-Hashemite et al. 2003; Kwiatkowski and Manning 2014). This has been successfully exploited in the EXIST-1 trial where 117 TSC patients with SEGAs were randomized to everolimus or placebo (Franz et al. 2013). Additionally, mTOR inhibitors are FDA-approved drugs used in the treatment of advanced RCC in the first- and second-line settings (Hudes et al. 2007; Motzer et al. 2010). Very few reports in the literature have explored the efficacy of mTOR inhibitors in TSC-associated RCC (Pressey et al. 2010; Kim et al. 2014). Although mTOR inhibitors remain FDA-approved for metastatic RCC in the first- and second-line settings, recent advances in the treatment of RCC have led to a justified decreased interest in the use of mTOR inhibitors as they showed less activity compared to more recently discovered anti-angiogenic agents

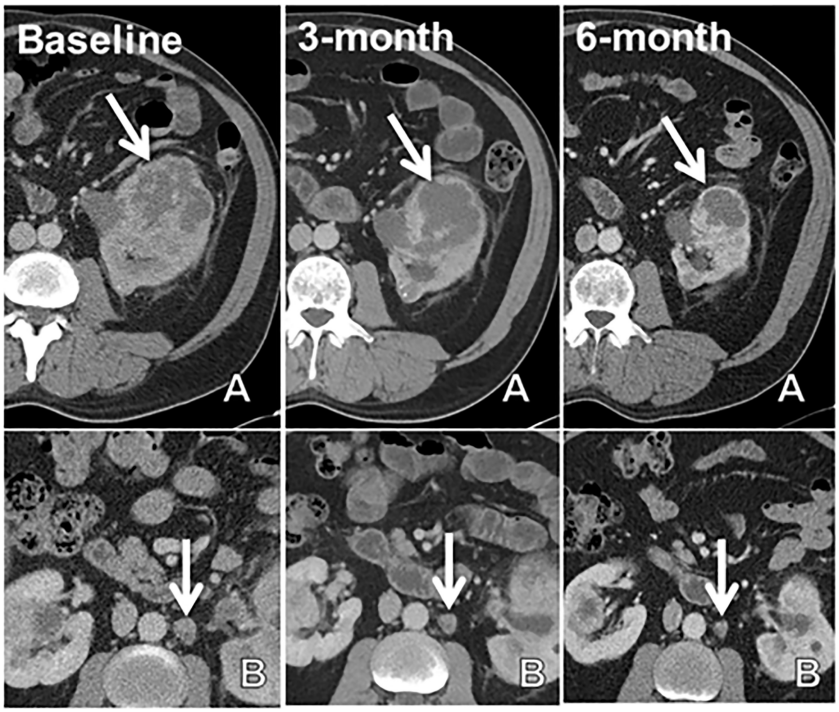

Figure 2. Exceptional response to everolimus seen in a patient with tuberous sclerosis (TSC)-associated RCC. (*A*) The shrinkage seen in the dominant left renal mass; (*B*) the reduction in size noted in the para-aortic adenopathy at 3- and 6-mo after starting therapy when compared with baseline. Response is sustained at 2-yr follow-up.

and anti PD-1 immunotherapy (Motzer et al. 2014; Choueiri et al. 2015; Motzer et al. 2015). We found two reports in the literature were found on the use of mTOR inhibitors in TSC-associated RCC. The first report was of a 7-yr-old child with TSC bilateral RCC and fibromatosis of the chest (Pressey et al. 2010). To spare a young patient bilateral nephrectomies, he was treated with the mTOR inhibitor sirolimus, and within 6 mo, the fibromatosis and RCC responded dramatically. The second interesting paper by Kim et al. (2014) is of a 49-yr-old woman with TSC and metastatic RCC who failed the TKI sunitinib and was subsequently started on everolimus. The patient had a meaningful clinical benefit and continued response to therapy (at least over a year) at the time of publication of the case report. More interestingly, the patient's other TSC manifestations (fibroadenomas, angiomyolipomas, cortical tubers, and SEGAs) were also ameliorated by everolimus, which further underlines the predominant role that mTOR signaling plays in the pathogenesis of TSC (Kim et al. 2014).

Our patient has remained progression-free for 2 yr into therapy so far. This is several-fold longer than the established durations of responses seen with mTOR inhibitors in RCC (Hudes et al. 2007; Motzer et al. 2014). Less activity and shorter progression-free survival (PFS) have been reported with mTOR inhibitors in the second-line setting (Choueiri et al. 2015; Motzer et al. 2015).

In summary, TSC is a genetic disorder that is associated with frequent tumor formation in various organs. Multiple studies, including randomized controlled trials, have shed light on the mTOR pathway as a major player in TSC-associated tumors and showed that mTOR inhibition represents a vital strategy in counteracting these tumors. Although mTOR inhibitors are not routinely used nowadays in the first-line treatment of metastatic RCC, we believe they are highly active in TSC-associated RCC as uninhibited mTOR signaling appears to be the

driving phenomenon. In this report, we showed a case of metastatic RCC in a TSC patient who had an exceptional and durable response to everolimus. Based on these findings, everolimus should be considered for treatment of RCC and other tumors associated with TSC. Further studies of somatic mutations in extended responders to mTOR inhibitors will help personalize therapy for these patients (Voss et al. 2014).

METHODS

Genetic Testing

Tumor-based genetic testing was obtained through the Foundation Medicine's commercial platform of FoundationONE assay. As per the company's description of the genomic profiling, the FoundationONE is designed to test at the time of patient's testing 315 genes as well as introns of 28 genes involved in rearrangements. The details regarding the coverage, sensitivity, and specificity of the assay are publicly available and well published (https://www.foundationmedicine.com/genomic-testing/foundation-one). The test recently also was approved by the Food and Drug Administration (FDA) as well for certain solid tumors and indications.

ADDITIONAL INFORMATION

Data Deposition and Access

The variants were submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers SCV000693713 and SCV000693714. Sequence data were unable to be submitted to publicly available databases because these data were collected by the clinical reference testing institution without patient consent to do so.

Ethics Statement

The patient provided informed consent for us to publish the case report. The patient's tumor-based genetic testing was done as part of his clinical care and not research. The publication of the findings noted as a case report does not require an IRB approval.

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

P.M.K. identified and has been taking care of this patient from a management standpoint. S.A. compiled the data into an initial draft. P.M.K. extensively revised the manuscript and suggested further edits. S.A. completed further simultaneous edits. P.M.K. finished the final draft. Both authors approved the final draft for submission to the journal.

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