Published OnlineFirst October 25, 2019; DOI: 10.1158/1535-7163.MCT-19-0642

Mutations and Response to Rapalogs in Patients with Metastatic Renal Cell Carcinoma

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ABSTRACT

We previously showed that alterations in mTOR pathway genes were correlated with response to rapalog therapy in metastatic renal cell carcinoma (mRCC), when the analysis focused on extremes of response. Herein, we expand on the prior cohort and examine genetic correlations with rapalog response in a dataset not selected for extremes of response. Tumors from 58 patients from the phase III trial of temsirolimus and 51 local patients with mRCC treated with rapalogs were studied. Somatic mutations were investigated using a targeted sequencing platform covering 27 genes. Clinical benefit (CB) was defined as patients with complete remission, partial response, or stable disease lasting at least 22 weeks. Mutational analyses focused on 5 mTOR pathway genes (*TSC1, TSC2, MTOR, PTEN, PIK3CA*) and 6 genes commonly mutated in RCC (*BAP1, KDM5C, PBRM1 SETD2, TP53*, and *VHL*). Among the 109 patients, 93 (85%) patients had clear cell histology, and 31 (28%) showed CB. Nine of 30 (30%) patients harboring mTOR pathway mutations in their tumor achieved CB versus 22 of 79 (28%) in the wild-type group. There was no distinct association between any individual or combination of mTOR pathway gene mutations and CB. Three of 7 patients with *TSC1* mutations showed CB. In addition, none of the 6 genes commonly mutated in RCC showed a mutation pattern that correlated with CB. Overall, in this large and diverse population of patients with mRCC, there is no suggestion of a correlation between response to rapalog therapy and mutation status for mTOR pathway genes.

Introduction

Over a decade ago, tyrosine kinase inhibitors and mTOR inhibitors transformed the therapeutic landscape of metastatic renal cell carcinoma (mRCC; ref. 1). mTOR inhibitors gained interest in RCC due to their relatively simple dosing schemes, and emerging data on the role of the mTOR protein complex in cell growth. mTOR is a conserved serine/threonine kinase required for cell growth, metabolism, survival, and angiogenesis (2). mTOR is found in two multi-subunit protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), that have nonoverlapping downstream targets (3). mTORC1 consists of the proteins mTOR, raptor, and mLST8 (4). TSC1 and TSC2, with TBC1D7, form the TSC protein complex, which functions as an upstream regulator of mTORC1, acting as a GTPase-activating protein (GAP) for RHEB (5, 6). In turn, the GAP activity of the TSC protein complex is regulated by many cellular sensors that phosphorylate regions of TSC1 and TSC2 (7).

In tumor cells, mTORC1 activation can occur through multiple mechanisms. Activating mutations in either *MTOR*, or inactivating mutations or deletions of *TSC1* or *TSC2*, lead to high-level activation of mTORC1. Furthermore, activating mutations in the catalytic subunit of PI3K (encoded by *PIK3CA*), and/or mutation or loss of *PTEN*, also lead to downstream mTOR pathway hyperactivity (8, 9). Upregulated mTORC1 causes multiple metabolic and anabolic perturbations that contribute to cell growth, including synthesis of nucleotides, lipids, amino acids, and ribosome biogenesis (7, 10, 11).

In RCC, alterations in *MTOR, TSC1*, or *TSC2* have been reported in 10% of clear cell tumors, 5% of papillary, and 9% of chromophobe RCC subtypes (12–14). Large, randomized clinical trials on mTOR inhibitors in the mRCC setting led to the FDA approval of temsirolimus and everolimus (15, 16). Temsirolimus is metabolized *in vivo* into rapamycin. Both everolimus and rapamycin bind to the cyclophilin FK506-binding protein (FKBP-12), and the complex of rapalog-FKBP-12 then interacts with an allosteric site on mTORC1 causing mTOR to dissociate from the mTORC1 subunit raptor and thus blocking mTORC1's kinase activity toward its downstream effectors (2, 17).

Previous studies have highlighted the importance of genomic alterations in mTOR pathway genes as potential determinants of response to mTOR inhibitors in various cancers (7, 18, 19), including our own previous analysis of mRCC patients selected for extremes of response (7). However, other studies have suggested that such mutations have no important association with response to rapalogs in mRCC (20). Furthermore, it has been suggested that *PBRM1* mutations in mRCC are associated with response to and improved survival from rapalogs (21). Here we sought to clarify the association between mTOR pathway mutations and response to rapalog therapy in mRCC. In addition, a limitation of our previous study was that the focus on extremes of response did not permit determination of the predictive power of mTOR pathway mutation for rapalog response in an unselected cohort of patients with RCC. To address this limitation,

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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Mol Cancer Ther 2020;19:690–6

doi: 10.1158/1535-7163.MCT-19-0642

Published OnlineFirst October 25, 2019; DOI: 10.1158/1535-7163.MCT-19-0642

here we assembled a cohort to include all patients treated with rapalog on the Global ARCC trial and at our institution, for whom mutation analysis was possible, enabling a population-wide analysis.

