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ORIGINAL REPORT

Phase II Trial and Correlative Genomic Analysis of Everolimus Plus Bevacizumab in Advanced Non–Clear Cell Renal Cell Carcinoma

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ABSTRACT

Purpose

The decreased effectiveness of single-agent targeted therapies in advanced non–clear cell renal cell carcinoma (ncRCC) compared with clear cell renal cell carcinoma (RCC) supports the study of combination regimens. We evaluated the efficacy of everolimus plus bevacizumab in patients with metastatic ncRCC.

Patients and Methods

In this single-center phase II trial, treatment-naive patients received everolimus 10 mg oral once per day plus bevacizumab 10 mg/kg intravenously every 2 weeks. The primary end point was progression-free survival (PFS) at 6 months. Correlative analyses explored candidate tissue biomarkers through next-generation sequencing.

Results

Thirty-five patients were enrolled with the following histologic subtypes: chromophobe (n = 5), papillary (n = 5), and medullary (n = 2) RCC and unclassified RCC (uRCC, n = 23). The majority of patients had papillary growth as a major component (n = 14). For 34 evaluable patients, median PFS, overall survival, and objective response rate (ORR) were 11.0 months, 18.5 months, and 29%, respectively. PFS varied by histology ($P < .001$), and ORR was higher in patients with significant papillary (seven of 18) or chromophobe (two of five) elements than for others (one of 11). Presence of papillary features were associated with benefit, including uRCC, where it correlated with ORR (43% *v* 11%), median PFS (12.9 *v* 1.9 months), and overall survival (28.2 *v* 9.3 months; $P < .001$). Several genetic alterations seemed to segregate by histology. In particular, somatic mutations in *ARID1A* were seen in five of 14 patients with papillary features but not in other RCC variants. All five patients achieved treatment benefit.

Conclusion

The study suggests efficacy for this combination in patients with ncRCC characterized by papillary features. Distinct mutational profiles among ncRCCs vary according to specific histology.

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INTRODUCTION

Kidney cancer comprises several different malignancies that vary in pathobiology and sensitivity to approved systemic agents. Conventional clear cell renal cell carcinoma (RCC) comprises 60% to 80% of cases; these tumors are uniformly dependent on vascular endothelial growth factor (VEGF) signaling due to functional loss of the von Hippel Lindau protein.[1] The remainder of subtypes are summarized as non–clear cell RCC (ncRCC), but constitute a diverse mixture of heterogeneous malignancies, including papillary, chromophobe, medullary, and collecting duct RCC. Cases that do not meet all criteria for these well-defined subtypes are categorized as unclassified RCC (uRCC).[2]

Although large randomized trials have standardized the therapeutic approach to metastatic clear cell RCC, phase III data to guide the management of metastatic ncRCC are limited to unplanned subgroup analyses.[3] Phase II studies have reported some efficacy with VEGF- and

mammalian target of rapamycin (mTOR)–directed agents across the majority of ncRCC variants.[4-8] However, the antitumor effect observed has been more modest and less durable than what is seen in clear cell RCC. Likely, lower response rates to sunitinib and other antiangiogenic drugs reflect the lack of von Hippel-Lindau loss in ncRCC, with a more heterogeneous underlying molecular biology. Such a hypothesis would favor the use of combination regimens to treat ncRCC and underscores the need to better categorize patients, ideally by integrated histopathologic and molecular criteria, in the development of effective treatment approaches.

Everolimus, a rapalog-type inhibitor of the mTOR complex 1 (mTORC1), and bevacizumab, a recombinant humanized monoclonal antibody directed against VEGF-A, are both approved for the treatment of advanced RCC.[9-11] The combination of everolimus and bevacizumab has been studied in clear cell RCC and was reported as tolerable when both drugs were given concurrently at standard doses.[12] We conducted a single-center phase II study of everolimus plus bevacizumab in treatment-naive patients with advanced ncRCC. Correlative end points were included to refine our definitions of ncRCC variants and explore biomarkers that could enable rational patient selection for future studies.

PATIENTS AND METHODS

Eligibility

Patients age 18 years or older were required to have histologically confirmed, advanced ncRCC, including papillary, chromophobe, collecting duct, and medullary RCC and uRCC, per review at Memorial Sloan Kettering Cancer Center (MSKCC). Advanced disease was defined as unresectable, locally recurrent, or metastatic. Other inclusion criteria were no prior systemic therapy with a VEGF or mTORC1 inhibitor; measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1[13]; Karnofsky performance status $\geq$ 70%; adequate renal, hepatic, and hematopoietic function at baseline; adequately controlled blood pressure; and absence of active brain metastases. The study was approved by the institutional review board at MSKCC; all patients provided written informed consent.

Study Design and Treatment

This was a single-institution, phase II study of everolimus plus bevacizumab in treatment-naive patients with advanced ncRCC sponsored by Novartis (Basel, Switzerland). Patients received concurrent therapy with everolimus (standard dose of 10 mg by mouth once per day) and bevacizumab (standard dose of 10 mg/kg intravenously every 14 days) until disease progression, intolerable toxicity, or withdrawal of consent. Archival tumor tissue and peripheral blood were collected for correlative analyses, including immunohistochemistry (IHC) and next-generation sequencing (NGS), from tumor and germline DNA.

