

Research Report

Genomic Alterations and Outcomes with VEGF-Targeted Therapy in Patients with Clear Cell Renal Cell Carcinoma

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Abstract.

Background: Mutations in *VHL*, *PBRM1*, *SETD2*, *BAP1*, and *KDM5C* are common in clear cell renal cell carcinoma (ccRCC), and presence of certain mutations has been associated with outcomes in patients with non-metastatic disease. Limited information is available regarding the correlation between genomic alterations and outcomes in patients with metastatic disease, including response to VEGF-targeted therapy.

Objective: To explore correlations between mutational profiles and cancer-specific outcomes, including response to standard VEGF-targeted agents, in patients with metastatic ccRCC.

Methods: A retrospective review of 105 patients with metastatic ccRCC who had received systemic therapy and had targeted next-generation sequencing of tumors was conducted. Genomic alterations were correlated to outcomes, including overall survival and time to treatment failure to VEGF-targeted therapy.

Results: The most frequent mutations were detected in *VHL* (83%), *PBRM1* (51%), *SETD2* (35%), *BAP1* (24%), *KDM5C* (16%), and *TERT* (14%). Time to treatment failure with VEGF-targeted therapy differed significantly by *PBRM1* mutation status ($p=0.01$, median 12.0 months for MT versus 6.9 months for WT) and *BAP1* mutation status ($p=0.01$, median 6.4 months for MT versus 11.0 months for WT). Shorter overall survival was associated with *TERT* mutations ($p=0.03$, median 29.6 months for MT versus 52.6 months for WT) or *BAP1* mutations ($p=0.02$, median 28.7 months for MT versus not reached for WT).

Conclusions: Genomic alterations in ccRCC tumors have prognostic implications in patients with metastatic disease. *BAP1* and *TERT* promoter mutations may be present in higher frequency than previously thought, and based on this data, deserve further study for their association with poor prognosis.

Keywords: Clear cell renal cell carcinoma, VEGF-targeted therapy, genomics, prognosis

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KEY MESSAGE

The most frequent mutations in metastatic clear cell renal cell carcinoma (ccRCC) are similar to those in localized ccRCC. Certain genetic alterations, such as *BAP1*, *PBRM1* and *TERT* promoter mutations, may have prognostic implications in metastatic ccRCC and should be further investigated.

INTRODUCTION

Clear cell renal cell carcinoma (ccRCC) is the most common subtype of kidney cancer, estimated to account for about 80% of the nearly 62,700 cases diagnosed annually in the United States [1, 2]. The majority of patients are cured by surgical resection, but a considerable portion is either diagnosed with metastatic disease or develops recurrence after surgery. Once the disease is metastatic, it is usually incurable with limited overall survival (OS) [2].

Over the last decade, approved therapies for metastatic ccRCC have expanded considerably. The growing number of available agents includes vascular-endothelial growth factor (VEGF)-directed compounds, mammalian target of rapamycin (mTOR) inhibitors, and recently, checkpoint inhibitors [3–7]. Lack of predictive biomarkers, however, prevents rational sequencing strategies for these regimens. This stands in contrast to other malignancies such as non-small cell lung cancer or melanoma, where validated molecular markers predict response to approved targeted agents [8–10].

Large scale sequencing efforts have helped unveil the genomic landscape of ccRCC [11, 12]. In addition to confirming *VHL* alterations as linchpin events in the pathogenesis of ccRCC, they uncovered other recurrent genomic events, including alterations in the tumor suppressors *PBRM1*, *SETD2*, *BAP1* and *KDM5C*. These analyses, however, were mostly carried out in patients with early stage disease. Similarly, prior reports associating some of these genomic alterations with outcome were limited to non-metastatic ccRCC [13–15].

For patients with metastatic disease, key questions remain unanswered, such as the incidence of genomic alterations and the association of alterations with clinical outcomes, including response to targeted therapies. In this study, we retrospectively analyzed patients with metastatic ccRCC treated with systemic therapy that had undergone targeted exome sequencing of cancer-related genes. The aim was to explore

correlations between mutational profiles and cancer-specific outcomes, including response to standard VEGF-targeted agents.

