VOLUME 34 · NUMBER 14 · MAY 10, 2016

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Randomized Open-Label Phase II Trial of Apitolisib (GDC-0980), a Novel Inhibitor of the PI3K/Mammalian Target of Rapamycin Pathway, Versus Everolimus in Patients With Metastatic Renal Cell Carcinoma

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Published online ahead of print at www.jco.org on March 7, 2016.

Supported by Genentech, South San Francisco, CA.

Presented in part at the 50th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, May 30-June 3, 2014, and at the American Association for Cancer Research Special Conference: Targeting the PI3K-mTOR Network in Cancer, Philadelphia, PA, September 14-17, 2014.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Clinical trial information: NCT01442090.

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0732-183X/16/3414w-1660w/$20.00

DOI: 10.1200/JCO.2015.64.8808

ABSTRACT

Purpose

To the best of our knowledge, this study is the first to compare dual inhibition of PI3K/mammalian target of rapamycin (mTOR) by apitolisib (GDC-0980) against single inhibition of mTORC1 by everolimus in metastatic renal cell carcinoma (mRCC).

Patients and Methods

Patients with clear-cell mRCC who progressed on or after vascular endothelial growth factor–targeted therapy were randomly assigned to apitolisib 40 mg once per day or to everolimus 10 mg once per day. End points included progression-free survival, safety, overall survival, and objective response rate. Biomarker assessments were conducted.

Results

Eighty-five patients were randomly assigned. After 67 events, stratified analysis revealed that median progression-free survival was significantly shorter for apitolisib than for everolimus (3.7 *v* 6.1 months; hazard ratio, 2.12 [95% CI, 1.23 to 3.63; $P < .01$]); apitolisib was not favored in any stratification subgroup. Median overall survival was not significantly different but trended in favor of everolimus (16.5 *v* 22.8 months; hazard ratio, 1.77 [95% CI, 0.97 to 3.24; $P = .06$]). The objective response rate was 7.1% for apitolisib and 11.6% for everolimus. Patients administered apitolisib with a greater incidence of grade 3 to 4 adverse events were more likely to discontinue treatment (31% *v* 12% for everolimus). No drug-related deaths were observed. Apitolisib in comparison with everolimus was associated with substantially more high-grade hyperglycemia (40% *v* 9%) and rash (24% *v* 2%). Apitolisib pharmacokinetics suggested a relationship between exposure, and rash and hyperglycemia. Retrospective biomarker analyses revealed a relationship between *VHL* mutation status and outcome with everolimus but not with apitolisib. High hypoxia-inducible factor 1α protein expression was associated with better outcome in both arms.

Conclusion

This study demonstrated that dual PI3K/mTOR inhibition by apitolisib was less effective than was everolimus in mRCC, likely because full blockade of PI3K/mTOR signaling resulted in multiple on-target adverse events. *VHL* mutation and hypoxia-inducible factor 1α expression may be predictive of an mTOR inhibitor benefit, although prospective validation is required.

J Clin Oncol 34:1660-1668. © 2016 by American Society of Clinical Oncology

INTRODUCTION

Targeted therapies directed towards key signaling pathway components, including vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR), are currently the standard of care for metastatic clear-cell renal cell carcinoma (RCC), although their absolute clinical benefit remains limited.[1,2] Up to 70% of RCC cases are of the clear cell type, and approximately 90% of patients with clear-cell RCC exhibit somatic loss of *VHL* gene expression through genetic/epigenetic mechanisms.[3,4] This results in dysregulation of hypoxia-inducible factor (HIF) 1α protein ubiquitination, elevated HIF1α and HIF2α levels, and

up-regulation of VEGF expression and signaling,[5] indicating angiogenesis seems to play a central role in clear-cell RCC.[2] The activity of mTORC1 also contributes to angiogenesis through regulation of HIF1α transcription and its cap-dependent translation.[6,7] Thus, loss of *VHL* expression and activation of mTORC1 signaling converge on enhanced HIF expression, thereby fueling angiogenic signaling in clear-cell RCC.

The rapalogs everolimus and temsirolimus have proven clinical efficacy in advanced and metastatic RCC (mRCC).[8,9] They were designed to inhibit two structurally and functionally distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), both of which stabilize the expression of HIF1α; mTORC2 also stabilizes HIF2α.[10] A limitation is that although mTORC1 is sensitive to rapalogs, mTORC2 generally is not.[11] Furthermore, inhibition of mTORC1 alone results in the loss of negative feedback inhibition of mTORC2 by mTORC1.[12] The subsequent increased activation of mTORC2 not only stabilizes HIF2α but also enhances PI3K/AKT-mediated proliferation and cell survival.[13,14] Preclinical studies including in vitro and in vivo experiments on RCC cell lines suggest that dual inhibition of PI3K/mTOR induces growth arrest and antitumor activity more effectively than does inhibition of mTORC1 alone.[15] Together, these observations support an approach to concurrently target mTORC1, mTORC2, and PI3K in mRCC to improve the efficacy of rapalogs.