Materials and Methods

Study design and patients

We identified two cohorts, a primary cohort of 58 patients with mRCC treated on the Global ARCC trial of temsirolimus versus interferon (IFNα) versus both drugs (NCT00065468; ref. 15) and a second cohort of 54 patients with mRCC treated with everolimus or temsirolimus at the Dana-Farber Cancer Institute (DFCI, Boston, MA). We included all subjects from the temsirolimus arm of the Global ARCC trial, for whom formalin-fixed paraffin-embedded (FFPE) tissue was available and sufficient DNA was obtained for sequencing analysis. Of note, the 58 patients included from the temsirolimus arm of the Global ARCC trial represented 28% (58/209) of entire cohort accrued. Among the 58 patients, 28 had been reported in previous work by our group (7).

The DFCI cohort included all patients with RCC ($n = 54$) treated with temsirolimus or everolimus at our institution during the period indicated, for whom FFPE tissue was available, and sufficient DNA was available for sequencing analysis. Three patients were excluded from further analysis due to sequencing failure. Of the 51 patients, 27 were part of the prior study we had reported (7). The additional cohort ($n = 24$) consisted of patients with intermediate responses including patients with stable disease lasting less than 22 weeks. Clinical data from the Global ARCC cohort was obtained from the trial through a data transfer agreement and included assessment of response using Response Evaluation Criteria in Solid Tumors version 1.0. (RECIST v1.0). Patients treated at DFCI were assessed by the same criteria by a board-certified radiologist (K.M. Krajewski). Consistent with prior reports, patients that derived clinical benefit (CB) were defined as those that achieved complete remission (CR), partial response (PR), or stable disease (SD) lasting at least 22 weeks (15, 22). Patients with no clinical benefit (NCB) were those with progressive disease (PD) by 22 weeks. Patients who were taken off drug after less than 6 months due to toxicity were excluded from this analysis, as they were judged unable to assess response. Institutional Review Board approval was obtained at DFCI prior to tissue processing and collection of clinical data and was conducted in accordance with the Declaration of Helsinki. Approved written informed consent was obtained from all patients. Clinical variables that were assessed included: gender, age at diagnosis of metastatic disease, histology, prognostic risk score (MSKCC), and treatment regimen.

Tissue collection and DNA extraction

Core biopsy and/or surgical resection specimens were reviewed by a genitourinary pathologist (S. Signoretti) to confirm the diagnosis, histologic subtype, tumor grade, and stage. Tumor regions consisting of at least 50% tumor cells were selected for DNA extraction. DNA was then isolated using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's instructions. DNA quantification was performed by both NanoDrop and PicoGreen assays.

Tumor-targeted gene sequencing

Massively parallel sequencing (MPS) was performed by standard methods (7). For 95 patients, a custom bait-set was used covering the exonic regions of 27 genes, including core mTOR pathway genes or genes mutated at a significant rate in any of the three major types of RCC based on TCGA analyses (12–14). A total of 17 to 200 ng of tumor DNA per patient was subjected to targeted exon capture and sequencing at the DFCI Center for Cancer Genome Discovery. Sequencing libraries were prepared using the KAPA Hyper kit (Kapa Biosystems item KK8504) and sequenced on a HiSeq 2500 sequencer (Illumina). Sequencing data were then demultiplexed and converted to FASTQ files using Picard tools, aligned against the human reference genome GRCh37/hg19 using Burrows-Wheeler Aligner (23), and filtered to remove low quality and duplicate reads. Samples were then analyzed for indels and single-nucleotide variants (SNV) using the Genome Analysis Toolkit (GATK; ref. 24). A custom analysis pipeline was employed to detect SNVs and small indels. All read calls at each nucleotide position of interest were captured using SAMtools v1.2 Pileup (25). Base call frequencies at each position were then determined for the 27 genes. Candidate SNVs were identified using the following criteria: observed in at least two reads, including at least 1 read in each orientation; and allele frequency (AF) of $\geq 4\%$. Candidate insertion and deletion (indel) variants were identified as having an AF $\geq 4\%$ and observed in at least two reads. All candidate variants were reviewed using Integrative Genomics Viewer (26) to exclude sequencing and alignment artifacts. Germline DNA analyses were not performed as part of this study. Hence, SNVs and small indels observed at any frequency in the ExAC Exome Aggregation Consortium browser (27) were excluded as they were thought likely to be germline or artifacts. Intronic variants within 15 base pairs of exon boundaries were retained, while others were excluded.