End Points and Clinical Assessments

Cycle length was 28 days; cross-sectional imaging was repeated every two cycles for efficacy assessment per RECIST 1.1.[13] Clinical and laboratory assessment were performed twice during cycle 1 and once during subsequent cycles. Urinalysis and fasting blood draw were conducted every two cycles. Toxicities were assessed after 14 days of study treatment then once per subsequent cycle and graded per Common Terminology Criteria for Adverse Events version 4.0.[14] The protocol guided dose modifications for everolimus toxicity (5 mg once per day and 5 mg every other day). No dose reductions were recommended for bevacizumab, but dosing could be delayed or permanently discontinued if held $>$ 8 weeks. In the event of bevacizumab discontinuation, everolimus treatment could be continued.

Correlative Analyses

Archival tumor tissue was not a requirement for eligibility, but efforts were maximized to obtain banked specimens for all patients. Samples were reviewed by a genitourinary pathologist (Y.-B.C.), who selected areas of high tumor content for analysis.

IHC staining for phosphorylated 4E-BP1 (p4E-BP1), a downstream effector of mTORC1,[15] and ERG, a transcriptional regulator of angiogenesis and vascular homeostasis,[16,17] were performed. Five-micrometer sections were stained with a p4E-BP1 (Thr37/46; 236B4) rabbit monoclonal antibody (Cell Signaling Technologies, Danvers, MA) or an ERG (EPR3864) rabbit monoclonal antibody (Ventana Medical Systems, Tucson, AZ) by using an automated platform (DISCOVERY XT; Ventana Medical Systems). A p4E-BP1 H score (range, 1 to 300) was calculated for each patient by multiplying the percentage of positive cells by the corresponding staining intensity (1 to 3). For ERG staining, an average count of positively stained endothelial cells per square millimeter (approximately five high-power fields) was documented.

For NGS analysis, we used the MSKCC IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) platform as previously described.[18] In the version used here, the assay achieves pull-down capture with target-specific probes for exons from 341 cancer-related genes, including oncogenes, tumor suppressor genes, and components of pathways deemed actionable by targeted therapies (Appendix Table A1, online only, provides a full list). Deep-coverage NGS is then performed on an Illumina HiSeq system (San Diego, CA) across all coding sequences of these genes of interest. Coverage rate of targeted coding sequences and tumor-to-normal coverage ratios are used to investigate copy number alterations for individual genes.

Statistical Analysis

The primary end point was progression-free survival (PFS) after 6 months, per RECIST 1.1.[13] Secondary end points were objective response rate (ORR), overall survival (OS), and treatment-emergent adverse events.

With a single-stage design for 34 patients, the regimen was to be considered promising if 22 or more patients were progression free at 6 months. Patients who left the study sooner without documented progression or as a result of death were conservatively treated as events for primary end point analysis. The design discriminates between true 6-month PFS rates $\leq$ 50% and $\geq$ 70%, with type I and II error rates of 6% and 19%, respectively. A 6-month PFS rate of 50% was chosen on the basis of phase II data for single-agent sunitinib in the same target population,[4] the agent most frequently chosen for the treatment of advanced ncRCC outside of clinical trials.

PFS and OS were also calculated by using time-to-event methods to account for censoring. PFS was defined as time from treatment start to disease progression or death within 28 days of treatment end. Patients who did not progress or die within 28 days of treatment end were censored at the date of treatment end or last clinic visit. OS was defined as time from treatment start to death as a result of any cause. PFS and OS were calculated by using the Kaplan-Meier method, with the log-rank test used for subgroup comparisons. The ORR was calculated for the entire cohort and specific histologic subgroups. Correlation between IHC scores and achievement of 6-month PFS was investigated by Wilcoxon rank sum test. The NGS analysis was descriptive without formal statistical design. Statistical analysis was performed with SAS 9.4 (SAS Institute, Cary, NC) and R 3.1.0 (survival and Hmisc packages) software. Data cutoff was November 2015.

RESULTS

Baseline Characteristics

Thirty-five patients were enrolled and treated in the trial; clinical and pathologic features are summarized in Table 1. The majority of patients were categorized as favorable (29%) or

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Table 1. Characteristics of the Study Population

Characteristic	No. (%)
No. of patients	35 (100)
Median age, years (range)	54 (19-75)
Sex	
Female	8 (23)
Male	27 (77)
Karnofsky performance status	
80%	14 (40)
90%	21 (60)
Histology subtype	
Unclassified	23 (66)
Unclassified, papillary features	14 (40)
Unclassified, other	9 (26)
Papillary	5 (14)
Chromophobe	5 (14)
Medullary	2 (6)
Nephrectomy	
Yes	27 (77)
No	8 (23)
MSKCC risk group	
Favorable	10 (29)
Intermediate	24 (69)
Poor	1 (3)
Heng risk group	
Favorable	10 (29)
Intermediate	21 (60)
Poor	4 (11)

Abbreviation: MSKCC, Memorial Sloan Kettering Cancer Center.

intermediate (69%) risk according to MSKCC criteria.[19] Histologic subtypes were uRCC (n = 23) and papillary (n = 5), chromophobe (n = 5), and medullary (n = 2) RCC. Patients with papillary RCC did not meet sufficient criteria to be further divided into type 1 or 2 subgroups. Within the large uRCC subgroup, several tumors were noted to have prominent papillary architectural features yet did not fulfill other criteria needed to establish a diagnosis of papillary RCC. During the course of the study, treatment benefit was noted for several patients within this subgroup. Consequently, after accrual was complete, a designated committee of genitourinary pathologists reviewed the specimens again for all 23 patients with uRCC. Blinded to treatment outcome, the committee divided cases of uRCC into two categories: uRCC with multinodular, intracystic papillary growth as a major component (uRCC with papillary features) or uRCC with various growth patterns without these specific papillary features specifically (uRCC without papillary features). Papillary features were seen in 14 of 23 patients with uRCC, and dedicated analyses for this subgroup were performed.