Apitolisib is a small molecule pan-PI3K and mTOR (mTORC1/2) inhibitor that potently blocks PI3K/mTOR pathway signaling in cancer cell lines and has demonstrated significant antitumor activity in tumor xenografts.[16] A phase I study of apitolisib demonstrated encouraging preliminary clinical activity at the recommended phase II dose of 40 mg.[17] Here, we present the results of what we believe is the first randomized trial of a dual PI3K/mTOR inhibitor against an approved mTORC1 inhibitor, everolimus, in clear-cell mRCC. Comprehensive exploratory biomarker analysis of the PI3K/mTOR pathway and key angiogenesis regulators was also undertaken.

PATIENTS AND METHODS

Study Design and Participants

Study PIM4973 (ROVER) was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was obtained from all patients before enrollment in agreement with approved protocols from the ethics committees at each study site.

This phase II, multicenter, international, open-label, randomized (1:1) trial was designed to evaluate apitolisib versus everolimus in patients with VEGF-refractory mRCC. Eligible patients included those 18 years of age or older, with metastatic clear-cell RCC and progression of disease after exposure to at least one VEGF pathway-targeted (VEGF-targeted) therapy, including but not limited to, pazopanib, sunitinib, sorafenib, and bevacizumab. Exposure to mTOR inhibitors was an exclusion criterion. Patients were required to have measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1[18] and to be randomly assigned within 6 months of stopping VEGF-targeted therapy. A Karnofsky performance status of at least 70%, as well as adequate bone marrow and liver/renal function, were required. Untreated brain metastases and unstable medical conditions such as cardiac disease and diabetes were exclusion criteria.

Random Assignment and Masking

Random assignment of eligible patients using a dynamic hierarchical randomization algorithm[19] was performed through an interactive voice response system. Patients were stratified according to Memorial Sloan Kettering Cancer Center (MSKCC) prognostic score[20] (favorable, intermediate, or poor) and time to progression after starting their first VEGF-targeted therapy (progressive disease [PD] $\leq$ 6 months or $>$ 6 months).

Procedures

Each treatment cycle was 28 days. Patients received once-per-day oral doses of apitolisib 40 mg or everolimus 10 mg. Study treatment continued until disease progression, intolerable toxicity, elective patient withdrawal from the study, or study completion/termination. Crossover was not permitted within the study. Dose interruptions of up to 28 days and reductions (5 mg for everolimus; 30 mg or 20 mg for apitolisib) were permitted. Clinical safety assessments occurred every 4 weeks until 30 days after the last dose of study treatment. Tumors were assessed every 8 weeks until progression of disease (RECIST v1.1) or patient withdrawal from the study, and survival status was collected until death or patient withdrawal from the study. Adverse events (AEs) were graded according to the National Cancer Institute's Common Terminology Criteria for AEs, v3.0. An internal monitoring committee convened on a regular basis to review all available safety data.

Pharmacokinetic and Biomarker Analyses

For patients receiving apitolisib, pharmacokinetic (PK) analysis was performed on plasma samples collected at day 1 of week 1 (predose and 1, 2, 4, and 24 hours after dose), week 3 (predose and 2 hours after dose), and week 9 (predose and 2 hours after dose). For patients receiving everolimus, whole-blood samples were collected at day 1 (weeks 1 and 9, predose and 2 hours after dose). Biomarker analyses were conducted on archival tumor tissue using a targeted next-generation sequencing platform, MMP-seq, as described previously.[21] The assay used a tiled polymerase chain reaction–based enrichment strategy to amplify 963 amplicons covering 88 oncogenes and tumor suppressors, followed by sequencing on the Illumina GAIIx platform. The assay gave complete coverage (including all exons and intron-exon junctions) for *PIK3CA*, *PTEN*, *VHL*, *PBRM1*, and numerous other PI3K- and mTOR-related genes relevant to this study. PTEN and HIF1α protein levels in tumor samples were quantitated by standard methods of immunohistochemistry, using antibody clones CST 138G6 and BD 54, respectively. PTEN was scored using the H-Score method, in which the percentage of tumor cells at four staining intensities results in a score from 0 (no staining) to 300 (high staining in 100% of tumor cells). PTEN low was defined as H-Score $\leq$ 150. HIF1α was scored qualitatively as either low (0% to 5% of tumor cells stained) or high ($>$ 5% of tumor cells stained). mRNA expression analysis for a panel of 96 genes, including PI3K/mTOR pathway, angiogenesis, and RCC-related genes (Appendix Fig A1, online only), was performed using the Fluidigm platform. Assays were validated for specificity and linearity as described previously.[22,23]

Statistical Analysis

This phase II study was designed to provide preliminary evidence of the activity of apitolisib versus everolimus and was only able to detect a large benefit with reasonable precision. With 60 events, the 95% CI at a target hazard ratio (HR) of 0.6 would be 0.39 to 0.92. The primary end point was progression-free survival (PFS), defined as the time from random assignment to disease progression, as assessed by the investigator (RECIST v1.1), or death from any cause. Secondary end points included confirmed objective response rate, which was based on investigator assessment, and overall survival (OS), defined as the time from random assignment until death by any cause. The Kaplan-Meier method was used to estimate median PFS and OS. A stratified log-rank test was used to test the difference in PFS and OS between treatment arms. The stratified Cox proportional hazard model was used to calculate HRs and 95% CIs of treatment effects. Stratification factors (MSKCC prognostic score and time to progression after starting first VEGF-targeted therapy) derived from source-verified data were presented in the analysis and may not be identical to the values used for random assignment because of data inconsistencies. Descriptive statistics were used to compare groups.

