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## Genomic Biomarkers of a Randomized Trial Comparing First-line Everolimus and Sunitinib in Patients with Metastatic Renal Cell Carcinoma

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### Abstract

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**Background**—Metastatic renal cell carcinoma (RCC) patients are commonly treated with vascular endothelial growth factor (VEGF) inhibitors or mammalian target of rapamycin inhibitors. Correlations between somatic mutations and first-line targeted therapy outcomes have not been reported on a randomized trial.

**Objective**—To evaluate the relationship between tumor mutations and treatment outcomes in RECORD-3, a randomized trial comparing first-line everolimus (mTOR inhibitor) followed by sunitinib (VEGF inhibitor) at progression with the opposite sequence in 471 metastatic RCC patients.

**Design, setting, and participants**—Targeted sequencing of 341 cancer genes at ~540× coverage was performed on available tumor samples from 258 patients; 220 with clear cell histology (ccRCC).

**Outcome measurements and statistical analysis**—Associations between somatic mutations and median first-line progression free survival (PFS1L) and overall survival were determined in metastatic ccRCC using Cox proportional hazards models and log-rank tests.

**Results and limitations**—Prevalent mutations (> 10%) were *VHL* (75%), *PBRM1* (46%), *SETD2* (30%), *BAP1* (19%), *KDM5C* (15%), and *PTEN* (12%). With first-line everolimus, *PBRM1* and *BAP1* mutations were associated with longer (median [95% confidence interval {CI}] 12.8 [8.1, 18.4] vs 5.5 [3.1, 8.4] mo) and shorter (median [95% CI] 4.9 [2.9, 8.1] vs 10.5 [7.3, 12.9] mo) PFS1L, respectively. With first-line sunitinib, *KDM5C* mutations were associated with longer PFS1L (median [95% CI] of 20.6 [12.4, 27.3] vs 8.3 [7.8, 11.0] mo). Molecular subgroups of metastatic ccRCC based on *PBRM1*, *BAP1*, and *KDM5C* mutations could have predictive values for patients treated with VEGF or mTOR inhibitors. Most tumor DNA was obtained from primary nephrectomy samples (94%), which could impact correlation statistics.

**Conclusions**—*PBRM1*, *BAP1*, and *KDM5C* mutations impact outcomes of targeted therapies in metastatic ccRCC patients.

**Patient summary**—Large-scale genomic kidney cancer studies reported novel mutations and heterogeneous features among individual tumors, which could contribute to varied clinical outcomes. We demonstrated correlations between somatic mutations and treatment outcomes in clear cell renal cell carcinoma, supporting the value of genomic classification in prospective studies.

## Keywords

Genomic biomarker; Kidney cancer; Targeted therapy; Everolimus; Sunitinib; Tumor suppressor gene; *PBRM1*; *SETD2*; *BAP1*; *KDM5C*

## 1. Introduction

Clear cell renal cell carcinoma (ccRCC) is most common histological subtype and accounts for the most RCC-specific deaths. The genetic inactivation of *Von Hippel Lindau* (*VHL*) tumor suppressor gene was the only known prevalent oncogenic driver event in ccRCC for decades [1]. Recent analyses of ccRCC using next-generation sequencing (NGS) revealed novel, common mutations including *PBRM1*, *BAP1*, *SETD2*, and *KDM5C* [2]. These genes

encode proteins that regulate chromatin [3] and most reported somatic mutations result in loss of function, indicating that these proteins function as tumor suppressors. Thus far, analyses of published cohorts encompassing Stages I–IV kidney cancer patients have suggested prognostic values of individual mutations [4,5]. However, large-scale mutation profiles of Stage IV kidney cancer are lacking.

Inhibitors of vascular endothelial growth factor (VEGF) or mammalian target of rapamycin (mTOR) signaling pathways are standard treatment options for patients with metastatic RCC (mRCC) [6]. RECORD-3 (Renal Cell Cancer Treatment With Oral RAD001 Given Daily) was a randomized phase 2 trial comparing sunitinib, a VEGF receptor-tyrosine kinase inhibitor, with everolimus, an mTOR inhibitor, in previously untreated patients with mRCC ( $N = 471$ ) [7]. After disease progression, patients crossed over to the alternative agent for second-line therapy. Most enrolled patients (~85%) had metastatic ccRCC. Median first-line progression-free survival (PFS1L; 7.9 mo, everolimus; 10.7 mo, sunitinib; hazard ratio [HR]: 1.4; 95% confidence interval [CI]: 1.2, 1.8) and final median overall survival (OS; 22.4 mo, everolimus-sunitinib; 29.5 mo, sunitinib-everolimus;  $HR_{EVE-SUN/SUN-EVE}$ : 1.1; 95% CI: 0.9, 1.4) [7,8] favored the standard sequence of sunitinib followed by everolimus [6,9]. Case studies involving cancer gene mutations of advanced (Stage IV or recurrent metastatic) ccRCC have indicated a potential correlation between mutations and treatment response to targeted therapy [10–12]; however, these associations have not been evaluated in a large clinical trial setting. To address these questions, we leveraged archived tumor samples collected from the RECORD-3 study, sequenced 341 cancer genes, and performed correlation analysis.

## 2. Materials and methods

### 2.1. Patients, study design, and treatment

The RECORD-3 trial design has been previously reported [7]. Patients received everolimus 10 mg/d or sunitinib 50 mg/d in a crossover design. Patients were randomly assigned 1:1 to sequentially receive either everolimus-sunitinib ( $n = 238$ ) or sunitinib-everolimus ( $n = 233$ ), and stratified by Memorial Sloan-Kettering Cancer Center (MSKCC) risk criteria [13]. Adult patients with measurable mRCC of any histology who had not previously received systemic therapy, and with a Karnofsky performance status  $\geq 70\%$  were included. All patients gave informed consent.

### 2.2. Tumor DNA and MSK-Integrated Mutation Profiling of Actionable Cancer Targets

Hematoxylin and eosin slides of available tumor tissue from RECORD-3 were reviewed by a dedicated genitourinary pathologist (YC). Unstained sections were microdissected to ensure tumor purity. DNA was purified using the DNeasy Blood and Tissue Kit and subjected to ultra-deep sequencing using the MSK-Integrated Mutation Profiling of Actionable Cancer Targets platform [14].

### 2.3. Statistical analysis

Associations between PFS1L (and OS), first-line treatment (treatment regimen), and gene alteration status (mutant type [MT] or wild type [WT]) were investigated. All

nonsynonymous mutations were considered while defining the alteration status. Median PFS1L (and OS) by first-line treatment (treatment regimen) and alteration status (MT vs WT) were determined by the Kaplan-Meier method. HR (95% CIs) are estimated from a Cox proportional hazards (PH) model for PFS1L (OS). The model included terms for mutation status, treatment arm, interaction between treatment arms and mutation status groups, with stratification by MSKCC risk groups and adjustment for baseline covariates (RCC histology when combining data from clear and nonclear cell, number of metastatic sites, baseline lactate dehydrogenase levels). Differences between survival curves of PFS1L (and OS) for each mutation status group and treatment arm were tested using the log-rank test. All *p* values were not adjusted for multiple testing. When exploring associations with OS, all ccRCC patients with NGS data were included based on the randomized treatment regimen, regardless of their crossover status, and no adjustments were performed for confounding effects of crossover. Patterns of mutual exclusivity or co-occurrence were explored via odds ratio, and statistical significance for the relationship between gene pairs was assessed using Fisher exact test [15].