METHODS

Patients and samples

Patients were identified from an institutional database of RCC patients who had previously undergone next-generation targeted exome sequencing of archival tumor tissue. Subjects with metastatic ccRCC who had received at least one line of systemic therapy at Memorial Sloan Kettering Cancer Center (MSKCC) were included in the analysis. Demographics, clinical characteristics and details on treatment effect were collected for each case via retrospective review of clinical records. This study was approved by the MSKCC Institutional Review Board, and all patients consented to a tissue procurement protocol.

Tissue acquisition and next-generation sequencing (NGS) analysis

Formalin-fixed, paraffin-embedded (FFPE) tissue specimens from either the primary or a metastatic site were reviewed by genitourinary pathologists to select areas of maximum tumor content for DNA extraction; blood samples were used for matched comparison of germline DNA. For NGS analysis, the MSKCC IMPACT™ (Integrated Mutation Profiling of Actionable Cancer Targets) assay was employed as previously described [16]. IMPACT is designed to detect SNVs, short indels, copy number aberrations and structural rearrangements. NGS was performed using Illumina HiSeq, with deep coverage across all exonic sequences, plus select introns deemed relevant for fusion events. Variants are annotated with Annovar, then further filtered using several criteria, including previous literature, and technical characteristics of the variant call such as depth of coverage. Initial samples (20%) had 341 genes investigated per an earlier version of the assay, while 410 genes were tested for all subsequent samples (full gene list in Supplemental Table 1, both versions include all commonly mutated genes in ccRCC). Five samples underwent whole genome sequencing.

Statistical analysis

The relationship between mutation status and overall survival (OS) and time to treatment failure (TTF)

from treatment initiation of first therapy was evaluated using time to event methods. For OS, patients still alive at the time of analysis were censored at the last clinic date on which survival status could be confirmed. For TTF, an event was considered to be the time of treatment discontinuation, for any reason (progression or toxicity). Patients still on therapy at the time of analysis were censored at that point in time. Kaplan-Meier estimates for both endpoints were summarized by calculating the median with 95% confidence intervals and for OS 2 year estimates. Comparisons by each mutation were tested using the Log-Rank statistic. Our analyses are hypothesis generating and as such, we do not adjust for multiple comparisons. All analyses were performed in SAS v 9.4 and R v 3.1.1.

RESULTS

Patient characteristics

A total of 105 patients were identified; patient characteristics are summarized in Table 1. Median age was 57 years (range 33–81). MSKCC Risk Group was available for 89 patients starting first-line therapy (35% favorable, 52% intermediate and 14% poor risk). Thirty-six (34%) patients had received 1 line of therapy at time of analysis, 49 (47%) had received 2-3 lines, and 20 (19%) had received 4 or more lines.

Mutation analysis

Mutation frequencies detected in our analysis of 105 tumors (66% primary tumors, 34% metastatic lesions) are outlined in Fig. 1. Mean depth of coverage across the cohort was 608x (range 147–1028). The majority of samples (90.5%) were collected prior to initiation of treatment with VEGF-targeted agents. The most frequent mutations were *VHL* (83%), *PBRM1* (51%), *SETD2* (35%), *BAP1* (24%), *KDM5C* (16%), and *TERT* (14%). *KDM5C* and *BAP1* mutations were mutually exclusive, while 15 of 17 tumors with *KDM5C* alterations also harbored mutations in *PBRM1*. Only one tumor had mutations in both *BAP1* and *TERT* (Fig. 1). All but one of the *TERT* mutations were in the promoter region. When ranking mutation frequencies separately from primary tumors vs. metastatic sites, the top 4 mutated genes (*VHL*, *PBRM1*, *SETD2*, *BAP1*) remained the same (Supplemental Table 2).