RESULTS

Patients

Between October 2011 and July 2012, 85 patients were randomly assigned (apitolisib: n = 42; everolimus: n = 43) at 21 sites across the United Kingdom, France, Spain, Germany, and the United States (Fig 1). All patients received at least one dose of the study drug. At the time of the final analysis (January 2014), median patient follow-up was 16.6 months. Patients' baseline demographics were generally balanced between the two groups but showed imbalances in key factors, including the median number of prior systemic treatments, the number of prior VEGF-targeted treatments received, and in the number of target lesions (Table 1).

Efficacy

The median PFS was significantly shorter for the apitolisib treatment arm compared with the everolimus treatment arm (3.7 months *v* 6.1 months; HR, 2.12 [95% CI, 1.23 to 3.63; $P < .01$]; Fig 2A). OS was also shorter for the apitolisib treatment arm, although the results did not reach statistical significance at the $\alpha = 0.05$ level (16.5 *v* 22.8 months; HR, 1.77 [95% CI, 0.97 to 3.24; $P = .06$]; Fig 2B). The objective response rate was not significantly different between the treatment arms (7.1% for apitolisib *v* 11.6% for everolimus; χ^2 $P = .48$).

In exploratory analyses, the impact on PFS of demographic and baseline characteristics, including the individual stratification variables (MSKCC score, time to PD after first VEGF-targeted therapy), sex, age, line of therapy, location and extent of baseline disease, and biomarker status (HIF1α and PTEN expression; *VHL*, *PBRM1*, and *PIK3CA* gene alterations) was examined (Fig 3). The biomarker data were available for only a subset of patients mainly because of tissue availability. There was no significant difference in the baseline characteristics or clinical outcomes for those patients with tissue for biomarker analysis and the intention to treat population. The treatment effect was similar across most subgroups, favoring the everolimus treatment arm. A trend toward a greater benefit from everolimus was seen in patients with putative inactivating mutations in *VHL1*, as described in more detail in Fig 3B.

Table 1. Patient Demographic and Baseline Characteristics

Characteristic	Apitolisib (n = 42)	Everolimus (n = 43)
Age, median (range), years	61 (46-76)	62 (39-90)
Male	33 (79)	31 (72)
Region		
Europe	35 (83)	33 (77)
Unites States	7 (17)	10 (23)
KPSS, ≥ 80%	40 (95)	42 (98)
MSKCC prognostic score*		
Favorable	12 (29)	16 (37)
Intermediate	27 (64)	20 (47)
Poor	3 (7)	7 (16)
Time to PD after first VEGF-targeted therapy*		
≤ 6 months	11 (26)	12 (28)
> 6 months	31 (74)	31 (72)
Prior radiotherapy	11 (26)	7 (16)
No. of prior systemic therapies ≥ 2	15 (36)	6 (14)
≥ 2 prior VEGF-targeted therapies	11 (26)	4 (9)
Prior sunitinib	22 (52)	16 (37)
≥ 3 target lesions at baseline	29 (69)	19 (44)
Lung metastases	33 (79)	28 (65)

NOTE. Data are presented as No. (%) unless indicated otherwise.
Abbreviations: KPSS, Karnofsky Performance Status Scale; MSKCC, Memorial Sloan Kettering Cancer Center; PD, progressive disease; VEGF, vascular endothelial growth factor.
*Protocol-defined MSKCC score and time to PD after first VEGF-targeted therapy, as calculated by the sponsor.