### 3. Results

#### 3.1. Study cohort

Among 258 successfully sequenced tumors, 220 were of cc histology (first-line everolimus, *n* = 109; first-line sunitinib, *n* = 111; Fig. 1). For a clear correlation analysis, we only included ccRCC patients. Our NGS ccRCC cohort (*n* = 220) reflects patient characteristics of the original 471 patients included in RECORD-3 (Table 1). Patient characteristics of the total biomarkers population (ccRCC and non-ccRCC) are shown (Supplementary Table 1).

#### 3.2. Somatic mutation profiling of RECORD-3 ccRCC tumors

The median sequencing coverage of the 341 cancer genes in our NGS ccRCC 220-patient cohort was ~540× (33–1378×), with 93% of exons at >100×. Germline DNA was sequenced for 171 patients (78%). DNA from 206 tumors (94%) was obtained from primary nephrectomy samples. Within the NGS ccRCC cohort, 217 tumors (99%) had ≥1 somatic mutation and 161 tumors (73%) had <10 mutations; the median number of mutations was five (Fig. 2A). There were 18 genes with mutation frequencies detected at ≥5%. Six genes—*VHL*, *PBRM1*, *SETD2*, *BAP1*, *KDM5C*, and *PTEN*—were mutated at ≥10% in our cohort, and there was no association between these mutations and MSKCC risk groups (Supplementary Table 2). Most mutations were truncating (*VHL*, 64%; *PBRM1*, 79%; *SETD2*, 89%; *BAP1*, 69%; *KDM5C*, 75%; *PTEN*, 81%), consistent with previous reports [2,3,16]. Compared with recent large-scale ccRCC genomic series that encompass primary tumors of ccRCC from Stages I–V, the RECORD-3 ccRCC NGS cohort exhibited comparable mutation frequencies in *VHL* (75% vs 49–82%), *PBRM1* (46% vs 29–41%), and *MTOR* (6–6%), whereas the frequency was higher in *SETD2* (30% vs 8–12%), *BAP1* (19% vs 7–10%), *KDM5C* (15% vs 4–8%), and *PTEN* (12% vs 2–4%; Fig. 2B).

#### 3.3. Correlations between mutations and PFS1L within treatment arms

The interaction terms between treatment and mutation status groups in the Cox PH models were not statistically significant. However HRs (MT vs WT) within each treatment group are

presented to determine if the data suggest a differential impact of the mutation on the treatment response which warrants further investigation in independent studies. In the everolimus arm, patients with *PBRM1* MT appear to have a lower risk of progression on first-line than WT tumors (HR: 0.53; 95% CI: 0.3, 0.8; median PFS1L [95% CI], 12.8 [8.1, 18.4] vs 5.5 [3.1, 8.4] mo), whereas in the sunitinib arm, the mutation status of *PBRM1* appears to have no effect (HR: 0.79; 95% CI: 0.5, 1.3; Table 2). Conversely, in the everolimus arm, patients with *BAP1* MT tumors appear to have a higher risk of progression than those with WT tumors (HR: 1.84; 95% CI: 1.1, 3.2; median PFS1L [95% CI], 4.9 [2.9, 8.1] vs 10.5 [7.3, 12.9] mo). In the sunitinib arm patients with *BAP1* MT tumors appear to have a higher risk of progression than WT tumors, although the HR is not statistically significant (HR: 1.69; 95% CI: 0.9, 3.2; median PFS1L [95% CI], 8.1 [3.1, 11.3] vs 11.0 [8.3, 13.8]). The mutation status of *KDM5C* appears to have no effect on PFS1L in the everolimus arm (HR: 1.06; 95% CI: 0.5, 2.1; median PFS1L [95% CI], 9.8 [2.2, 16.6] vs 8.2 [5.3, 10.9]); whereas patients in the sunitinib arm with *KDM5C* MT appear to have lower risk of progression than those with WT tumors (HR: 0.57; 95% CI: 0.3, 1.1; median PFS1L [95% CI], 20.6 [12.4, 27.3] vs 8.3 [7.8, 11.0]).

There were no statistically significant differences between PFS1L for the *VHL*, *SETD2*, and *PTEN* genotypes within either treatment arm. *TSC1* and *MTOR* were mutated in 13% of our cohort, and no clear association between PFS1L outcomes and everolimus (HR: 0.91; 95% CI: 0.4, 2.2) or sunitinib (HR: 1.24; 95% CI: 0.7, 2.4) was observed. Of note, seven patients with *MTOR* tumor mutations were treated with first-line everolimus. Three patients remained on everolimus and were censored at 13.4 mo, 21.8 mo, and 21.8 mo, and three patients progressed at 2.2 mo, 2.8 mo, and 13.9 mo (at primary analysis data cut-off) [7]. One patient was censored due to the addition of a new treatment.

### 3.4. Subgroup analyses of PFS1L by *PBRM1*, *BAP1*, and *KDM5C* mutation status

Consistent with reported PFS1L of the overall RECORD-3 population [7], patients in the NGS ccRCC population who received first-line everolimus versus sunitinib had shorter median PFS1L (median [95% CI], 8.3 [5.4–10.7] vs 10.8 [8.1–12.9] mo; Table 1). However, when PFS1L was examined within genetic mutation subgroups, heterogeneous clinical outcomes were observed, although the heterogeneity did not meet the thresholds for statistical significance (Table 2, Supplementary Table 3). Among patients with *KDM5C* tumor mutations, although not statistically significant, patients receiving first-line everolimus have a high risk of progression than those receiving sunitinib (HR: 2.23; 95% CI: 0.9, 5.5; median PFS1L [95% CI], 9.8 [2.2, 16.2] vs 20.6 [12.4, 27.3] mo; Fig. 3A). There was no significant difference in the risk of progression and median PFS1L among patients with *PBRM1* tumor mutations who received first-line everolimus versus sunitinib (HR: 1.02; 95% CI: 0.6, 1.7; median PFS1L [95% CI] 12.8 [8.1, 18.4] vs 11.0 [8.3, 13.8] mo; Fig. 3B). The shortest median PFS1L was found among patients with *BAP1* tumor mutations in both treatment arms (median [95% CI], sunitinib 8.1 [3.1, 11.3] vs everolimus 4.9 [2.9, 8.1] mo; Fig. 3C).

Patients randomized to first-line sunitinib versus everolimus had longer median PFS1L irrespective of *VHL*, *SETD2*, or *PTEN* genetic status. Median PFS1L and mutation status of

patients in the total biomarkers population (ccRCC and non-ccRCC) are shown (Supplementary Table 4). Given that 85% patients of the whole NGS cohort had ccRCC, comparable statistics data were observed (Table 2, Supplementary Table 4).

### 3.5. Correlation of genomic subgroups based on mutation status of *PBRM1*, *BAP1*, and *KDM5C* and OS on targeted therapies

Our data presented thus far demonstrated correlations between individual somatic mutations, including *PBRM1*, *BAP1*, and *KDM5C*, and the PFS1L of two distinct targeted therapies, everolimus and sunitinib. Acknowledging the existence of confounding factors such as the cross-over design and the 48–54% cross-over rate (Fig. 1), we explored the correlation between individual mutations and OS (Supplementary Table 5).