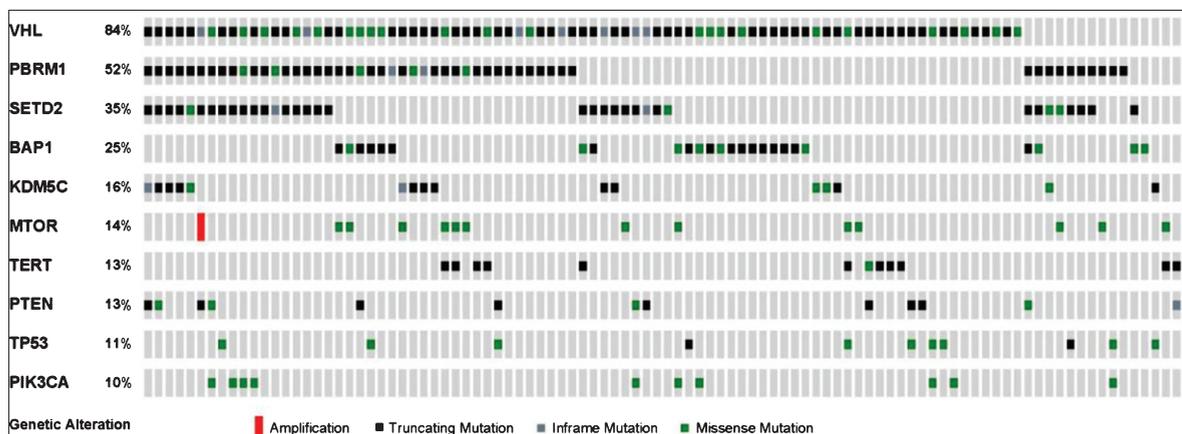
Table 1
Patient characteristics (N = 105)

| | |
|---|------------|
| Age at diagnosis (median, range) | 57 (33–81) |
| <i>Sex</i> | |
| F | 28 (26.7%) |
| M | 77 (73.3%) |
| <i>Nephrectomy</i> | |
| Yes | 95 (90.5%) |
| No | 10 (9.5%) |
| <i>Type of Nephrectomy</i> | |
| Localized Disease | 44 (46.3%) |
| Cytoreductive | 51 (53.7%) |
| <i>Sarcomatoid Features</i> | |
| Yes | 30 (28.6%) |
| No | 65 (61.9%) |
| NA | 10 (9.5%) |
| <i>Grade</i> | |
| G1 | 0 (0.0%) |
| G2 | 14 (13.3%) |
| G3 | 37 (35.2%) |
| G4 | 45 (42.9%) |
| NA | 9 (8.6%) |
| <i>Stage at Time of Diagnosis</i> | |
| I | 11 (10.5%) |
| II | 10 (9.5%) |
| III | 26 (24.8%) |
| IV | 57 (54.3%) |
| NA | 1 (1.0%) |
| <i>MSKCC Risk Group</i> | |
| Favorable | 31 (34.8%) |
| Intermediate | 46 (51.7%) |
| Poor | 12 (13.5%) |
| NA | 16 (18.0%) |
| <i>Received a VEGF-Targeted Agent</i> | |
| Yes | 95 (90.5%) |
| No | 10 (9.5%) |
| <i>Therapies Prior to VEGF-Targeted Agent</i> | |
| None | 94 (89.5%) |
| mTOR inhibitor | 6 (5.7%) |
| Interferon | 5 (4.8%) |
| <i>First VEGF-Targeted Agent</i> | |
| Sunitinib | 60 (57.2%) |
| Pazopanib | 31 (29.5%) |
| Bevacizumab | 1 (1.0%) |
| Axitinib | 0 (0.0%) |
| Sorafenib | 1 (1.0%) |
| Sunitinib/bevacizumab ^a | 2 (1.9%) |

^aThis regimen was given as part of a clinical trial [32]. *VEGF, vascular endothelial growth factor; NA, not applicable; MSKCC, Memorial Sloan Kettering Cancer Center.

Mutation status and OS

With a median follow-up of 26 months, median OS from the initiation of treatment for the entire cohort was 50 months (95% CI 36.3 – not reached). Correlation of mutation status in individual genes of interest with OS is summarized in Table 2. Patients with *TERT* mutant (MT) tumors had a significantly shorter OS compared to *TERT* wild-type (WT) tumors (median 29.6 vs. 52.6 months; $p=0.03$). Similar adverse



*5 patients with whole genome sequencing were excluded

Fig. 1. Frequency of genetic alterations.