For the remaining 14 patients, targeted gene sequencing was performed using an institutional analytic platform, OncoPanel, which is certified for clinical use and patient reporting under the Clinical Laboratory Improvement Amendments (CLIA) act. Genomic DNA from each tumor sample was subjected to targeted exon capture and sequencing using one of three versions of the OncoPanel assay (V1–V3) in the Department of Pathology at Brigham and Women's Hospital (BWH, Boston, MA). The OncoPanel gene panel includes capture probes for 275–447 cancer-associated genes, as well as intronic portions of 60 genes for rearrangement detection (28). Targeted capture was performed using a solution-phase Agilent SureSelect hybrid capture kit and custom bait-sets. Sequencing libraries were prepared from captured DNA as described in detail elsewhere (28). Paired-end sequencing was performed on an Illumina HiSeq 2500. Reads were demultiplexed using Picard tools (http://picard.sourceforge.net) and aligned to the human reference genome GRCh37/hg19 using the Burrows-Wheeler Aligner (ref. 23; http://bio-bwa.sourceforge.net/bwa.shtml). Low quality reads and duplicates were filtered and eliminated using Picard. We focused our mutational analysis on the 27 genes that were common with our custom bait-set (Supplementary Table S1.2). SNVs and indels were analyzed using MuTect v.1 0.27200 (https://gdac.broadinstitute.org/ display/CGATools/MuTect; accessed May 2013) and annotated using Oncotator (https://software.broadinstitute.org/cancer/cga/oncotator; accessed May 2013).

Variant assessment

The functional effect of all variants identified, both SNVs and indel variants, was considered. For tumor suppressor genes, all loss-of-function variants were considered deleterious, including nonsense mutations, frameshift indels, and splice site alterations affecting consensus nucleotides. Special consideration was given to missense variant assessment. Missense variants in *TSC1/TSC2* were reviewed in the tuberous sclerosis database (i.e., Leiden Open Variation Database, LOVD) to assess their pathogenicity and association with clinical diagnosis of tuberous sclerosis. Missense variants in *MTOR* were

Published OnlineFirst October 25, 2019; DOI: 10.1158/1535-7163.MCT-19-0642

Table 1. Clinicopathologic characteristics of 109 patients with metastatic RCC.

	Global ARCC Cohort			DFCI Cohort			Overall (combined cohort)		
	No CB	CB		No CB	CB		No CB	CB	
Characteristics	$n = 44$ (%)	$n = 14$ (%)	Total ($n = 58$) (%)	$n = 34$ (%)	$n = 17$ (%)	Total ($n = 51$) (%)	$n = 78$ (%)	$n = 31$ (%)	Total ($n = 109$) (%)
Gender									
Female	16 (36)	4 (29)	20 (34)	7 (21)	3 (18)	10 (20)	23 (29)	7 (23)	30 (28)
Male	28 (64)	10 (71)	38 (66)	27 (79)	14 (82)	41 (80)	55 (71)	24 (77)	79 (72)
Age at diagnosis of metastatic disease (years)									
Median	58	57	58	61	60	60	59	58	58
(Min, Max)	(35, 78)	(42, 79)	(35, 79)	(44, 80)	(42, 77)	(42, 80)	(35, 80)	(42, 79)	(35, 80)
Histology									
Clear cell RCC	39 (89)	14 (100)	53 (91)	28 (82)	12 (71)	40 (78)	67 (86)	26 (84)	93 (85)
Non–clear cell RCC	3 (7)	0 (0)	3 (5)	6 (18)	5 (29)	11 (22)	9 (12)	5 (16)	14 (13)
Unclassified RCC	2 (5)	0 (0)	2 (3)	0 (0)	0 (0)	0 (0)	2 (3)	0 (0)	2 (2)
Prognostic risk score (MSKCC)									
Good	0 (0)	0 (0)	0 (0)	5 (15)	2 (12)	7 (14)	5 (6)	2 (6)	7 (6)
Intermediate	15 (34)	6 (43)	21 (36)	18 (53)	13 (76)	31 (62)	33 (42)	19 (61)	52 (48)
Poor	29 (66)	8 (57)	37 (64)	6 (18)	1 (6)	7 (14)	35 (45)	9 (29)	44 (40)
Unknown	0 (0)	0 (0)	0 (0)	5 (15)	1 (6)	6 (12)	5 (6)	1 (3)	6 (6)
Treatment									
Everolimus	0 (0)	0 (0)	0 (0)	25 (74)	13 (76)	38 (75)	25 (32)	13 (42)	38 (35)
Temsirolimus	44 (100)	14 (100)	58 (100)	9 (26)	2 (12)	11 (22)	53 (68)	16 (52)	69 (63)
Everolimus/ Temsirolimus	0 (0)	0 (0)	0 (0)	0 (0)	2 (12)	2 (4)	0 (0)	2 (6)	2 (2)
Response status at 22 weeks									
PD	44 (100)	0 (0)	44 (76)	34 (100)	0 (0)	34 (67)	78 (100)	0 (0)	78 (72)
SD	0 (0)	9 (64)	9 (16)	0 (0)	12 (71)	12 (24)	0 (0)	21 (68)	21 (19)
PR	0 (0)	5 (36)	5 (9)	0 (0)	5 (29)	5 (10)	0 (0)	10 (32)	10 (9)