Mutual exclusivity [15] was detected between *PBRM1* and *BAP1* ( $p < 0.001$ ) mutations with only seven (3%) cases carrying both mutations in our NGS ccRCC cohort (Fig. 4A), which was also observed with reported ccRCC genomics encompassing Stage I–IV tumors [2,4,16,17]. As the mutation status of *PBRM1* and *BAP1* was shown to associate with RCC-specific survival among ccRCC patients of all stages without taking consideration of treatment information [4,5,17], we classified patients into three molecular groups based on their combined *PBRM1* and *BAP1* mutation status and assessed the impact of genotypes on OS of patients with ccRCC in each treatment arm. The *PBRM1* group was composed of *PBRM1* MT and *BAP1* WT (38%), the *BAP1* group was composed of *BAP1* MT and *PBRM1* WT/MT (17%), and the WT group was composed of *PBRM1* WT and *BAP1* WT (44%). Of note, patients with mutations in both *PBRM1* and *BAP1* have been shown to fare poorly [17], and were assigned to the *BAP1* group in our analysis. Among patients treated with sequential everolimus-sunitinib, the longest OS (median 95% CI: 43.5 [34.4, not estimated] mo) was observed with the *PBRM1* group, the shortest (9.8 [8.2, 22.4]) with the *BAP1* group, and an intermediate (18.1 [11.1, 29.6]) with the WT group (Fig. 4B). Among patients treated with sequential sunitinib-everolimus, median OS (95% CI) was 33.2 (25.8, 45.0) mo with the *PBRM1* group, 29.9 (8.9, not estimated) mo with the *BAP1* group, and 33.1 (.5, 39.5) mo with the WT group (Fig. 4C). Although the interaction between molecular groups and treatment was not significant in the Cox PH model, estimates of HR (95% CI) are presented within each treatment sequence, in order to assess if the data suggest there could be a differential impact of the molecular groups on treatment response. Risk for death appeared to be larger within the everolimus-sunitinib sequence when comparing the *BAP1* and *PBRM1* groups (HR: 2.2; 95% CI: 2.2 1.1, 4.3; log-rank  $p = 0.009$ ); however, the risk for death was not significant within the sunitinib-everolimus sequence (HR: 1.6; 95% CI: 0.8, 3.2; log-rank  $p = 0.4$ ).

As patients with *KDM5C* mutations appear to have longer PFS1L with first-line sunitinib but not with first-line everolimus (Fig. 3A), and *PBRM1* mutations exhibited comparable PFS1L (Fig. 3B), we examined whether mutation status affected OS in our cohort.

Estimates of HR (95% CI) between mutation groups, within each treatment sequence are presented, in order to assess if the data suggest there could be a differential impact of the molecular groups on treatment response; although it should be noted that the interaction between treatment and mutation groups was not significant for *KDM5C* or *PBRM1*. The risk



for death appears to be smaller when comparing *KDM5C* MT and WT patients within the sunitinib-everolimus sequence (HR: 0.39; 95% CI: 0.2, 0.9; log-rank  $p = 0.02$ ); whereas it appears not to differ between the *KDM5C* MT and WT patients within the everolimus-sunitinib sequence (HR: 0.55; 95% CI: 0.2, 1.3; log-rank  $p = 0.2$ ). Intriguingly, patients with *PBRM1* MT appear to have a lower risk of death compared with WT, within the everolimus-sunitinib sequence (HR: 0.50; 95% CI: 0.3, 0.8; log-rank  $p = 0.004$ ); while it appears not to differ between *PBRM1* MT versus WT groups, within the sunitinib-everolimus (HR: 0.73; 95% CI: 0.5, 1.2; log-rank  $p = 0.4$ ; Supplementary Table 5).

#### 4. Discussion

The implementation of targeted therapy with VEGF receptor and mTOR inhibitors for mRCC transformed an imminently lethal disease into a more manageable malignancy [18]. However, a wide range of clinical outcomes has been observed [19–21]. The discoveries of intratumor heterogeneity [22–24] and novel prevalent mutations in ccRCC suggest a potential genetic basis for observed diverse clinical outcome.

Consistent with previous studies, we did not detect an association between *VHL* mutation and clinical outcome in ccRCC. Unexpectedly, patients whose tumors carry *KDM5C* mutation derived longer median PFS1L benefit with sunitinib (20.6 mo; 95% CI: 12.4–27.3) but not with everolimus (9.8 mo; 95% CI: 2.2–16.6). *KDM5C* is located at the X-chromosome, and single-allele mutation of *KDM5C* in male patients would result in complete genetic loss of *KDM5C*. The extended benefit in patients (especially men) with *KDM5C* mutations who received VEGF inhibitor therapy was noted in a smaller study where statistical significance was not detected [12]. The role of *KDM5C* in antagonizing the hypoxia-inducible factor pathway was recently reported using cell-based assays [25], offering potential mechanistic basis.

Kidney cancer is a metabolic disease [26,27]. Outlier studies have shown that direct mTOR complex 1 (mTORC1) activation through either complete *TSC1* loss or *MTOR*-activating mutation [28] was associated with long-term therapeutic benefit from mTORC1 inhibitors [29]. Interestingly, in our NGS ccRCC cohort, four of six patients with mTOR mutations benefited from first-line everolimus with PFS1L >12 mo. The association of a *PBRM1* or *BAP1* mutation with better or worse PFS1L, respectively, with everolimus is surprising because higher mTORC1 activity was reported for patients with *BAP1* versus *PBRM1* mutant ccRCC [30]. Altogether, our data favor an indirect role of *BAP1* loss in mTORC1 activation.

In this study, when patients with metastatic ccRCC were stratified into three genotypic groups based on *PBRM1* and *BAP1* mutations, median OS (mo) was longer in the *PBRM1* group (43.5) than in the *BAP1* (9.8) or WT (18.1) group in the everolimus-sunitinib arm, whereas median OS was similar in all three groups (*BAP1*, 29.9; *PBRM1*, 33.2; WT, 33.1) in the sunitinib-everolimus arm. In the intent-to-treat population the final OS was 22.4 mo with sequential everolimus-sunitinib and 29.5 mo with sequential sunitinib-everolimus. Intriguingly, survival durations of patients in the *PBRM1* group of this study demonstrated a

median OS of 43.5 mo with sequential everolimus-sunitinib and 33.2 mo with sequential sunitinib-everolimus.

Our analysis puts common RCC mutations into clinical context for patients treated with VEGF and mTOR inhibitors in a clinical trial at the frontline setting and suggests correlative clinical outcome signals for sunitinib and everolimus that warrant further investigation. The importance of tumor mutations in predicting benefit of first-line targeted therapy suggested by the current study necessitates further validation. Nevertheless, we confirmed that *VHL*, *PBRM1*, *SETD2*, *BAP1*, and *KDM5C* are the most commonly mutated genes in ccRCC and found that *PBRM1* and *KDM5C* mutations could be associated with better clinical outcomes with everolimus and sunitinib, respectively.