Table 2
Overall survival (OS) by mutation status

| Mutation status | N | Median OS (months) | 95% CI | Log-rank p-value | 2 year OS | 95% CI |
|-----------------|----|--------------------|-----------|------------------|-----------|----------|
| <i>VHL</i> WT | 18 | 26.1 | (18.4-NA) | 0.13 | 62.0% | (37–100) |
| <i>VHL</i> MT | 87 | 49.9 | (37.3-NA) | | 77.0% | (67–87) |
| <i>PBRM1</i> WT | 52 | 36.3 | (29.6-NA) | 0.12 | 72.8% | (61–88) |
| <i>PBRM1</i> MT | 53 | NR | (49.9-NA) | | 77.6% | (66–92) |
| <i>SETD2</i> WT | 68 | 40.0 | (29.6-NA) | 0.29 | 72.7% | (62–86) |
| <i>SETD2</i> MT | 37 | NR | (37.3-NA) | | 79.3% | (66–96) |
| <i>BAP1</i> WT | 80 | NR | (41.2-NA) | 0.02 | 78.0% | (68–89) |
| <i>BAP1</i> MT | 25 | 28.7 | (23.9-NA) | | 65.4% | (48–90) |
| <i>KDM5C</i> WT | 88 | 40 | (32.3-NA) | 0.15 | 73.4% | (64–85) |
| <i>KDM5C</i> MT | 17 | NR | (41.2-NA) | | 84.0% | (66–100) |
| <i>TERT</i> WT | 90 | 52.6 | (40.0-NA) | 0.03 | 76.1% | (67–87) |
| <i>TERT</i> MT | 15 | 29.6 | (18.4-NA) | | 68.6% | (46–100) |

*MT, mutant; WT, wild-type; NA, not applicable.

association with outcome was apparent for mutation status in *BAP1* ($p=0.02$, median 28.7 months vs. not reached) (Fig. 2). There were no statistically significant OS differences when comparing MT vs. WT for *VHL*, *PBRM1*, *SETD2*, and *KDM5C* (Table 2).

Mutation status and TTF to VEGF-targeted therapy

Ninety-five patients had received VEGF-targeted agents; 20 were still on treatment at the time of this analysis and the remaining 75 had stopped therapy for progression ($n=59$), toxicity ($n=14$) or other reason ($n=2$). TTF on first VEGF-targeted therapy by mutation status is summarized in Table 3. TTF with first VEGF-targeted therapy differed significantly by *PBRM1* mutation status, where presence of acquired somatic mutation associated with more

favorable TTF ($p=0.01$; median 12.0 months vs. 6.9 months for WT). *BAP1* mutation status correlated adversely with TTF in VEGF-targeted therapy treated patients ($p=0.01$; median 6.4 months for MT vs. 11.0 months for WT). We found no significant correlation for patients harboring mutations in *VHL*, *SETD2*, *TERT*, and *KDM5C*.

DISCUSSION

With tumor genomic profiling increasingly being used in the diagnostic workup of RCC patients, knowledge of mutation spectrum has grown, but prior studies were largely limited to non-metastatic ccRCC [13–15]. This report represents an effort to correlate genomic profiling and outcomes with VEGF-targeted therapy in metastatic ccRCC patients.