Efficacy

One patient was taken off the trial after the first dose of bevacizumab because of the need for a surgical procedure unrelated to his cancer or the study treatment. He was not evaluable and thus replaced with another subject. Efficacy outcomes for the 34 evaluable subjects are summarized in Table 2. The median follow-up for patients who remained alive was 23.6 months. Eighteen (53%) subjects were alive and progression free after 6 months and 10 (29%) after 12 months; two were still receiving active study treatment at the time of report (30.4 and 20.2 months on treatment, respectively; Fig 1A).

PFS varied significantly by histology (log-rank $P < .001$), as did the rate of patients who achieved a PFS $\geq$ 6 months (three of five with chromophobe RCC, zero of two with medullary RCC, two of four with papillary RCC, 12 of 14 with uRCC with papillary features, and one of nine with uRCC without papillary features). Objective responses were observed in a sizable proportion of subjects with significant papillary (seven of 18) or chromophobe (two of five) tumor components but rarely in patients with uRCC without papillary features (one of nine) or those with medullary RCC (zero of two; Table 2; Fig 1B).

For patients with uRCC, the presence (n = 14) or absence (n = 9) of a major papillary component correlated strongly with ORR (43% *v* 11%), median PFS (12.9 *v* 1.9 months), and median OS (28.2 *v* 9.3 months; each log-rank for curves, $P < .001$; Fig 2). We compared baseline clinical features that could account for such discrepancies in outcome, including extent of disease, nephrectomy status, and MSKCC and International Metastatic Renal Cell Carcinoma Database Consortium risk status, but found no significant difference between the two subgroups (Appendix Table A2, online only). Only one subject with uRCC without papillary features remained on treatment > 6 months. In this subject, bevacizumab was discontinued after 95 days on study due to proteinuria. At the time, radiographic assessment was consistent with a partial response (PR). She then continued study treatment with everolimus monotherapy and ultimately achieved a complete response (CR). At the time of this report (20.2 months) this patient was still receiving treatment. As noted below, this subject's tumor analysis revealed the presence of a somatic mutation in the kinase domain region of *MTOR*.

Table 2. Summary Efficacy Analysis

Group	PR	CR	SD	PD	Median PFS (months)	95% CI	6-Month PFS* (%)
Full cohort (n = 34)†	9	1	15	8	11.0	3.8 to 19.3	60
Unclassified RCC with papillary features (n = 14)	6	0	8	0	12.9	10.9 to NA	92
Unclassified RCC without papillary features (n = 9)†	0	1	3	4	1.9	1.6 to NA	11
Chromophobe RCC (n = 5)	2	0	2	1	NR	1.9 to NA	75
Papillary RCC (n = 4)	1	0	2	1	13.8	1.4 to NA	75
Medullary RCC (n = 2)	0	0	0	2	1.7	1.6 to NA	0

Abbreviations: CR, complete response; NA, not applicable; NR, not reached; PD, progressive disease; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; SD, stable disease.
*Kaplan-Meier estimates.
†One patient with unclassified RCC experienced clinical disease progression and death related to cancer before radiographic disease reassessment during the trial could be obtained. Although the patient is evaluable for PFS analysis (per time of clinical progression/death), radiographic response assessment was not performed.

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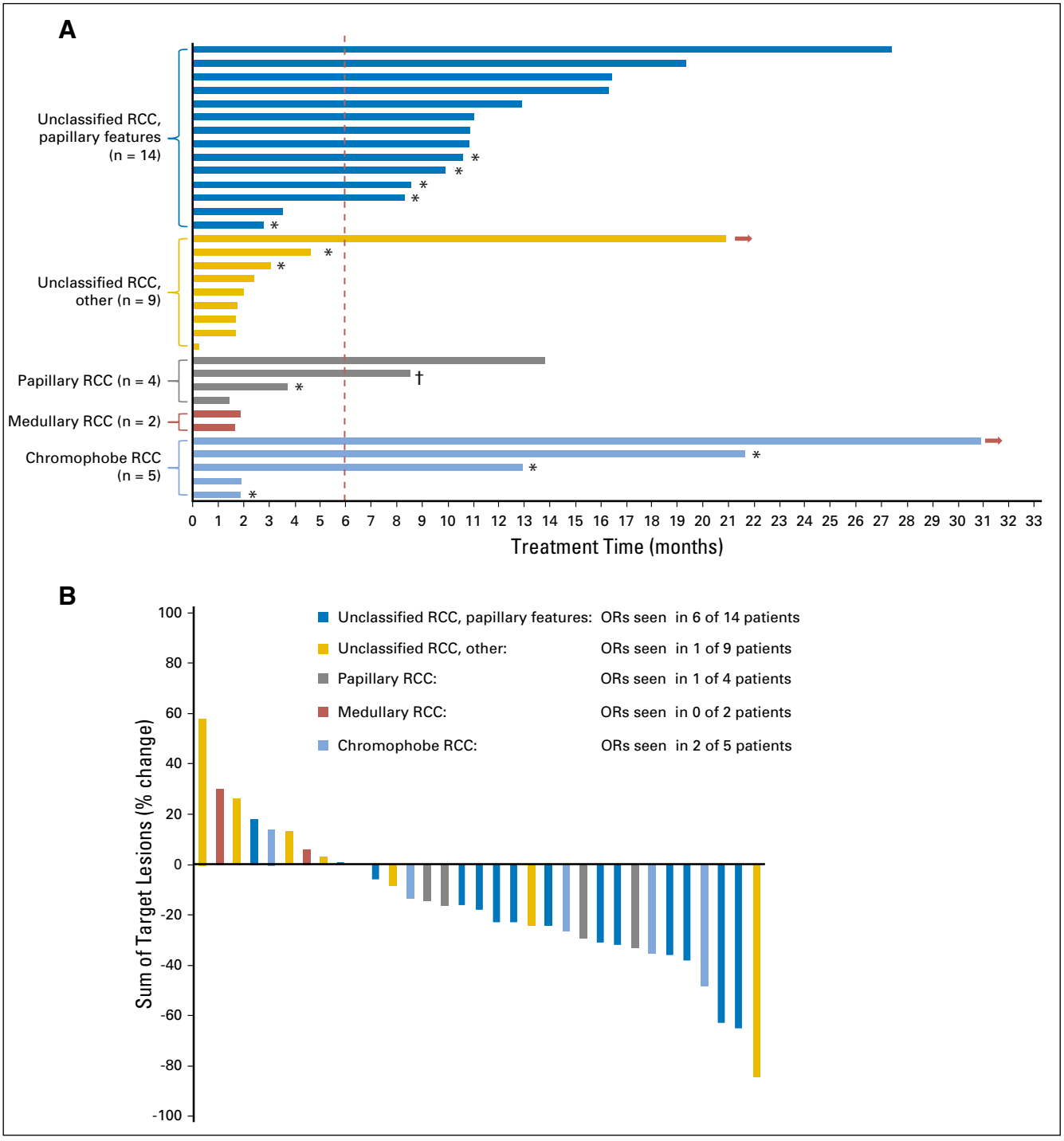