## 5. Conclusions

Our analysis encompassed a large and relatively homogenous population of patients (all metastatic ccRCC) and included a broad panel of genes. We evaluated standard end points of two classes of targeted agents and showed the impact of genetic alterations on treatment. Our results confirmed prevalent ccRCC mutations, demonstrated distinct mutation enrichment in metastatic ccRCC, showed mutual exclusivity between *BAP1* and *PBRM1* or *KDM5C* mutations, and suggested distinct molecular subtypes based on *PBRM1*, *BAP1*, and *KDM5C* mutations could potentially have different efficacy genomic biomarker values for patients with metastatic ccRCC treated with targeted therapies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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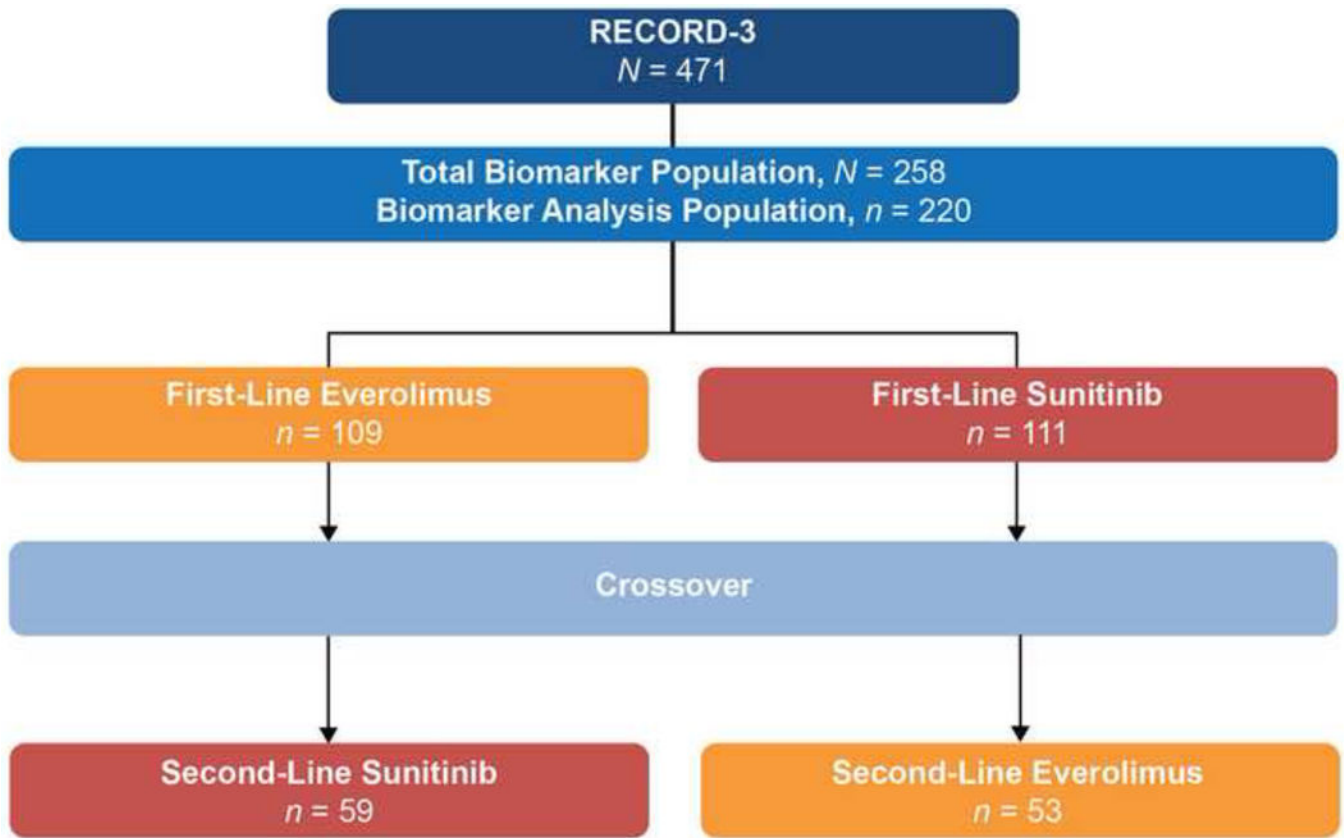
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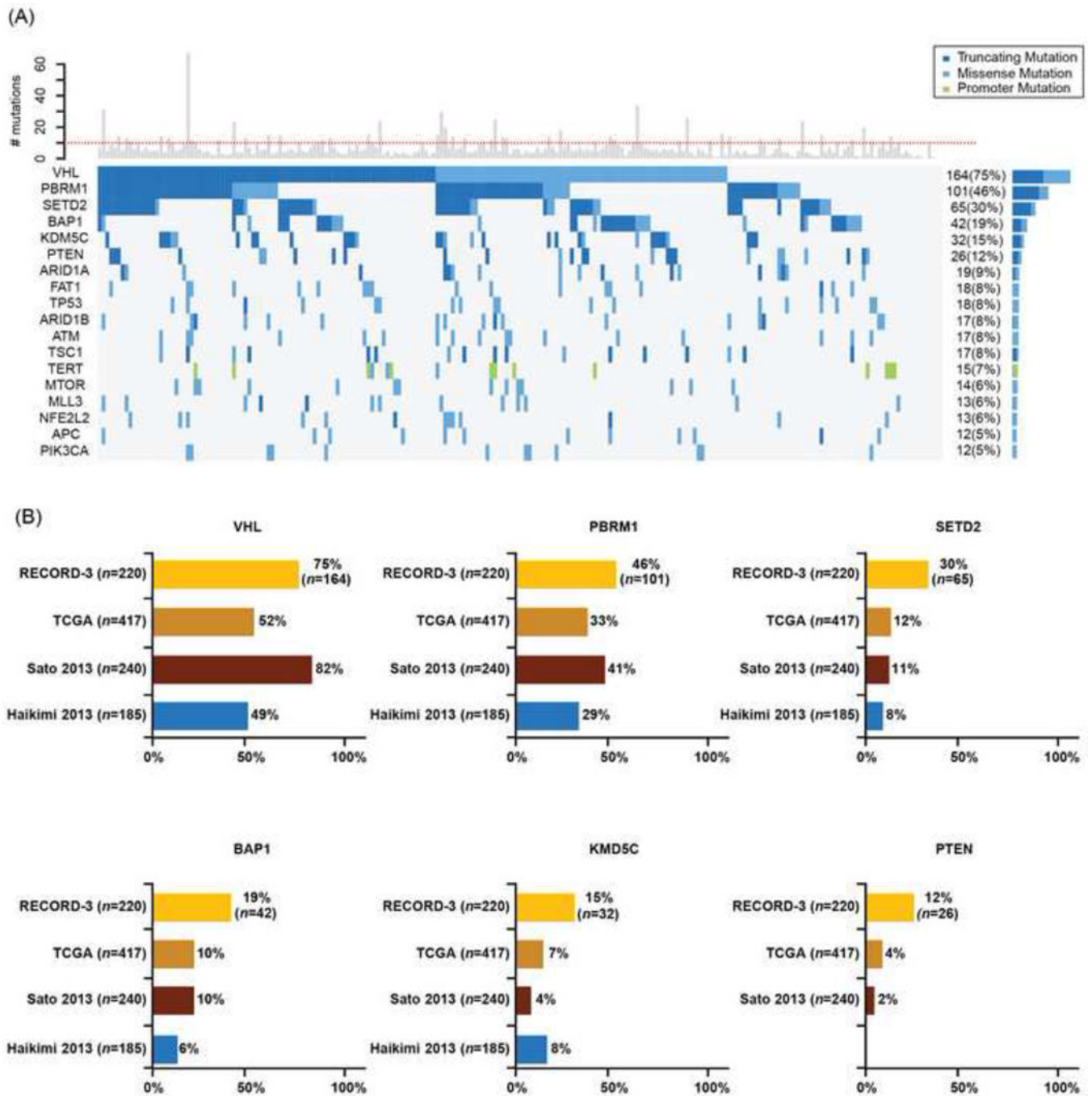
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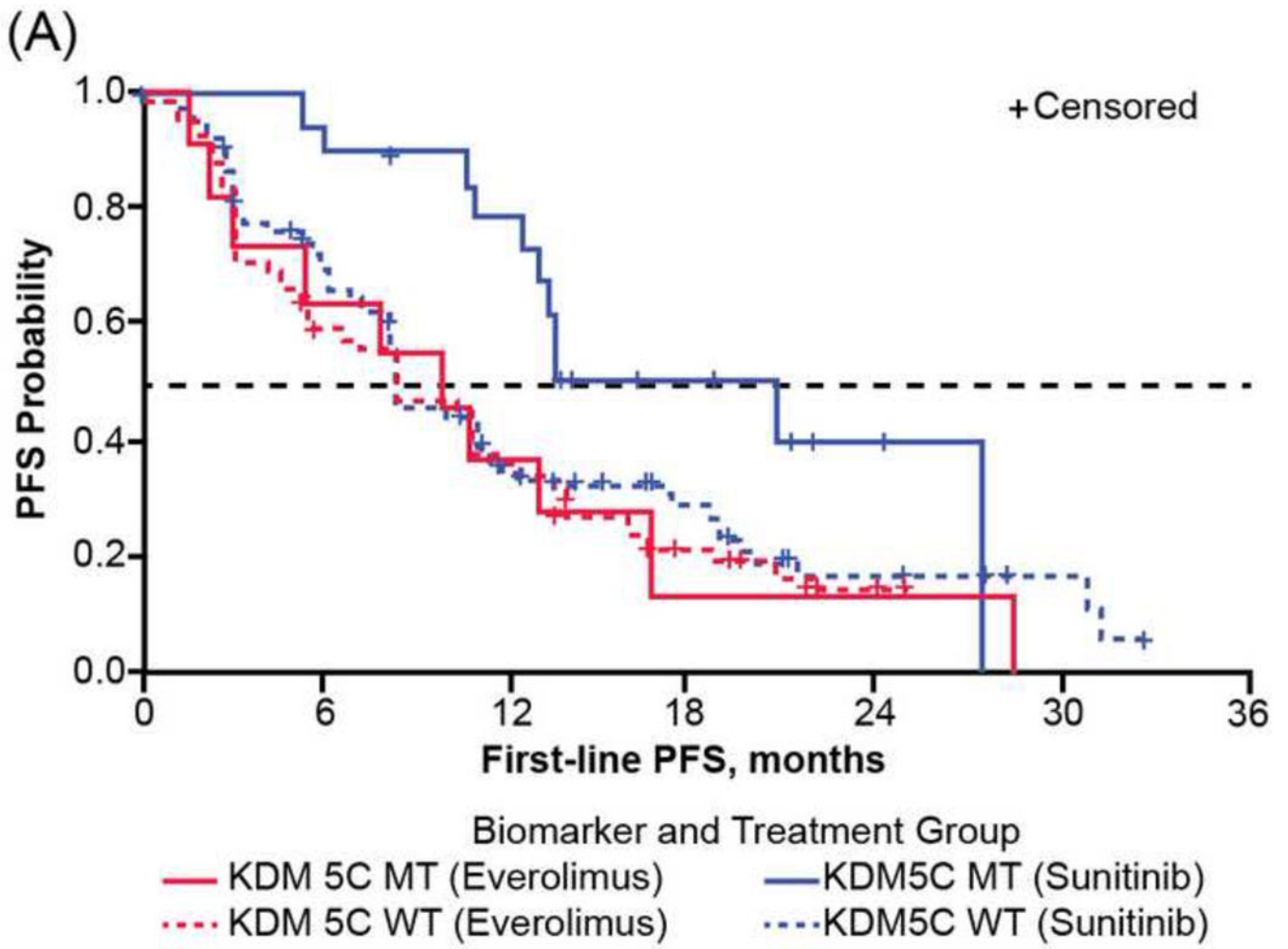
Targeted next-generation sequencing and clinical correlation analyses of a randomized metastatic renal cell carcinoma first-line targeted therapy trial (RECORD-3) demonstrated enrichment of tumor suppressor gene mutations, showed mutual exclusivity among mutations, and presented distinct molecular subtypes based on *PBRM1/BAP1/KDM5C* mutations that could have predictive/prognostic values.