Abbreviations: ARCC, Advanced Renal-Cell Carcinoma; MSKCC, Memorial Sloan Kettering Cancer Center.

compared with previously reported variants known to cause mTORC1 activation (29). For the remaining 24 genes analyzed, the functional impact of missense variants was determined by *in silico* functional analysis using SIFT (30) and Polyphen-2 (31). Missense mutations classified as "damaging" in SIFT and/or "probably damaging" in Polyphen-2 were considered deleterious for the initial analysis. In addition, we also employed a more stringent mutation filter for the tumor suppressor genes *PTEN, TSC1, TSC2*. For this second filter, we retained missense variants only if they were reported in the TCGA version of cBioPortal (32, 33) and were thought to be likely oncogenic.

Validation of tumor variant calls

Candidate SNVs and indels in the mTOR pathway with probable pathogenicity were validated by Sanger sequencing for variants with an AF $\geq$10%, and amplicon MPS (aMPS) for variants with < 10% AF, as described previously (7). Amplicons of 91–110 nucleotides around a variant of interest were designed and amplified on the tumor DNA from a patient and three control DNA samples. Amplicons were then pooled at equal molarity in separate pools for each identical amplicon, A-tailed, ligated to Illumina Y-branched adaptors, and subjected to limited amplification followed by a second limited amplification using pool-specific indexed primers. Libraries were then sequenced on a MiSeq v2 (Illumina). Reads were demultiplexed and aligned to the human genome as above Bam files were generated and converted to . sam files using SamTools 1.2, and the prevalence of wild-type and variant sequences were determined using custom python code. A read depth of 10,000 to 1,000,000 was achieved for each candidate variant. Candidate tumor-specific variants seen in this analysis at an AF less than two times that of the two control samples were considered not confirmed, while those with AF $\geq$ 2x that of the two control samples were considered confirmed.

Statistical analysis

All statistical analyses were performed using R 3.3.1 (https://www.r-project.org). Statistical tests included the χ^2 and Fisher exact tests for categorical variables. The primary objective was to examine the association between mutations in several genes from the mTOR pathway with CB to rapalog therapy. In an exploratory analysis, we also assessed the association of mutations in the commonly mutated RCC genes (*BAP1, KDM5C, PBRM1, SETD2, TP53*, and *VHL*) with CB.

Secondary objectives assessed the association between progression-free survival (PFS) and overall survival (OS) and the four following mutation categories: (i) mutation in *TSC1* or *TSC2*; (ii) mutation in *MTOR, TSC1*, or *TSC2*; (iii) mutation in *MTOR, TSC1, TSC2*, or *PTEN*; (iv) mutation in *MTOR, TSC1, TSC2, PTEN*, or *PIK3CA*. PFS was calculated from the start date of rapalog therapy to the date of progression, death, or last follow-up. Patients alive and progression-free at 35 months were censored. OS was calculated from the start date of rapalog therapy to the date of death or last follow-up. Patients alive at 35 months were censored. The follow-up was truncated at 35 months to ensure a consistent and comparable follow-up time for the two cohorts due to the significant difference in follow-up between the ARCC trial (28 months) and DFCI cohorts (70 months). Kaplan–Meier estimates of PFS and OS were calculated. Cox proportional hazards models were used to estimate HRs with 95% confidence intervals (CI) and log-rank test statistics for comparing clinical outcomes between the four mutational categories.