Fig 1. Efficacy assessment by subject (colors encode renal cell carcinoma [RCC] variants). (A) Swimmer plot depicts individual patients as lines. Arrows indicate patients who remained on therapy. The primary end point for the trial was progression-free survival at 6 months (vertical line). (B) The best radiographic response for 33 evaluable patients treated. Best response was assessed per Response Evaluation Criteria in Solid Tumors 1.1. OR, objective response. (*) Discontinuation due to toxicity. (†) Voluntary withdrawal from study.

Toxicity

Treatment-emergent adverse events are summarized in Table 3. Treatment was generally well tolerated, although low-grade toxicities were commonly seen. High-grade (Common Terminology Criteria for Adverse Events grade 3 or greater) events were infrequent with the exception of hyperglycemia (11%), hypertriglyceridemia (14%), lymphopenia (20%), hypertension (29%), and proteinuria (18%), all established, class-specific events for mTORC1 or VEGF inhibitors. Eleven (32%) subjects required everolimus dose reductions, all for class-specific toxicities, including mucositis, cytopenias, and fatigue. Proteinuria developed in > 70% of patients (grade 2 or less for most). Nephrotic-range proteinuria developed in three cases, and eight (24%)

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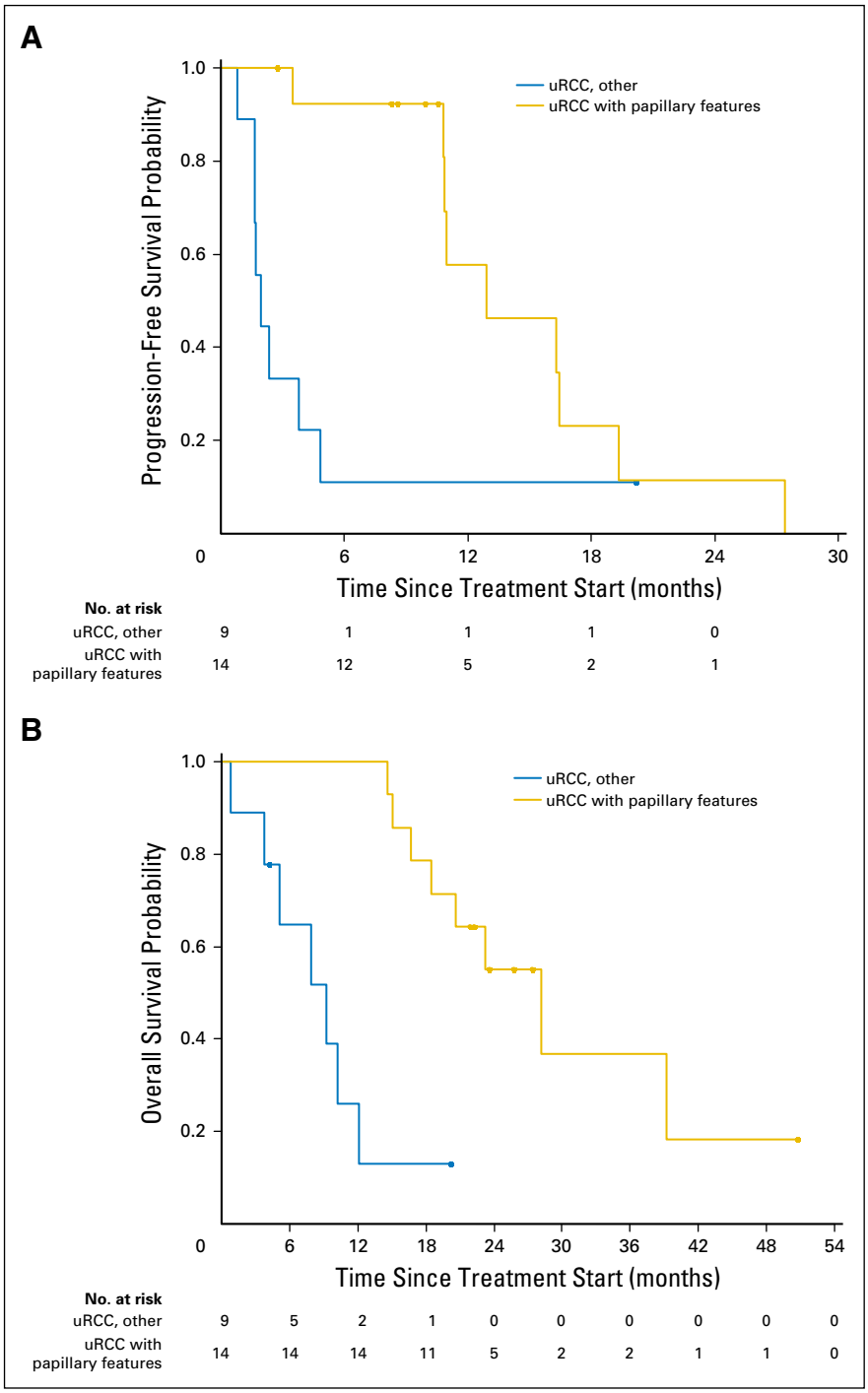