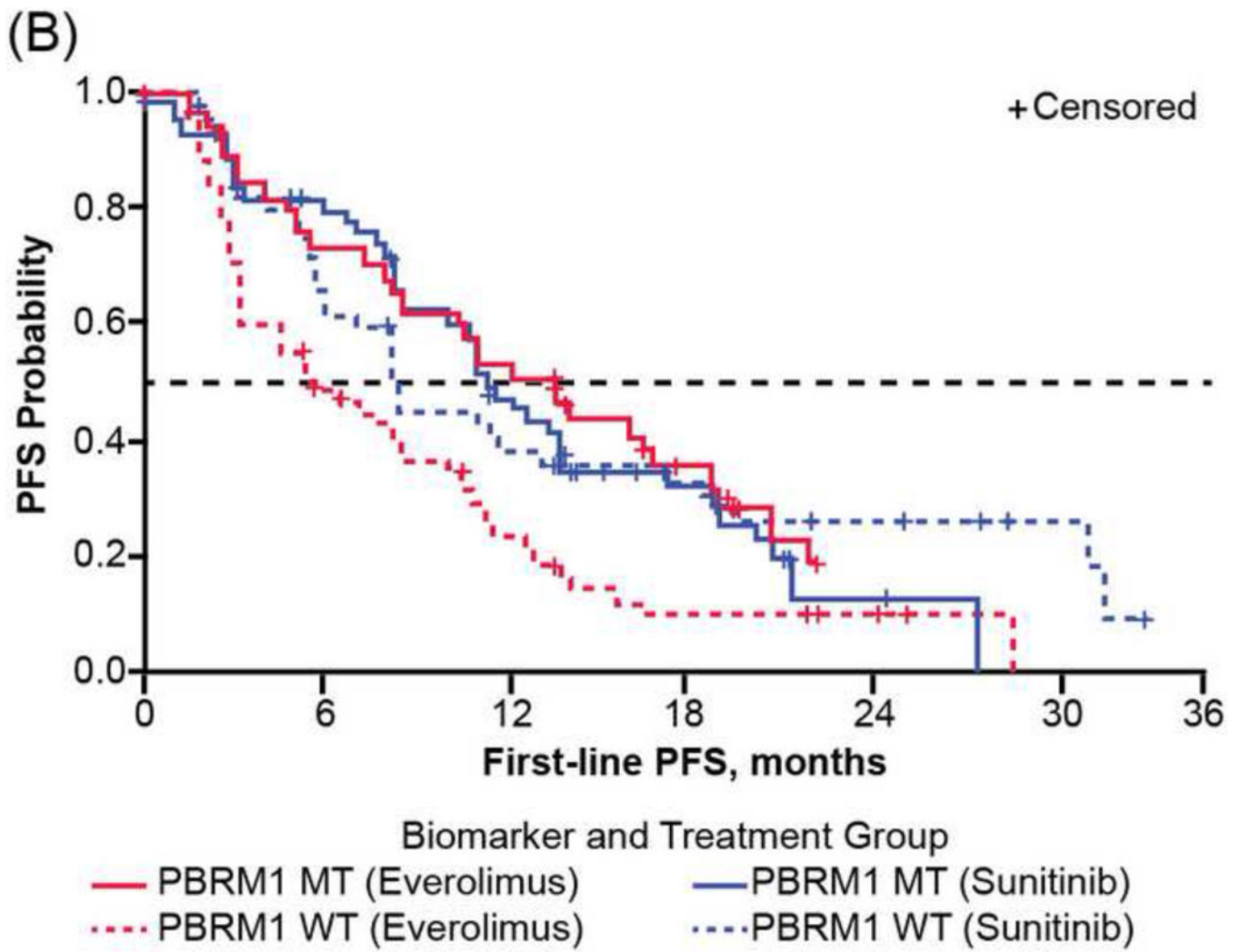
**Fig 1.** RECORD-3 clear cell renal cell carcinoma next-generation sequencing cohort.