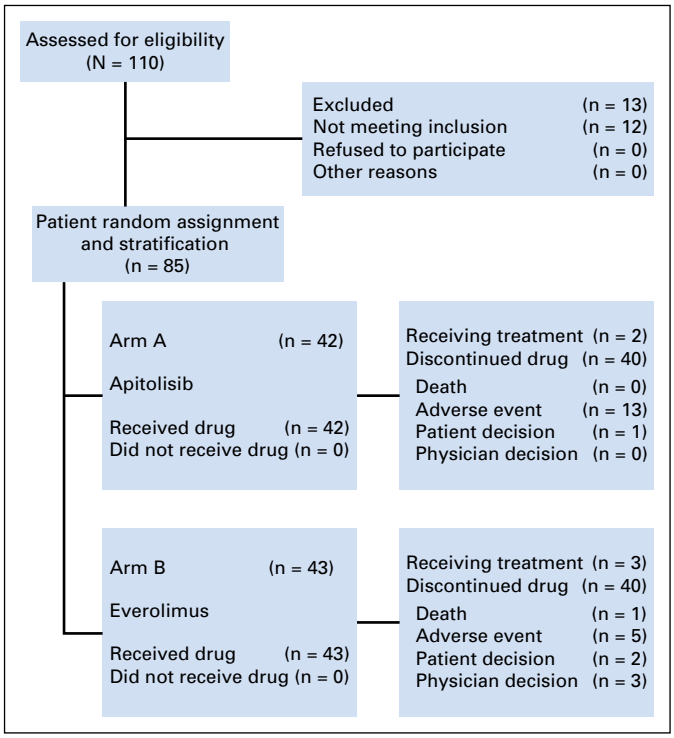


Fig 1. CONSORT diagram.

Disposition and Safety

At the time of analysis, 80 patients (94%) had discontinued study treatment (apitolisib: 40 patients [95.2%]; everolimus: 40 patients [93.0%]), most commonly because of disease progression (apitolisib: 26 patients [61.9%]; everolimus: 29 patients [67.4%]). There was a notable difference in the rate of discontinuation because of AEs (apitolisib: 13 patients [31%]; everolimus: five patients [12%]).

The most common treatment-related AEs (all grades) were rash (55% *v* 61%), hyperglycemia (57% *v* 21%), diarrhea (41% *v* 51%), mucosal inflammation (26% *v* 47%), nausea (45% *v* 28%), and fatigue (21% *v* 35%) for the apitolisib and everolimus arms, respectively (Table 2). Grade 3 or worse AEs were more frequent in patients receiving apitolisib compared with everolimus (74% *v* 44%), and this difference was caused primarily by differences in the rates of rash (24% *v* 2%) and hyperglycemia (40% *v* 9%).

Dose reductions were common in both arms (45% for apitolisib; 40% for everolimus), although the median time to first dose reduction or treatment discontinuation was double in the everolimus arm (apitolisib: 28 days; everolimus: 56 days; Appendix Fig A2).