Fig 2. Kaplan-Meier curves that summarize outcomes for patients with unclassified renal cell carcinoma (uRCC). Separate curves depict the comparison between subjects with uRCC with (n = 14; red line) and without (n = 9; black line) significant papillary features. (A) Median progression-free survival differed significantly between groups (12.9 *v* 1.9 months; log-rank $P = .01$). (B) Median overall survival was significantly longer for uRCC with papillary features than for other uRCC variants (28.2 *v* 9.3 months; log-rank $P < .001$).

had to discontinue bevacizumab permanently because of a persistent urine protein/creatinine ratio > 2. They continued everolimus as monotherapy until criteria for removal from trial were met. Two patients died while on study, both from a gastrointestinal hemorrhage. One death was related to progressive disease and the other possibly to bevacizumab.

Correlative Analysis

For 28 subjects, archival tissue was analyzed with deep targeted sequencing across 341 cancer-related genes; matched germline comparison from healthy cells was included for 17 of these. Average depth of coverage was 558 times. Findings are summarized in Figure 3. Although limited by small numbers, specific genetic alterations seemed to segregate by histology. Recurrent events included *TP53* mutations in three of five patients with chromophobe RCC. Tumors from five of 14 subjects with a major papillary component (including papillary and uRCC variants) harbored mutations in *ARID1A*, whereas none were detected in the other RCC variants tested. All patients with *ARID1A* mutations achieved the primary end point of 6-month

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Table 3. Treatment-Emergent Adverse Events of Interest

Toxicity	No. (%)	
	All Grades	Grade 3/4
Constipation	17 (49)	1 (3)
Cough	15 (43)	0 (0)
Diarrhea	20 (57)	0 (0)
Dyspnea	20 (57)	2 (6)
Edema, limbs	13 (37)	1 (3)
Epistaxis	24 (69)	0 (0)
Fatigue	29 (83)	2 (6)
Hypertension	25 (74)	10 (29)
Mucositis, oral	28 (80)	1 (3)
Nausea/vomiting	21 (60)	0 (0)
Pain	25 (71)	4 (11)
Peripheral sensory neuropathy	13 (37)	0 (0)
Pleural effusion	11 (31)	0 (0)
Proteinuria	26 (77)	6 (18)
Rash	22 (63)	0 (0)
Sore throat	7 (20)	0 (0)
ALP increase	20 (57)	0 (0)
AST/ALT increased	26 (74)	3 (9)
Creatinine increased	19 (54)	0 (0)
Hyperglycemia	30 (86)	4 (11)
Hyperkalemia	11 (31)	1 (3)
Hypernatremia	10 (29)	0 (0)
Hypertriglyceridemia	24 (69)	5 (14)
Lymphocyte count decreased	7 (20)	7 (20)
Platelet count decreased	20 (57)	0 (0)
WBC decreased	17 (49)	0 (0)

Abbreviation: ALP, alkaline phosphatase.

PFS, three of five with radiographic PR. Functional loss of fumarate hydratase was detected in tumors from four patients with uRCC with papillary features (all meeting the primary PFS end point) and one patient with uRCC without papillary features (who did not meet the PFS end point). As aforementioned, one patient with uRCC without papillary features achieved a durable CR and remains on study treatment; NGS analysis of her tumor tissue revealed the presence of an *MTOR* L2427R mutation that maps to the gene's kinase domain. Functional study confirmed this mutation to be activating.

IHC testing for p4E-BP1 and ERG was performed on archival samples from 19 subjects. Six-month PFS correlated neither with p4E-BP1 IHC score ($P = .96$; Appendix Fig A1, online only) nor with microvessel density per number of ERG-positive cells per square millimeter ($P = .38$; Appendix Fig A1).