Published OnlineFirst October 25, 2019; DOI: 10.1158/1535-7163.MCT-19-0642

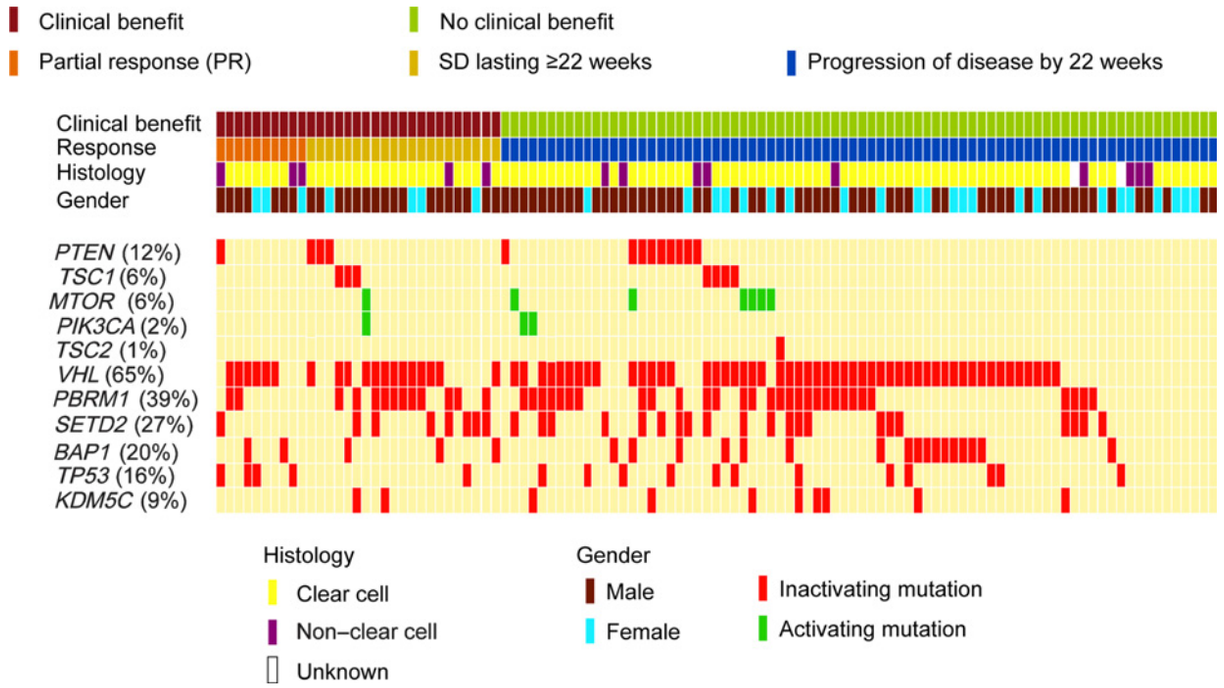

Figure 1.

Comutation plot of genes with ≥5% mutation frequency in a cohort of 109 patients with RCC. Mutation types and clinical features are indicated. Clinical benefit (CB) included patients that achieved partial response (PR) or stable disease (SD) lasting at least 22 weeks. No clinical benefit (NCB) included patients with progressive disease (PD) by 22 weeks.

Results

Patient and treatment characteristics

A total of 109 patients with mRCC treated with rapalog therapy were included in this study. The majority of tissue specimens were primary nephrectomies (103 of 109, 94%) while the remaining 6 were metastatic biopsies. Fifty-eight patients had been treated with temsirolimus as part of a randomized trial (July 2003 to April 2005; ref. 15), while 51 had been treated with rapalogs at DFCI between October 2007 and December 2017. The median age at diagnosis of metastatic disease was 58 years (range, 35–80 years). The majority of the patients were male (72%). Sixty-nine (63%) were treated with temsirolimus and 38 (35%) received everolimus (**Table 1**; Supplementary Table S1.1). The majority of samples had clear cell renal cell carcinoma histology (93/109, 85%). Thirty (28%) patients had CB in response to rapalog therapy, while 79 (72%) did not, as assessed by RECIST criteria (**Table 1**). Patient characteristics and clinical outcomes for the 58 patients from the Global ARCC trial were similar to the entire set of those enrolled in that trial (Global ARCC trial subset: PR = 5/58, 9%; Global ARCC trial overall cohort: PR = 18/209, 8.6%; ref. 15).

Mutation findings and CB

Five mTOR pathway genes and 6 commonly mutated RCC genes were analyzed for mutations in tumor samples from all subjects (Supplementary Tables S1.2 and S1.3). Mutation cooccurrence and frequency is displayed in **Fig. 1**.

Considering the 11 genes individually, *TSC1* mutation was associated with the highest rate of response to rapalog therapy (3/7, 43%), but this was not statistically significant. All other genes showed a similar response rate in those with mutations compared with those without mutations (**Table 2A**; Supplementary Table S1.4; all *P* values were not significant).