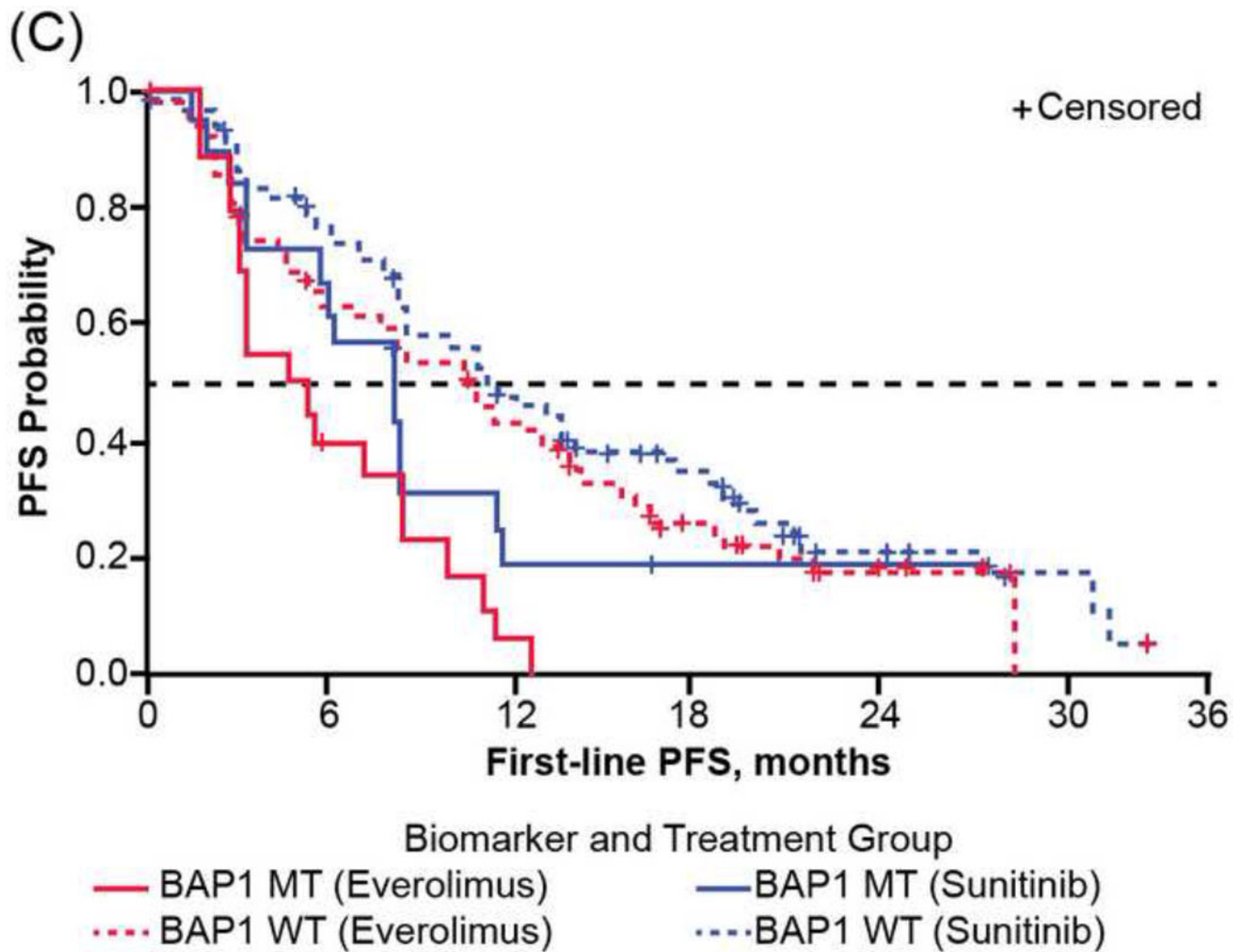


**Fig. 2.** Mutation profiles of RECORD-3 clear cell renal cell carcinoma next-generation sequencing cohort. (A) Oncoprint of frequently mutated genes (≥ 5%) from RECORD-3 patients with clear cell metastatic renal cell carcinoma. (B) Frequently mutated genes from RECORD-3 and other published data. TCGA = The Cancer Genome Atlas.