DISCUSSION

We tested everolimus plus bevacizumab in patients with advanced ncRCC, a heterogenous group of diseases with poorly defined standards of care.[20] Although the trial did not meet its primary end point, a striking signal was observed for defined histologic subgroups, specifically those with a significant papillary tumor component. The ORR in this group was 39%, which is comparable with those reported for VEGF-targeted therapies in phase II trials of clear cell RCC.[21,22] The majority of responses were durable, with 14 (78%) of 18 patients progression free at 6 months and the median PFS eclipsing 1 year (12.9 months). These outcomes compare favorably with those reported for first-line sunitinib in the largest efforts to date, the phase II ASPEN (Randomized Phase II Study of Afinitor [RAD001] vs. Sutent [Sunitinib] in Patients With Metastatic Non-Clear Renal Cell Carcinoma) and ESPN (Everolimus Versus Sunitinib Prospective Evaluation in Metastatic Non-Clear Cell Renal Cell Carcinoma) trials (median PFS, 8.3 and 4.1 months, respectively, among subjects with papillary histology).[7,8,23]

Activity was observed for patients with chromophobe RCC. Three of five remained on treatment for > 12 months, which adds to prior reports of benefit with rapalog therapies in this subgroup.[6,7,23,24] Subjects with other variants (medullary RCC and uRCC without papillary features), achieved little or no benefit from everolimus plus bevacizumab. Novel treatment approaches beyond VEGF- and mTOR-directed therapy are needed for such patients.

We had conducted a phase I study of sunitinib plus everolimus in 20 treatment-naive patients, including seven with ncRCC.[25] Three of the seven (papillary, chromophobe,

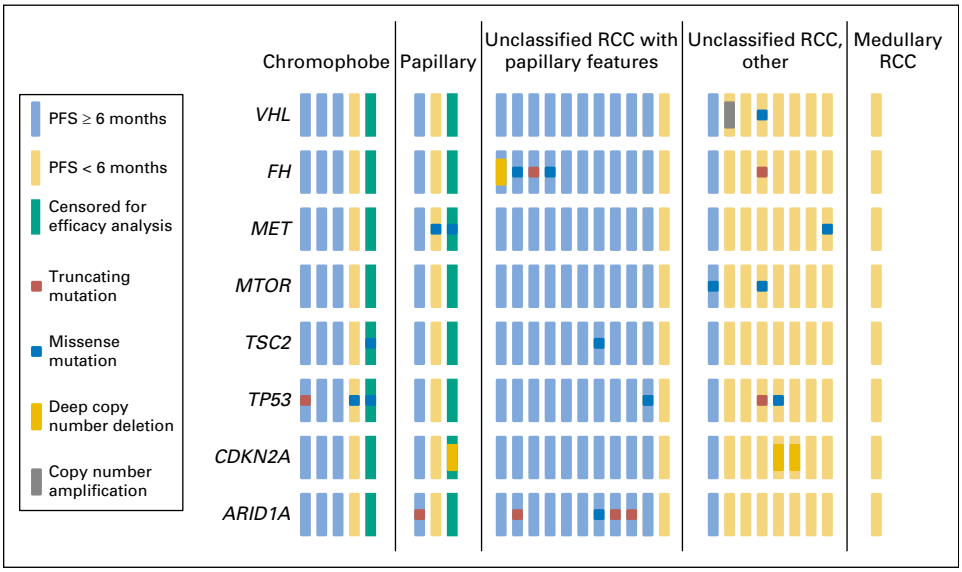

Fig 3. Oncogenomic changes detected by deep-sequence analysis from archival tumor specimens across 28 subjects. Columns represent individual subjects; rows represent selected genes of interest examined for each sample. Subjects who reached the primary efficacy goal of 6 months progression-free survival (PFS) are marked blue; those who did not are marked gold. Subjects censored within their first 6 months of the trial are marked gray. Colored squares mark the presence of somatic alterations detected by sequence analysis. *ARID1A* mutations were seen in five of 14 tumors with major papillary features; no *ARID1A* mutations were seen in all other renal cell carcinoma (RCC) variants. For patients with *ARID1A* mutations, PFS was > 6 months in five of five, and three of the five achieved a partial response.

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chromophobe) achieved a PR, and all three remained on protocol for > 1 year. The current findings lend further support to combining mTOR- and VEGF-directed therapy in the papillary and chromophobe variants.

This prospective study is the first to our knowledge to report on defined subgroups of uRCC and to identify histologic hallmarks that seem to correlate with treatment benefit. Most uRCC tumors in this study (14 of 23) contained multinodular, intracystic papillary growth as a major component (uRCC with papillary features). These subjects were significantly more likely to benefit from treatment, as reflected by superior ORR, PFS, and OS (Fig 2). Of note, nine of 14 subjects with uRCC with papillary features were referred for management from outside institutions, where the majority had been classified as papillary RCC. Pathologic re-review at MSKCC determined that they did not meet sufficient criteria for a formal diagnosis of papillary RCC, and they were recategorized as having uRCC with papillary features. Others have investigated whether papillary RCC and uRCC with papillary morphology originate from a common cell of origin but were unable to demonstrate a unifying genomic background.[26] Moreover, recent work suggested a number of oncogenomic variants within the spectrum of papillary RCC.[26,27] The Southwest Oncology Group recently opened SWOG 1500 (NCT02761057), the largest prospective effort in papillary RCC to date, which incorporates not only a randomized comparison of various targeted treatment strategies, but also various correlative end points to better define the landscape of papillary RCC.