We then considered associations between grouped mTOR pathway gene mutations and clinical benefit using both an initial and a more stringent set of criteria to define functional significance for missense mutations (see Methods). Four mutation categories were considered: (i) mutation in *TSC1* or *TSC2*; (ii) mutation in *MTOR, TSC1*, or *TSC2*; (iii) mutation in *MTOR, TSC1, TSC2*, or *PTEN*; (iv) mutation in *MTOR, TSC1, TSC2, PTEN*, or *PIK3CA*. There was no significant association with clinical benefit among any of the 4 mutation categories using both the initial and the more stringent criteria to assess mutations (**Table 2B**; Supplementary Table S1.5).

mTOR pathway mutations and survival

Despite the lack of association between response and mutation status, we considered the possibility that mutation status for one or more of these genes might show association with duration of response and overall survival. Because these patient populations were very different with respect to prior treatment history, we analyzed PFS and OS in the two cohorts separately (**Fig. 2**). Subjects with a mutation in *MTOR, TSC1, TSC2*, or *PTEN* experienced significantly shorter OS than those without such mutations in the Global ARCC cohort but not in the DFCI cohort [Global ARCC, HR = 2.08 (1.11–3.91), *P* = 0.02; DFCI, HR = 1.32 (0.59–2.96), *P* = 0.5; **Fig. 2A** and **B**, respectively; Supplementary Table S1.6]. The other three mutation categories were not

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Table 2A. Mutation rates for mTOR pathway and common RCC genes and associations with clinical benefit.

Combined analysis[a] of DFCI and Global ARCC cohorts	Total ($N = 109$) *N* (%)	No CB ($n = 78$) *N* (%)	CB ($n = 31$) *N* (%)
mTOR	7 (6)	5 (6)	2 (6)
PIK3CA	3 (3)	2 (3)	1 (3)
PTEN	13 (12)	9 (12)	4 (13)
TSC1	7 (6)	4 (5)	3 (10)
TSC2	1 (1)	1 (1)	0 (0)
ATM	4 (4)	4 (5)	0 (0)
BAP1	22 (20)	17 (22)	5 (16)
KDM5C	10 (9)	8 (10)	2 (6)
PBRM1	43 (39)	30 (38)	13 (42)
SETD2	29 (27)	21 (27)	8 (26)
TP53	17 (16)	12 (15)	5 (16)
VHL	71 (65)	52 (67)	19 (61)

[a]Analysis of both Global ARCC and DFCI cohorts.

significantly associated with OS in either cohort. In contrast, subjects with mutations in any of *MTOR, TSC1*, and *TSC2* experienced shorter PFS in the DFCI cohort but not the Global ARCC cohort [DFCI, HR = 2.63 (1–6.94), $P = 0.04$; Global ARCC, HR = 1 (0.48–2.05), $P = 0.99$; **Fig. 2C** and **D**, respectively; Supplementary Table S1.7]. None of the other mutation categories correlated with PFS.

Discussion

Multiple studies have reported conflicting data regarding the association between tumor mutations and response to rapalog therapy in mRCC. The earliest reported analysis identified *TSC1* and *MTOR* mutations in 3 of 5 patients with RCC that were exceptional responders to mTOR inhibitors (34). In our previous study (7), we confirmed that mutations in *MTOR, TSC1*, and *TSC2* were associated with response to rapalog therapy in a cohort of 79 RCC selected to be either extreme responders or nonresponders. Later, a subsequent report by the same investigators as those reporting the first study failed to validate this association in a large set of 220 patients with clear cell RCC. In contrast, they noted that that *PBRM1* and *BAP1* mutations correlated with longer and shorter PFS, respectively, in patients treated with first-line everolimus (21). In a more recent report, this same group of investigators reported that loss of PTEN expression by IHC was strongly associated with improved PFS in patients treated with everolimus compared with those with intact PTEN IHC staining (Intact PTEN IHC staining, median PFS of 5.3 months; loss of PTEN IHC staining, median PFS of 10.5 months; ref. 20). Interestingly, PTEN loss by IHC did not correlate with the presence of mutations in the PI3K pathway, and was much higher than the mutation frequency in *PTEN* in RCC in most past series (12–14, 35, 36), similar to what was seen here. In addition, although epigenetic silencing of *PTEN* had been reported in cancers in the past (37), the promoter of *PTEN* is mostly unmethylated in kidney cancer (38). Moreover, epigenetic silencing assessed by CpG methylation was a rare phenomenon in the comprehensive TCGA analysis of RCC (39). Nonetheless, it is possible that suppression of PTEN expression is occurring by other means, and further investigation is required.

In our previous study, we had chosen subjects to be outliers of response through an international recruitment effort; "intermediate" responders were excluded. In this study, we expanded the prior study to include all subjects for whom pathologic material was available to us irrespective of response status. A large group of patients included here were those treated with rapalog in whom PD was detected by 22 weeks on drug, but not before 3 months, who had not been included in the earlier analysis. With this larger and less selective patient cohort, we find that mTOR pathway mutations are not associated with improved clinical outcomes to rapalog therapy in RCC. This work thus illustrates the importance of follow-up studies that characterize clinical status irrespective of the type of response, accounting for responses that are of "intermediate" quality.