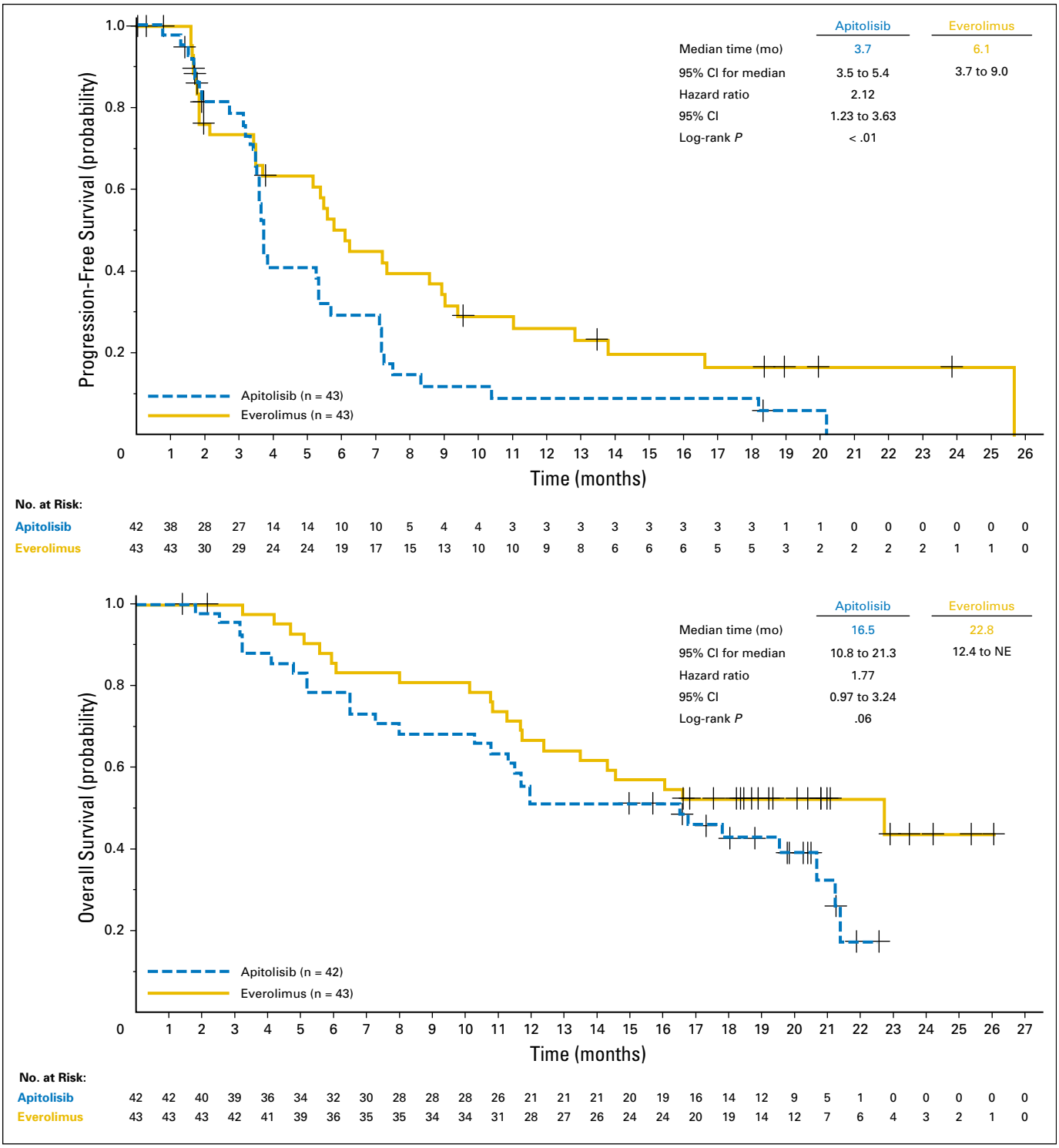


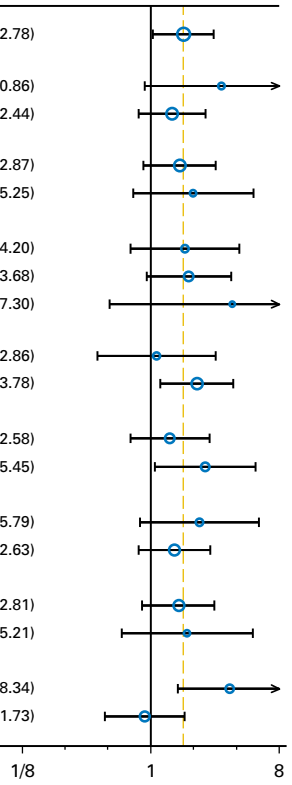
Fig 2. Kaplan-Meyer estimates of progression-free survival (A) and overall survival (B) for all randomly assigned patients. Hazard ratios estimated by Cox proportional hazards regression stratified by calculated Memorial Sloan Kettering Cancer Center risk score and time to progression on first vascular endothelial growth factor–targeted therapy. NE, not evaluable.

PK and Biomarker Analyses

Given that only sparse PK data were available for ROVER, population PK model simulations were performed to estimate apitolisib exposure (patient steady-state area under the curve). Generally, the PK simulations for apitolisib were similar to those observed in other subjects treated with apitolisib (data not shown). Exposure-response analyses for both safety and efficacy were conducted using this simulated exposure data. No exposure-efficacy relationships were found. Although there was a trend towards higher apitolisib exposure (simulated steady-state area under the curve) among patients who

A

Baseline Risk Factors	Total n	Everolimus (n = 43) n	Everolimus (n = 43) Median (months)	Apitolisib (n = 42) n	Apitolisib (n = 42) Median (months)	Hazard Ratio	95% Wald CI
Description of planned arm	85	43	6.1	42	3.7	1.70	(1.04 to 2.78)
Sex							
Female	21	12	6.1	9	3.7	3.14	(0.91 to 10.86)
Male	64	31	5.8	33	3.7	1.42	(0.82 to 2.44)
Age group 65							
< 65	59	31	6.1	28	3.6	1.60	(0.89 to 2.87)
≥ 65	26	12	5.6	14	5.3	1.99	(0.76 to 5.25)
MSKCC prognostic score*							
Favorable	28	16	6.3	12	7.1	1.74	(0.72 to 4.20)
Intermediate	47	20	6.1	27	3.6	1.86	(0.94 to 3.68)
Poor	10	7	5.6	3	3.5	3.74	(0.51 to 27.30)
PDTime on first VEGF-targeted therapy*							
PD ≤ 6 mo	23	12	5.8	11	5.3	1.10	(0.42 to 2.86)
PD > 6 mo	62	31	6.3	31	3.7	2.11	(1.17 to 3.78)
Prior sunitinib							
No	47	27	5.6	20	3.7	1.36	(0.72 to 2.58)
Yes	38	16	8.6	22	3.7	2.42	(1.07 to 5.45)
Site of metastatic disease, lung							
No	24	15	7.2	9	3.2	2.21	(0.84 to 5.79)
Yes	61	28	6.1	33	3.7	1.47	(0.82 to 2.63)
Number of previous systemic regimens							
1	64	37	6.3	27	3.7	1.57	(0.87 to 2.81)
≥ 2	21	6	5.8	15	3.7	1.80	(0.62 to 5.21)
Number of target lesions at baseline							
< 3	37	24	8.6	13	3.3	3.59	(1.55 to 8.34)
≥ 3	48	19	3.4	29	3.7	0.91	(0.47 to 1.73)