Our NGS analysis, although limited by sample size, yielded a number of thought-provoking findings. Acquired mutations in *ARID1A* were detected in five of 14 tumors with major papillary components (Fig 3), with all five subjects achieving benefit with a PFS > 6 months. No *ARID1A* mutations were detected in patients with PFS < 6 months, nor were any seen in tumors without papillary components. *ARID1A* is a member of the switch/sucrose nonfermentable chromatin-remodeling complex, which modulates DNA accessibility to other cellular machinery (repair, transcription, duplication) through regulation of nucleosome repositioning. Somatic mutations in *ARID1A* are frequently seen across human malignancies, and a haploinsufficient effect that promotes oncogenesis has been described.[28,29] The exact mechanisms of its cancer-promoting effects remain largely unknown. Recent preclinical reports indicate that loss of *ARID1A* function and abnormal phosphatidylinositol 3-kinase (PI3K) pathway signaling can converge in tumorigenesis, which provides a rationale for targeting the PI3K/mTORC1 signaling axis in *ARID1A* mutant tumors.[30] However, more recent data from in vitro breast cancer models suggest that loss of *ARID1A* through activated annexin A1 expression may confer resistance to mTOR inhibition.[31] Although the current results support the former hypothesis, the small sample size prohibits conclusions from being drawn. Furthermore, concurrent bevacizumab may play an important role that is not accounted for in these models. NGS analysis of papillary RCC in The Cancer Genome Atlas project identified mutations in members of the switch/sucrose nonfermentable complex in 20% and 27% of papillary type 1 and 2 tumors, respectively.[27] Altogether, the data suggest that *ARID1A* merits further study for its functional role in papillary RCC variants and as a candidate biomarker for future study of everolimus plus bevacizumab.

Prior reports have linked alterations in PI3K pathway components, particularly *TSC1*, *TSC2*, and *MTOR*, to exceptional benefit in patients with RCC treated with rapalog monotherapy[32] and mTORC1/VEGF-directed combination therapy.[33] Detection of an *MTOR* kinase domain mutation (L2427R) in a subject with uRCC, who achieved a CR with single-agent everolimus (after early discontinuation of bevacizumab) adds to these data. A subject with an R1369W missense mutation in *TSC2* continued to receive study treatment > 6 months, but a concurrent *ARIDA1* mutation may have also accounted for his treatment response. One subject with uRCC who harbored a Y1151C missense mutation in *MTOR* did not benefit from the study treatment most likely because the alteration did not affect a functionally relevant portion of the gene.

In summary, this study demonstrates a benefit of everolimus plus bevacizumab in ncRCC, particularly in variants with significant papillary elements. Future studies are warranted and should narrow histologic entry criteria. The data also illustrate distinct mutational profiles among ncRCCs that vary according to specific histology and identify *ARID1A* as a potential marker of papillary architecture and as a candidate predictive biomarker in future trials of this promising regimen.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Phase II Trial and Correlative Genomic Analysis of Everolimus Plus Bevacizumab in Advanced Non–Clear Cell Renal Cell Carcinoma

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Appendix

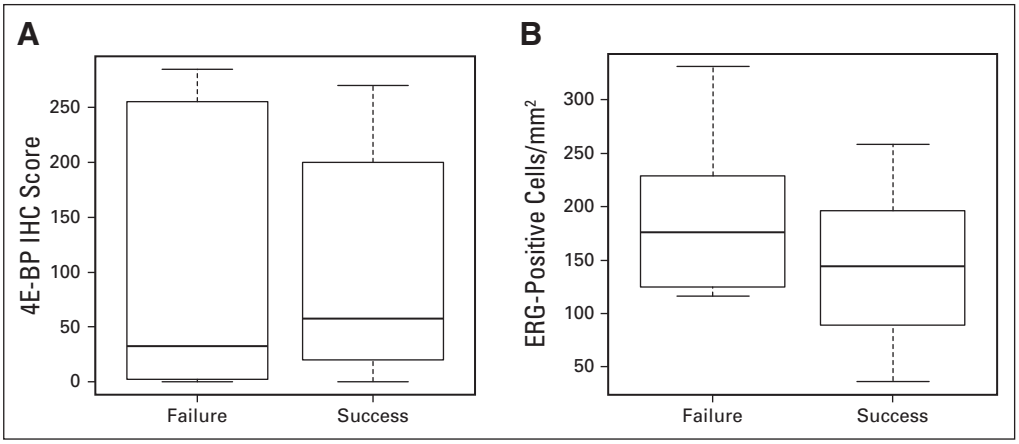

Fig A1. Analysis of archival tissue by immunohistochemistry (IHC) failed to demonstrate correlates of treatment benefit. (A) Box plot of median and range of 4E-BP1 IHC scores in patients who experienced disease progression or died within $<$ 6 months of starting the study treatment (failure) versus those alive and free from disease progression at $\geq$ 6 months (success; median score, 32.5 *v* 57.5; Wilcoxon rank sum $P = .96$). (B) Box plot of median and range of ERG-positive cells/mm^2 in patients who experienced disease progression or died within $<$ 6 months of starting study treatment (failure) versus those alive and free from disease progression (success; median score, 176 *v* 144; Wilcoxon rank sum $P = .38$).