There are significant limitations to this study. As only tumor samples were analyzed, we could not distinguish somatic from germline variants with certainty. Second, in this analysis we did not consider copy number variation, as we felt that it was not reliably determined in this sample set. Copy number variation is known to be important for many genes in RCC located on chromosome 3p. However, none of the mTOR pathway genes considered here is located on chromosome 3p, or commonly subject to copy number variation in RCC. Third, RNA-seq data was not available. Fourth, intratumor heterogeneity likely confounded our ability to detect important mutational drivers in some cases. However, our analysis of a single tumor specimen reflects routine clinical practice in that multiple biopsies from different sites are rarely available. Fifth, we used both SIFT/Polyphen-2 to assess the

Table 2B. Associations of mutation category status with response status.

mTOR Mutation category	Mutational status	Mutations, *n* (%)	No CB ($n = 78$), *N* (%)	CB ($n = 31$), *N* (%)	OR (95% CI)	Fisher exact, *P*
TSC1, TSC2	No (ref)	101 (93)	73 (94)	28 (90)	1.56 (0.35–6.98)	0.686
	Yes	8 (7)	5 (6)	3 (10)		
mTOR, TSC1, TSC2	No (ref)	94 (86)	68 (87)	26 (84)	1.31 (0.41–4.19)	0.759
	Yes	15 (14)	10 (13)	5 (16)		
mTOR, TSC1, TSC2, PTEN	No (ref)	81 (74)	59 (76)	22 (71)	1.27 (0.5–3.23)	0.633
	Yes	28 (26)	19 (24)	9 (29)		
mTOR, TSC1, TSC2, PTEN, PIK3CA	No (ref)	79 (72)	57 (73)	22 (71)	1.11 (0.44–2.79)	0.816
	Yes	30 (28)	21 (27)	9 (29)		

Abbreviation: CI, confidence interval.

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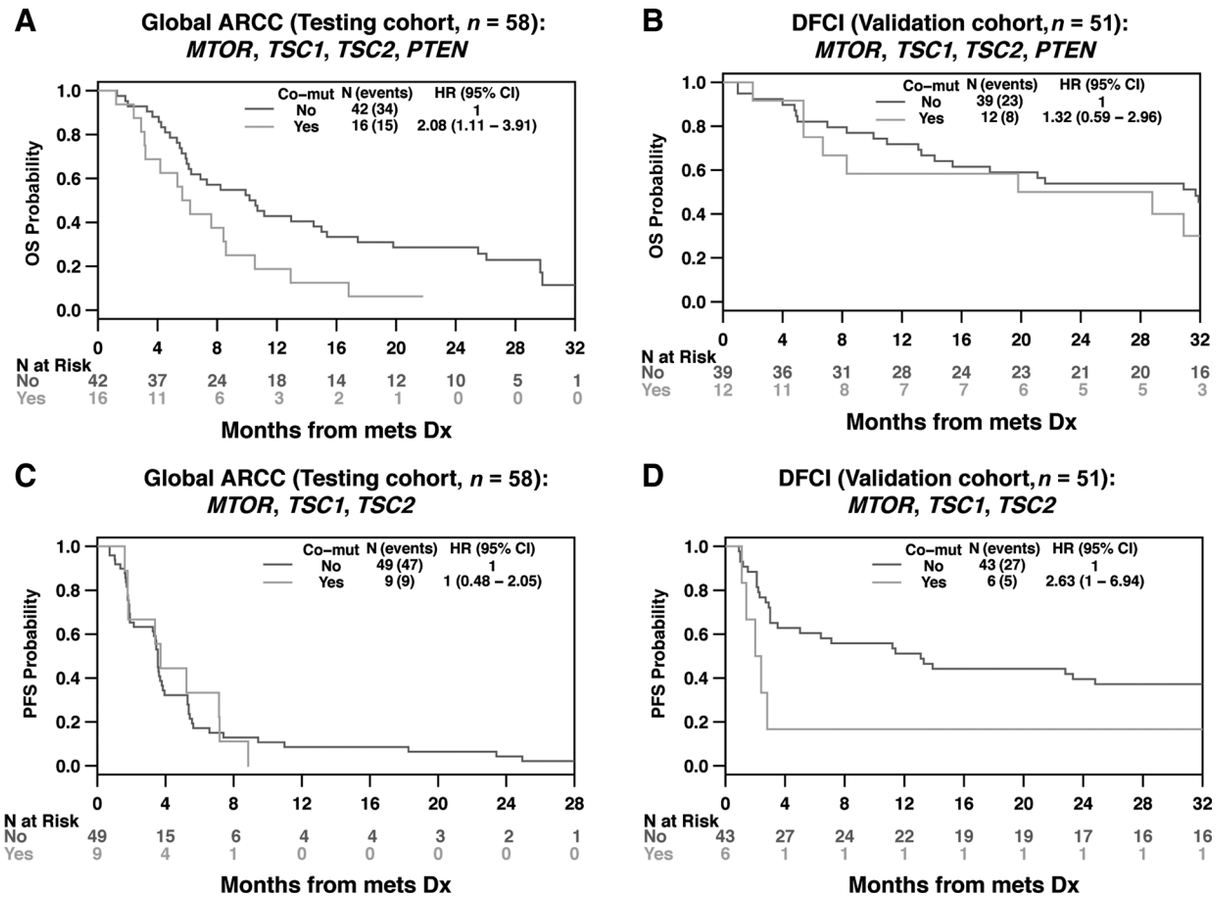