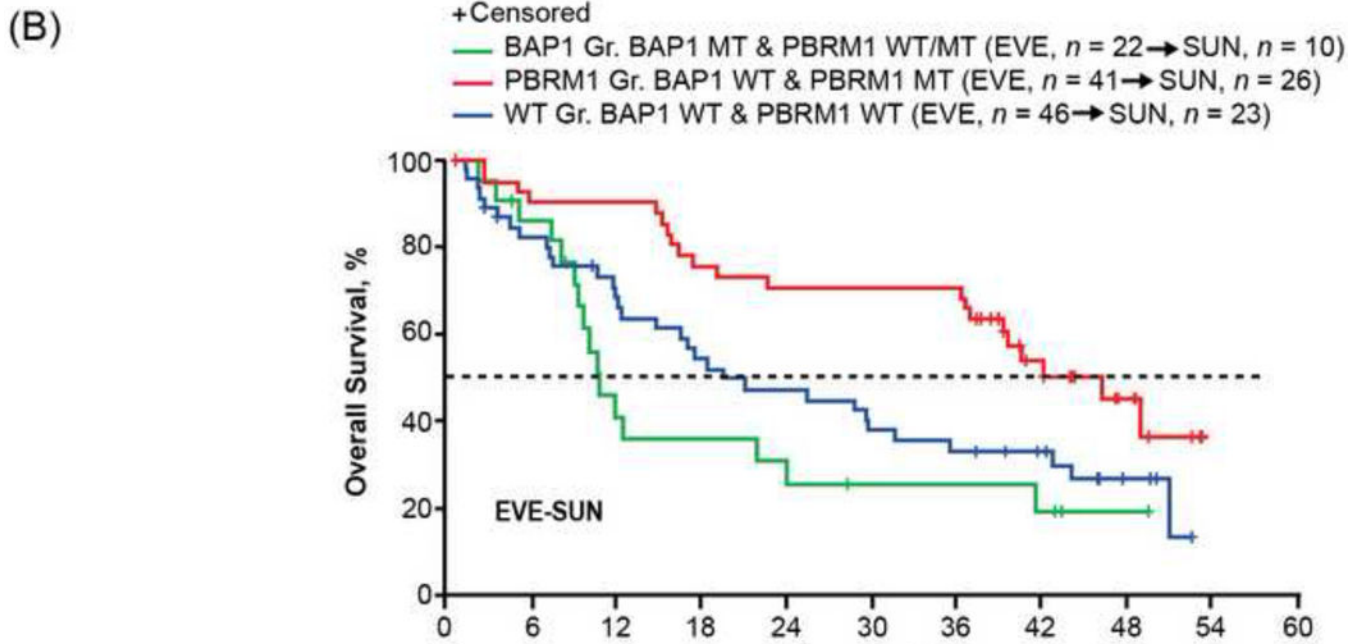
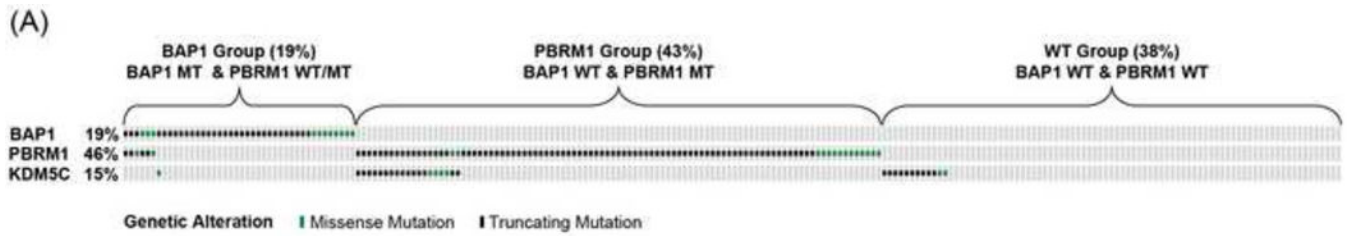








**Fig. 3.** First-line progression-free survival (PFS) of RECORD-3 patients with metastatic clear cell renal cell carcinoma. First-line PFS of patients with (A) *KDM5C*, (B) *PBRM1*, and (C) *BAP1* mutations. MT = mutant type; WT = wild type.

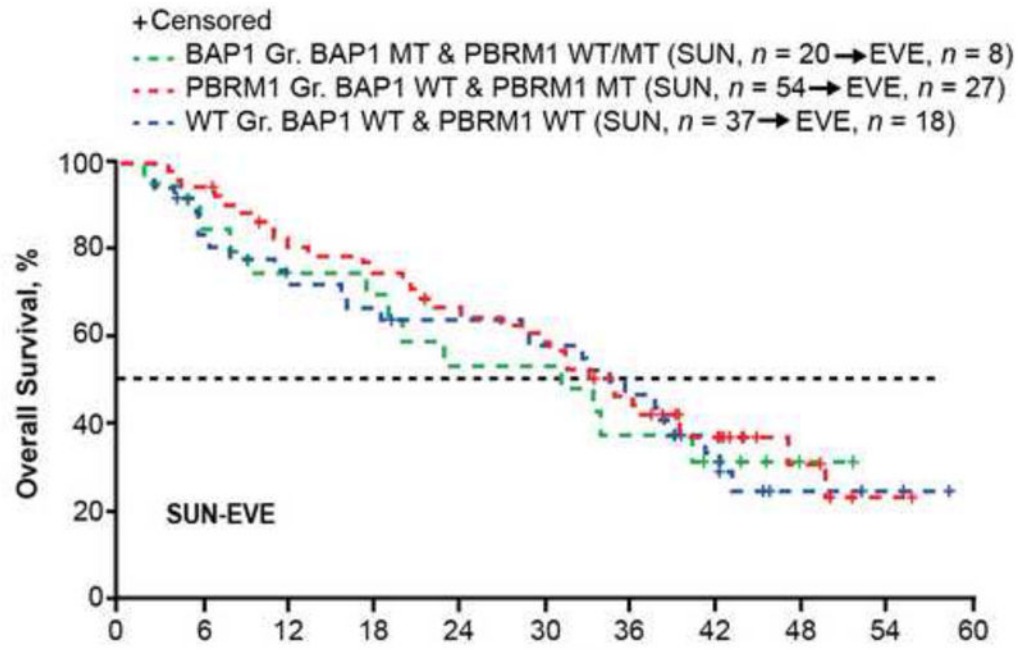


**Number of patients at risk**

	0	6	12	18	24	30	36	42	48	54
BAP1 MT & PBRM1 WT/MT	22	18	7	7	5	4	4	1	0	
BAP1 WT & PBRM1 MT	41	37	37	30	29	29	24	14	6	1
BAP1 WT & PBRM1 WT	46	36	27	22	19	15	13	8	1	

Group	<i>n</i>	Median OS	95% CI	
BAP1 Gr. BAP1 MT & PBRM1 WT/MT	22	9.78	8.2	22.4
PBRM1 Gr. BAP1 WT & PBRM1 MT	41	43.5	34.4	NE
WT Gr. BAP1 WT & PBRM1 WT	46	18.1	11.1	29.6

(C)