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Table A1. Target-Specific Probes for Exons From 341 Cancer-Related Genes and Components of Pathways Deemed Actionable by Targeted Therapies

	CCND2	EPHB1	HGF	MDC1	PBRM1	RET	TGFBR2
ABL1	CCND3	ERBB2	HIST1H1C	MDM2	PDCD1	RFWD2	TMEM127
AKT1	CCNE1	ERBB3	HIST1H2BD	MDM4	PDGFRA	RHOA	TMPRSS2
AKT2	CD274	ERBB4	HIST1H3B	MED12	PDGFRB	RICTOR	TNFAIP3
AKT3	CD276	ERCC2	HNF1A	MEF2B	PDPK1	RIT1	TNFRSF14
ALK	CD79B	ERCC3	HRAS	MEN1	PHOX2B	RNF43	TOP1
ALOX12B	CDC73	ERCC4	ICOSLG	MET	PIK3C2G	ROS1	TP53
APC	CDH1	ERCC5	IDH1	MITF	PIK3C3	RPS6KA4	TP63
AR	CDK12	ERG	IDH2	MLH1	PIK3CA	RPS6KB2	TRAF7
ARAF	CDK4	ESR1	IFNGR1	MLL	PIK3CB	RPTOR	TSC1
ARID1A	CDK6	ETV1	IGF1	MLL2	PIK3CD	RUNX1	TSC2
ARID1B	CDK8	ETV6	IGF1R	MLL3	PIK3CG	RYBP	TSHR
ARID2	CDKN1A	EZH2	IGF2	MPL	PIK3R1	SDHA	U2AF1
ARID5B	CDKN1B	FAM123B	IKBKE	MRE11A	PIK3R2	SDHAF2	VHL
ASXL1	CDKN2A	FAM175A	IKZF1	MSH2	PIK3R3	SDHB	VTCN1
ASXL2	CDKN2B	FAM46C	IL10	MSH6	PIM1	SDHC	WT1
ATM	CDKN2C	FANCA	IL7R	MTOR	PLK2	SDHD	XIAP
ATR	CHEK1	FANCC	INPP4A	MUTYH	PMAIP1	SETD2	XPO1
ATRX	CHEK2	FAT1	INPP4B	MYC	PMS1	SF3B1	YAP1
AURKA	CIC	FBXW7	INSR	MYCL1	PMS2	SH2D1A	
AURKB	CREBBP	FGF19	IRF4	MYCN	PNRC1	SHQ1	
AXIN1	CRKL	FGF3	IRS1	MYD88	POLE	SMAD2	
AXIN2	CRLF2	FGF4	IRS2	MYOD1	PPP2R1A	SMAD3	
AXL	CSF1R	FGFR1	JAK1	NBN	PRDM1	SMAD4	
B2M	CTCF	FGFR2	JAK2	NCOR1	PRKAR1A	SMARCA4	
BAP1	CTLA4	FGFR3	JAK3	NF1	PTCH1	SMARCB1	
BARD1	CTNNB1	FGFR4	JUN	NF2	PTEN	SMARCD1	
BBC3	CUL3	FH	KDM5A	NFE2L2	PTPN11	SMO	
BCL2	DAXX	FLCN	KDM5C	NKX2-1	PTPRD	SOCS1	
BCL2L1	DCUN1D1	FLT1	KDM6A	NKX3-1	PTPRS	SOX17	
BCL2L11	DDR2	FLT3	KDR	NOTCH1	PTPRT	SOX2	
BCL6	DICER1	FLT4	KEAP1	NOTCH2	RAC1	SOX9	
BCOR	DIS3	FOXA1	KIT	NOTCH3	RAD50	SPEN	
BLM	DNMT1	FOXL2	KLF4	NOTCH4	RAD51	SPOP	
BMPR1A	DNMT3A	FOXP1	KRAS	NPM1	RAD51B	SRC	
BRAF	DNMT3B	FUBP1	LATS1	NRAS	RAD51C	STAG2	
BRCA1	DOT1L	GATA1	LATS2	NSD1	RAD51D	STK11	
BRCA2	E2F3	GATA2	LMO1	NTRK1	RAD52	STK40	
BRD4	EED	GATA3	MAP2K1	NTRK2	RAD54L	SUFU	
BRIP1	EGFL7	GNA11	MAP2K2	NTRK3	RAF1	SUZ12	
BTK	EGFR	GNAQ	MAP2K4	PAK1	RARA	SYK	
CARD11	EIF1AX	GNAS	MAP3K1	PAK7	RASA1	TBX3	
CASP8	EP300	GREM1	MAP3K13	PALB2	RB1	TERT	
CBFB	EPCAM	GRIN2A	MAPK1	PARK2	RBM10	TET1	
CBL	EPHA3	GSK3B	MAX	PARP1	RECQL4	TET2	
CCND1	EPHA5	H3F3C	MCL1	PAX5	REL	TGFBR1	

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Table A2. Baseline Clinical Features for Patients With uRCC With and Without Papillary Features

Feature	uRCC, No. (%)			*P**
	All Cases	With Papillary Features	Without Papillary Features	
No. of patients	23 (100)	14 (100)	9 (100)	
KPS				.36
80%	7 (30)	3 (21)	4 (44)	
90%	16 (70)	11 (79)	5 (56)	
Nephrectomy				.99
Yes	18 (78)	11 (79)	7 (78)	
No	5 (22)	3 (21)	2 (22)	
MSKCC risk group				.59
Favorable	6 (26)	4 (29)	2 (22)	
Intermediate	16 (70)	10 (71)	6 (67)	
Poor	1 (4)	0	1 (11)	
IMDC risk group				.99
Favorable	6 (26)	4 (29)	2 (22)	
Intermediate	15 (65)	9 (64)	6 (67)	
Poor	2 (9)	1 (7)	1 (11)	
Metastatic sites				.41
1	6 (26)	5 (36)	1 (11)	
2	6 (26)	4 (29)	2 (22)	
≥ 3	11 (48)	5 (36)	6 (67)	

Abbreviations: IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; KPS, Karnofsky performance status; MSKCC, Memorial Sloan Kettering Cancer Center; uRCC, unclassified renal cell carcinoma.
*Fisher exact test.

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