B

Baseline Risk Factors	Total n	Everolimus (n = 43) n	Everolimus (n = 43) Median (months)	Apitolisib (n = 42) n	Apitolisib (n = 42) Median (months)	Hazard Ratio	95% Wald CI
Description of planned arm	85	43	6.1	42	3.7	1.70	(1.04 to 2.78)
HIF1α IHC							
Low	19	9	6.3	10	3.7	1.75	(0.60 to 5.12)
High	56	28	8.6	28	5.3	1.57	(0.84 to 2.93)
PTEN IHC							
Low	40	21	6.3	19	3.7	1.90	(0.91 to 3.97)
High	34	17	6.1	17	3.7	1.61	(0.72 to 3.60)
PTEN low or PIK3CA							
Not present	32	17	6.1	15	5.3	1.45	(0.63 to 3.34)
Present	42	21	6.3	21	3.6	2.04	(0.99 to 4.20)
VHL							
Absence of deleterious alteration	29	16	5.5	13	3.6	0.93	(0.39 to 2.21)
Deleterious alteration	24	15	8.6	9	3.5	2.96	(1.01 to 8.67)
PBRM1							
Absence of deleterious alteration	39	22	5.5	17	3.6	1.71	(0.81 to 3.62)
Deleterious alteration	11	6	13.8	5	3.6	1.11	(0.20 to 6.16)

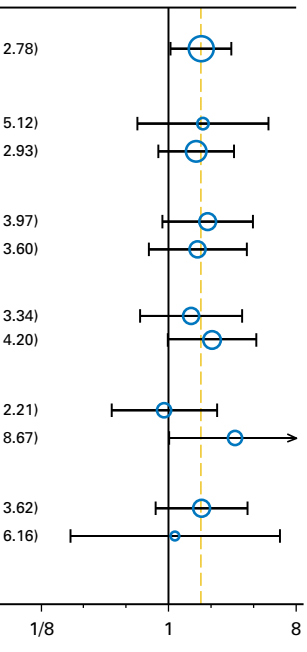


Fig 3. Forest plot of progression-free survival by (A) selected clinical baseline factors and (B) selected biomarkers (B). HIF, hypoxia-inducible factor; IHC, immunohistochemistry; MSKCC, Memorial Sloan Kettering Cancer Center; PD, progressive disease; VEGF, vascular endothelial growth factor.

Table 2. Treated-Related Adverse Events in ≥ 20% of Patients

	Apitolisib (n = 42)		Everolimus (n = 43)	
Adverse Event (CTCAE v4.0)	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3
Any adverse event	42 (100)	31 (74)	40 (93)	19 (44)
Rash*	23 (55)	10 (24)	26 (61)	1 (2)
Hyperglycemia	24 (57)	17 (40)	9 (21)	4 (9)
Diarrhea	17 (41)	3 (7)	22 (51)	1 (2)
Mucosal inflammation	11 (26)	4 (9)	20 (47)	4 (9)
Nausea	19 (45)	—	12 (28)	1 (2)
Fatigue	9 (21)	1 (2)	15 (35)	3 (7)
Decreased appetite	14 (33)	1 (2)	8 (19)	—
Vomiting	14 (33)	2 (5)	5 (12)	1 (2)
Infection†	8 (19)	2 (5)	13 (30)	—
Cough	4 (10)	—	11 (26)	—
Epistaxis	3 (7)	—	11 (26)	—
Dyspnea	2 (5)	—	11 (26)	—
Asthenia	10 (24)	3 (7)	9 (21)	2 (5)
Dry skin	6 (14)	2 (5)	10 (23)	1 (2)
Pruritus	9 (21)	1 (2)	7 (16)	—
Constipation	6 (14)	—	9 (21)	—

NOTE. Data are presented as No. (%).
Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events.
*Includes preferred terms of rash, rash maculopapular, rash pruritic, rash erythematous, and rash papular.
†Includes preferred terms of nasopharyngitis, lung infection, gingivitis, rhinitis, urinary tract infection, abscess, bronchitis, cystitis, ear infection, eczema impetiginous, gastroenteritis, lower respiratory tract infection, oral herpes, paronychia, penile infection, sinusitis, staphylococcal infection, tooth infection, and upper respiratory tract infection.

had best responses of partial response versus PD (Fig 4A), data were limited, and overall there was no apparent correlation between exposure and tumor response (Fig 4B). Kaplan-Meier analysis also showed no significant difference in PFS between patients with apitolisib exposures above the median versus below the median (data not shown). However, the exposure-safety relationship showed a trend in that patients who discontinued because of an AE were more likely to have higher exposure to apitolisib (Fig 4C). Everolimus whole-blood trough levels were similar to those reported previously.[24]