Figure 2.

Survival outcomes of mTOR pathway–mutated mRCC tumors in relation to rapalog therapy. **A,** Kaplan–Meier curve for overall survival in Global ARCC patients with mRCC with any mutation in *MTOR, TSC1, TSC2*, and *PTEN* compared with patients with mRCC whose tumors had no mutations in those genes. **B,** Kaplan–Meier curve for overall survival in DFCI patients with mRCC with any mutation in *MTOR*, *TSC1, TSC2*, and *PTEN* compared with patients with mRCC whose tumors had no mutations in those genes. **C,** Kaplan–Meier curve for progression-free survival in Global ARCC patients with mRCC with any mutation in *MTOR, TSC1*, and *TSC2* compared with patients with mRCC whose tumors had no mutations in those genes. **D,** Kaplan–Meier curve for progression-free survival in DFCI patients with mRCC with any mutation in *MTOR, TSC1*, and *TSC2* compared with patients with mRCC whose tumors had no mutations in those genes. Mets Dx, metastatic diagnosis.

pathogenicity of missense variants, and did a second filtration using TCGA cBioPortal data to help to determine the functional effects of missense variants in *PTEN, TSC1*, and *TSC2*. However, both of these methods may lead to erroneous inclusion of some "nonsignificant" mutations while excluding other significant ones. However, the majority of variants in *TSC1/TSC2/PTEN* were clearly inactivating (16/22, 73%; Supplementary Table S1.3), and the missense variants in *PIK3CA* and *MTOR* were selected to be known activating mutations. In addition, we validated all mTOR pathway variants in this study by a second round of sequencing.

Overall, we demonstrated that patients with RCC with mutations in mTOR pathway genes are not enriched for those responding to rapalog therapy. Nonetheless, it is possible that such variants do correlate to a limited degree with long-term durable responses to rapalogs.

Disclosure of Potential Conflicts of Interest

S. Signoretti is a consultant at AstraZeneca, Bristol-Myers Squibb, and Merck, is a senior editor, CCR at AACR, is a co-chair renal task force at NCI, reports receiving a commercial research grant from Bristol-Myers Squibb, AstraZeneca, Exelixis, and Novartis, and has ownership interest (including patents) in Biogenex. T.K. Choueiri reports receiving a commercial research grant from Bristol-Myers Squibb, Exelixis, and Merck, Pfizer, Novartis, and GSK, and is a consultant/advisory board member for Merck, BMS, Exelixis, Pfizer, Novartis, and GSK. D.J. Kwiatkowski reports receiving a commercial research grant from Novartis. No potential conflicts of interest were disclosed by the other authors.

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Published OnlineFirst October 25, 2019; DOI: 10.1158/1535-7163.MCT-19-0642

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Acknowledgments

This work was supported in part by Novartis Pharmaceuticals Corporation CRAD001LUS276T (to T.K. Choueiri), NIH NCI Kidney SPORE P50CA101942-12 (to T.K. Choueiri and D.J. Kwiatkowski), the Trust Family (to T.K. Choueiri), Michael Brigham Funds for Kidney Cancer Research at Dana-Farber Cancer Institute (to T.K. Choueiri), and Loker Pinard Funds for Kidney Cancer Research at Dana-Farber Cancer Institute (to T.K. Choueiri). We thank all of the research assistants who assisted with this study.

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Received June 28, 2019; revised September 8, 2019; accepted October 21, 2019; published first October 25, 2019.

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Published OnlineFirst October 25, 2019; DOI: 10.1158/1535-7163.MCT-19-0642

Molecular Cancer Therapeutics

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Mol Cancer Ther 2020;19:690-696. Published OnlineFirst October 25, 2019.