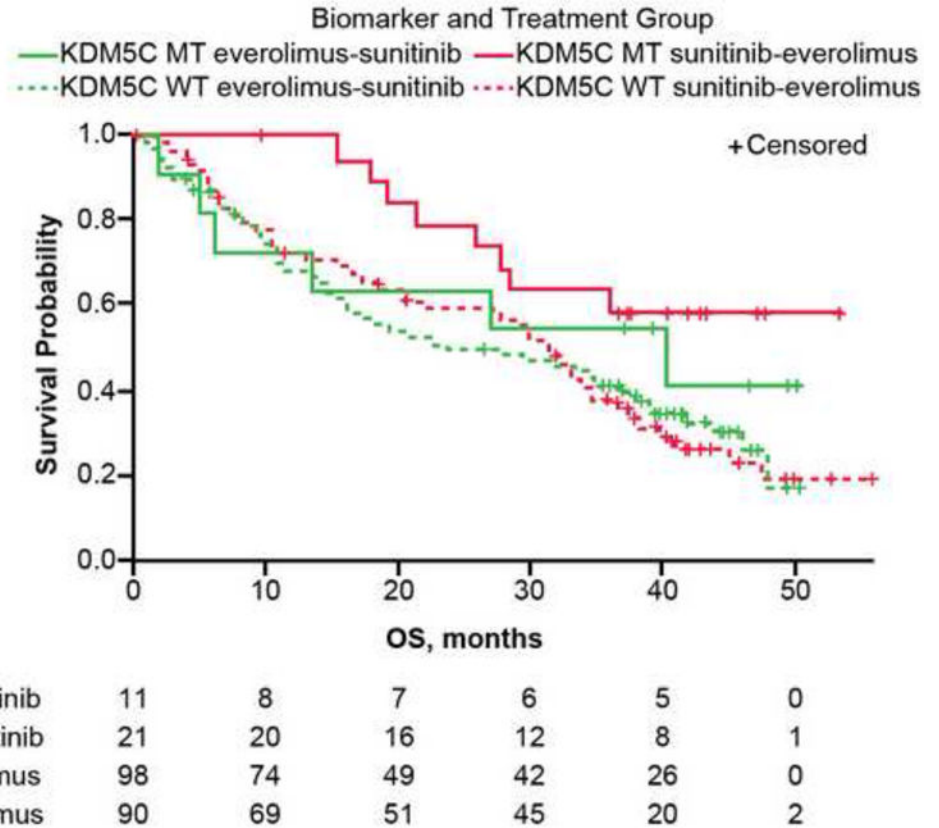


Number of patients at risk

	Time, months										
	0	6	12	18	24	30	36	42	48	54	60
BAP1 MT & PBRM1 WT/MT	20	17	14	13	10	9	7	3	1		
BAP1 WT & PBRM1 MT	54	50	41	38	32	28	19	9	2	0	
BAP1 WT & PBRM1 WT	37	30	26	23	22	20	16	5	3	1	

Group	n	Median OS	95% CI	
BAP1 Gr. BAP1 MT & PBRM1 WT/MT	20	29.9	8.9	NE
PBRM1 Gr. BAP1 WT & PBRM1 MT	54	33.2	25.8	45.0
WT Gr. BAP1 WT & PBRM1 WT	37	33.1	15.5	39.5

(D)



**Fig. 4.** Overall survival (OS) of RECORD-3 patients with metastatic c clear cell renal cell carcinoma. (A) *BAP1*, *PBRM1*, and *KDM5C* mutation status. OS and *BAP1/PBRM1* tumor mutations in the (B) everlimus-sunitinib (EVE-SUN) arm and the (C) SUN-EVE arm. (D) OS and *KDM5C* tumor mutation status.

CI = confidence interval; Gr = group; MT = mutant type; WT = wild type

**Table 1**

Baseline characteristics of patients with clear cell renal cell carcinoma included in the next-generation sequencing analysis

	<b>First-line everolimus N = 109</b>	<b>First-line sunitinib N = 111</b>	<b>Biomarker analysis set N = 220</b>
Sex, <i>n</i> (%)			
Male	80 (73)	88 (79)	168 (76)
Female	29 (27)	23 (21)	52 (24)
Age, mean (range; yr)	62 (21–89)	62 (32–84)	62 (21–89)
Race, <i>n</i> (%)			
White	78 (72)	90 (81)	168 (76)
Black	1 (1)	3 (3)	4 (2)
Asian	20 (18)	10 (9)	30 (14)
Other	10 (9)	8 (7)	18 (8)
KPS, <i>n</i> (%)			
90	28 (26)	28 (25)	56 (25)
80	20 (18)	12 (11)	32 (14)
70	10 (9)	12 (11)	22 (10)
Missing	51 (47)	59 (53)	110 (50)
Nephrectomy, <i>n</i> (%)			
Yes	98 (90)	101 (91)	199 (90)
No. of metastatic sites, <i>n</i> (%)			
0	1 (<1)	2 (2)	3 (1)
1	21 (19)	28 (25)	49 (22)
2	87 (80)	81 (73)	168 (76)
MSKCC risk group, <i>n</i> (%)			
Favorable	41 (38)	53 (48)	94 (43)
Intermediate	56 (51)	50 (45)	106 (48)
Poor	12 (11)	8 (7)	20 (9)
LDH, median (range; U/l)	169 (102–860)	159 (106–669)	163 (102–860)
Median PFS1L (mo), (95% CI)	8.3 (5.4–10.7)	10.8 (8.1–12.9)	—

KPS = Karnofsky performance status; LDH = lactate dehydrogenase; MSKCC = Memorial Sloan Kettering Cancer Center; PFS1L = progression-free survival first-line.



**Table 2**

Median progression-free survival first-line (PFS1L) by first-line treatment and mutation status of patients with clear cell renal cell carcinoma ( $n = 220$ )

Biomarker	Treatment	Mutation status	Patients (n)	Events (n)	Median PFS1L, mo (95% CI)	Hazard ratio, MT vs WT <sup>a</sup> (95% CI)	Log-rank p value <sup>b</sup>
<i>VHL</i>	Everolimus	WT	29	22	6.5 (3.1–10.9)	0.77 (0.5–1.3)	0.2
		MT	80	62	8.4 (5.3–11.2)		
	Sumitinib	WT	27	17	10.9 (5.4–27.3)	0.88 (0.5–1.5)	0.5
		MT	84	59	10.8 (8.1–13.3)		
<i>PBRM1</i>	Everolimus	WT	64	53	5.5 (3.1–8.4)	0.53 (0.3–0.8)	0.002
		MT	45	31	12.8 (8.1–18.4)		
	Sumitinib	WT	55	36	8.3 (5.8–12.9)	0.79 (0.5–1.3)	0.3
		MT	56	40	11.0 (8.3–13.8)		
<i>SETD2</i>	Everolimus	WT	78	57	8.4 (6.5–11.1)	1.19 (0.7–1.9)	0.2
		MT	31	27	6.6 (3.0–12.1)		
	Sumitinib	WT	77	51	9.8 (8.1–12.4)	0.99 (0.6–1.6)	0.3
		MT	34	25	11.5 (8.1–16.9)		
<i>BAP1</i>	Everolimus	WT	87	65	10.5 (7.3–12.9)	1.84 (1.1–3.2)	0.02
		MT	22	19	4.9 (2.9–8.1)		
	Sumitinib	WT	91	62	11.0 (8.3–13.8)	1.69 (0.9–3.2)	0.06
		MT	20	14	8.1 (3.1–11.3)		
<i>KDM5C</i>	Everolimus	WT	98	74	8.2 (5.3–10.9)	1.06 (0.5–2.1)	0.5
		MT	11	10	9.8 (2.2–16.6)		
	Sumitinib	WT	90	65	8.3 (7.8–11.0)	0.57 (0.3–1.1)	0.03
		MT	21	11	20.6 (12.4–27.3)		
<i>PTEN</i>	Everolimus	WT	100	77	8.3 (5.5–10.7)	1.24 (0.6–2.8)	0.4
		MT	9	7	9.1 (2.1–28.4)		
	Sumitinib	WT	94	62	10.8 (8.1–13.8)	1.52 (0.8–2.8)	0.1
		MT	17	14	10.6 (5.8–12.4)		

CI = confidence interval; MT = mutant type; WT = wild type.

<sup>a</sup>HR for MT versus WT within a treatment arm is computed from a Cox proportional hazard model with terms for mutation status, treatment arm, interaction between treatment arms and mutation status groups, with stratification by Memorial Sloan Kettering Cancer Center risk groups and adjustment for baseline covariates (number of metastatic sites, baseline lactate dehydrogenase levels).

<sup>b</sup>The p values from the log-rank test for difference in survival curves of PFS1L between mutation status groups, within treatment arm are not adjusted for multiple testing.