Data from comprehensive molecular profiling consisting of targeted next-generation sequencing, mRNA expression, and immunohistochemistry for PTEN and HIF1α were evaluated for associations with best tumor response and PFS. Activating mutations in *PIK3CA* were rare in this patient population (9%), but inactivating mutations in *VHL* and high expression of HIF1α were relatively common (45% and 75%, respectively; Fig 5A). There was no apparent biomarker relationship with best tumor response; mutations in *VHL*, *PBRM1*, *PIK3CA*, and *PTEN* were found in similar proportions in patients who experienced stable disease/PD as opposed to partial response/complete response (Fig 5A). However, there was a trend toward an association between high expression of HIF1α and PFS in both treatment arms (everolimus HR, 0.54 [95% CI, 0.23 to 1.29]; apitolisib HR, 0.53 [95% CI, 0.23 to 1.20]) and between deleterious *VHL* gene alterations and PFS in the everolimus arm only (HR, 0.55 [95% CI, 0.24 to 1.25]; Figs 5B and 5C). Gene expression analyses were also performed on a panel of selected genes related to PI3K/mTOR biology and RCC. These genes were selected from the literature and validated in cell lines/tumor samples (Appendix Fig A1 and data not shown). This analysis identified three genes, including *MYC*, *SOSTDC1*, and the mTOR regulator *STK11*, associated with significantly better PFS for everolimus compared with apitolisib (Appendix Fig A3). Low expression of *STK11*, defined as below median expression, showed a PFS HR of 3.02. In contrast, no genes were preferentially associated with benefit from apitolisib.

DISCUSSION

The ROVER study is the first randomized phase II trial to directly compare simultaneous targeting of multiple nodes in the PI3K/mTOR signaling pathway with isolated mTORC1 inhibition. In preclinical studies, PI3K/mTOR dual inhibitors have shown superior activity to mTORC1-targeting rapalogs.[15] However, the results of the ROVER study failed to provide proof-of-concept for this hypothesis, with the PI3K/mTORC1/mTORC2 inhibitor, apitolisib, substantially underperforming the mTORC1 inhibitor, everolimus.

Several alternative hypotheses may help explain the outcome of this trial. First, mTORC1 may be the central node in the PI3K/AKT/mTOR pathway in mRCC, as demonstrated by both temsirolimus and everolimus. Inhibition of upstream PI3K and mTORC2 signaling incurs excess toxicity without adding significant benefit. The lack of frequent alterations in *PIK3CA* is consistent with this hypothesis. Second, resistance to mTORC1 inhibition emerges through activation of parallel signaling pathways; therefore, horizontal inhibition may succeed where vertical inhibition has failed. Some preclinical data suggest that PI3K/mTOR inhibition may lead to compensatory activation of HER family receptors and the MAP kinase pathway.[25] Combination of mTOR inhibitor with inhibitors of VEGF, EGFR, or MEK may achieve superior clinical efficacy by overcoming this bypass mechanism, although likely with increased toxicity as shown in two previous randomized trials.[26,27]

Several other factors may have contributed to the lower efficacy of apitolisib relative to everolimus. First, apitolisib, like other PI3K/mTOR dual inhibitors in development for solid tumors,[28] was poorly tolerated. Treatment exposure in patients was compromised substantially by high-grade rash (24%) and hyperglycemia (40%), which occurred early in the treatment course and resulted in one half of the patients requiring treatment modifications and nearly one