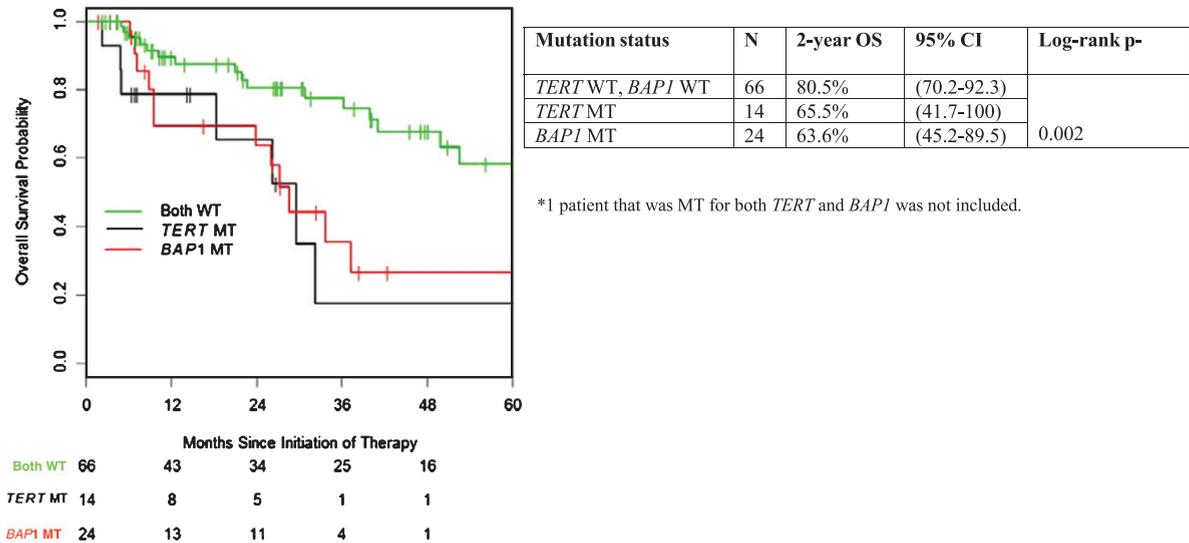
Fig. 2. Overall survival stratified by presence of *TERT* promoter and *BAP1* mutation.

Table 3
Time to failure (TTF) on first VEGF-targeted therapy
by mutation status

| Mutation status | N | Median TTF (months) | 95% CI | Log-rank p-value |
|-----------------|----|---------------------|-------------|------------------|
| <i>VHL</i> WT | 16 | 6.5 | (5.8-NA) | 0.82 |
| <i>VHL</i> MT | 79 | 10.6 | (7.4-16.6) | |
| <i>PBRM1</i> WT | 48 | 6.9 | (4.6-10.8) | 0.02 |
| <i>PBRM1</i> MT | 47 | 12.0 | (10.0-21.6) | |
| <i>SETD2</i> WT | 61 | 8.6 | (6.7-12.2) | 0.72 |
| <i>SETD2</i> MT | 34 | 10.0 | (6.5-19.5) | |
| <i>BAP1</i> WT | 73 | 11.0 | (8.4-19.3) | 0.01 |
| <i>BAP1</i> MT | 22 | 6.4 | (3.9-16.6) | |
| <i>KDM5C</i> WT | 78 | 8.6 | (6.5-12.2) | 0.90 |
| <i>KDM5C</i> MT | 17 | 11.4 | (9.7-24.3) | |
| <i>TERT</i> WT | 82 | 10.6 | (7.1-12.3) | 0.25 |
| <i>TERT</i> MT | 13 | 6.9 | (2.3-NA) | |

*MT, mutant; WT, wild-type; NA, not applicable.

In this cohort, recurrent alterations were detected across genes previously implicated in early stage disease, including *VHL*, *PBRM1*, *SETD2*, and *BAP1*, but at a higher frequency than in prior reports. For example, as compared to results from The Cancer Genome Atlas (TCGA), *PBRM1* mutations were identified in 51 vs. 33%, *SETD2* in 37 vs. 12%, and *BAP1* in 24 vs. 10%; this finding supports their suggested significance in the pathogenesis of this disease, including development of a metastatic phenotype [12]. Furthermore, recurrent alterations were detected in *TP53* (11%), and the *TERT* promoter region (13%), which were not reported as commonly altered genes in earlier non-metastatic datasets. These may represent late events in the evolution of metastatic disease.

The presence of *BAP1* or *TERT* promoter mutations was associated with a significantly worse OS. These two mutations were mostly mutually exclusive, with only one tumor harboring mutations in both genes. *BAP1* codes for a deubiquitinating enzyme involved in chromatin remodeling, and several studies in mostly localized RCC have found that loss of *BAP1* is associated with poor prognosis [13, 14]. Although *TERT* mutations were not described in the original TCGA study of ccRCC, a recent study of mostly non-metastatic cases identified approximately 10% prevalence in ccRCC tumors, and also showed that there may be an association with aggressive disease [17]. *TERT* promoter mutations are the most common somatic noncoding mutations in cancer, and have also been associated with adverse outcomes in several malignancies, including melanoma, thyroid cancer, and bladder cancer [18-24]. An exploratory analysis was performed to investigate if acquired mutations in *BAP1* or *TERT* were associated with MSKCC prognostic risk group or the presence of sarcomatoid features on histopathologic review. While there was no association with mutation status and risk group, there was a significantly higher rate of sarcomatoid features in MT vs. WT ($p=0.008$). Due to sample size constraints, we were unable to investigate whether these are independent risk factors for adverse outcomes; this should be analyzed in larger cohorts. Patients whose tumors displayed sarcomatoid features had worse overall survival regardless of mutation status, but among those without sarcomatoid features, *BAP1* or *TERT* MT had worse

OS than WT (Supplemental Figure 1; Supplemental Table 3).

In this study, mutations in *PBRM1* correlated with statistically longer TTF with VEGF-targeted therapy, with a trend towards superior OS (not reached for MT vs. 36.3 months for WT; $p=0.12$). Similar findings were previously reported for metastatic patients treated on the RECORD-3 trial, with a trend towards superior outcome for patients with *PBRM1* MT tumors, and in a recent analysis of ccRCC patients with extreme responses to VEGF-targeted therapy. In contrast to these findings in metastatic disease, early-stage studies have reported adverse association with cancer-specific outcome [14]. *PBRM1* is a component of the SWI/SNF chromatin remodeling complex, and mutations of *PBRM1* are an early event in ccRCC tumor development [25, 26]. Together, these findings may suggest that loss of *PBRM1* is biologically relevant for invasiveness and metastatic spread; but once tumor cells metastasize, those with loss of *PBRM1* may behave less aggressively than tumors which metastasize by other molecular means (e.g. mutations in *BAP1* or *TERT*) [14, 25, 27]. Such observations are limited by sample size, and without a control arm, it is not possible to determine whether *PBRM1* mutation status is a predictive or prognostic biomarker.

In the prior analysis of the RECORD-3 trial, there was a significant correlation between presence of *KDM5C* mutation and PFS in 111 patients receiving sunitinib (median PFS 8.3 for *KDM5C* WT vs. 20.6 months for MT; $p=0.03$), but not for 109 everolimus-treated patients (median PFS 8.2 for *KDM5C* WT vs. 9.8 months for MT; $p=0.03$). In the present analysis, TTF did not correlate significantly with *KDM5C* mutation status across 95 VEGF-targeted therapy treated patients (TTF 8.6 months for *KDM5C* WT vs. 11.4 months for MT; $p=0.9$). The inability to confirm the previously suggested correlation may relate to sample size and a more heterogeneous patient population.

This study has several other limitations. As a single-institution experience of limited sample size, findings will need to be corroborated in larger independent datasets. In addition, TTF, which is subject to individual decision making of treating physicians, was used as a measure of therapeutic benefit and limits comparison to more rigid assessment approaches, such as Response Evaluation Criteria in Solid Tumors (RECIST) [28]. Archival samples were derived from both primary tumor and metastatic sites, some having been collected long before the

initiation of targeted therapy. Finally, intratumor heterogeneity is a well-known phenomenon in RCC and limits our accuracy in assessing the true mutant allele frequency in each sample [25, 29]. Nevertheless, biology of metastatic sites should guide survival for most patients, and recent data shows a high correlation of mutation profiles in primary vs. metastatic sites [30].

The data presented here cannot inform the choice of agent for patients with metastatic ccRCC, although the ever expanding number of approved targeted therapies leaves prescribers with a clear need for predictive biomarkers in this space. Our findings can, however, help better inform our understanding of pathobiology and clinical course of this disease. Certain genetic alterations, such as *BAP1* and *TERT* promoter mutations, may be present in higher frequency than previously thought and, based on this data, deserve further study for their prognostic implications. Ultimately, they may prove useful in refining our current standards of assessing risk status in metastatic disease, and could inform surveillance strategies and future trial design [31].

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SUPPLEMENTARY MATERIAL

The supplementary table and figure are available in the electronic version of this article: <http://dx.doi.org/10.3233/KCA-160003>.

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