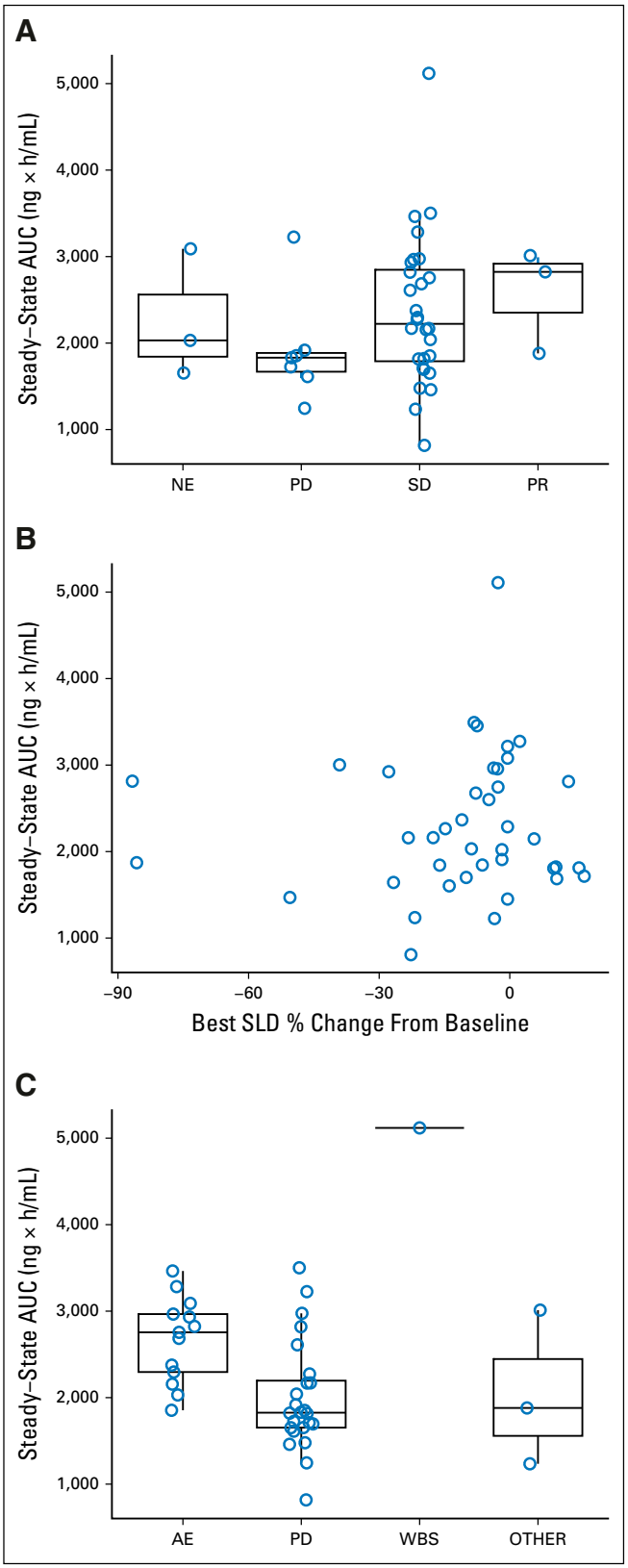


Fig 4. Relationships between steady-state AUC and (A) types of responders, (B) tumor size, and (C) reasons for patient discontinuation. AE, adverse event; AUC, area under the curve; NE, not evaluable; OTHER, other reasons for subject discontinuation; PD, progressive disease; PR, partial response; SD, stable disease; SLD, sum of the longest diameters of the target lesions; WBS, withdraw by subjects.

third discontinuing apitolisib permanently. Rash and hyperglycemia are common AEs observed with many pan-PI3K and dual PI3K mTOR inhibitors and likely represent on-target effects of pathway inhibition in normal tissues.[28] Second, this study enrolled quickly, so there was little time to apply the experience in managing apitolisib-specific toxicities from earlier patients to later patients, in contrast to the everolimus experience accumulated since its approval in 2009.

In addition, the treatment arms were imbalanced in terms of the median number of prior systemic treatments, the number of prior VEGF-targeted treatments, and the number of target lesions. Compared with the everolimus arm, more than twice as many patients in the apitolisib arm had two or more prior systemic therapies (36% *v* 14%), nearly three times as many patients in the apitolisib arm had more than one prior VEGF-targeted therapy (26% *v* 9%), and a higher percentage of patients in the apitolisib arm had three or more target lesions (69% *v* 44%). The greater number of prior treatments and target lesions in the apitolisib arm suggests that these patients may have had more advanced disease, which may have influenced outcomes, as seen in previous trials.[29] The multivariate analyses yielding adjusted HRs were generally consistent with the primary stratified Cox estimate provided in Results (adjusted HRs incorporating different combinations of variables ranged from 1.99 to 2.62).

To our knowledge, the ROVER study presents the most comprehensive biomarker assessment reported in an mRCC trial, consisting of next-generation sequencing for a panel of 88 cancer-related genes, coupled with focused biomarker assessment of key components of mTOR signaling and the vascular biology of RCC. However, the strength of the conclusions is limited by the small sample size and the retrospective nature of the analyses. Nonetheless, the findings support a model in which mTORC1 and VHL converge on regulation of HIF1α protein levels. Mutational inactivation of *VHL*, which is predicted to result in elevated HIF1α protein levels, was associated with longer PFS in the everolimus arm, although validation of this observation will require further clinical testing. Consistent with this interpretation, direct analysis of HIF1α protein levels by immunohistochemistry also showed an association between high levels of HIF1α and longer PFS in both arms of the study. In addition, low expression of the tumor suppressor STK11/LKB1, which acts upstream of mTORC1 and when lost potently activates mTOR signaling,[30] was preferentially associated with benefit to everolimus. The differences observed between everolimus and apitolisib may be a result of insufficient inhibition of mTORC1 by apitolisib. Overall, suppression of mTORC1 activity preferentially benefits patients who show aberrant activation of HIF1α resulting from *VHL* or *STK11* loss. These analyses were based primarily on archival tissue, and patients had received VEGF tyrosine kinase inhibitor therapy, which could have substantially influenced tumor biology. Although suggestive, our findings require rigorous prospective validation in randomized studies of mTOR inhibitors to determine whether these putative biomarkers can selected for patients who may benefit from mTORC1-targeted therapy in RCC.

As the first phase II trial to evaluate dual PI3K/mTOR inhibition in mRCC, the ROVER study confirms the significant clinical benefit of everolimus in VEGF-refractory disease and suggests that sustained dual inhibition of the PI3K/mTOR pathway in mRCC is severely limited by toxicity and a narrow therapeutic index, resulting in limited efficacy in RCC. The median OS for everolimus was 22 months, which is the longest reported for this drug to date. *VHL*

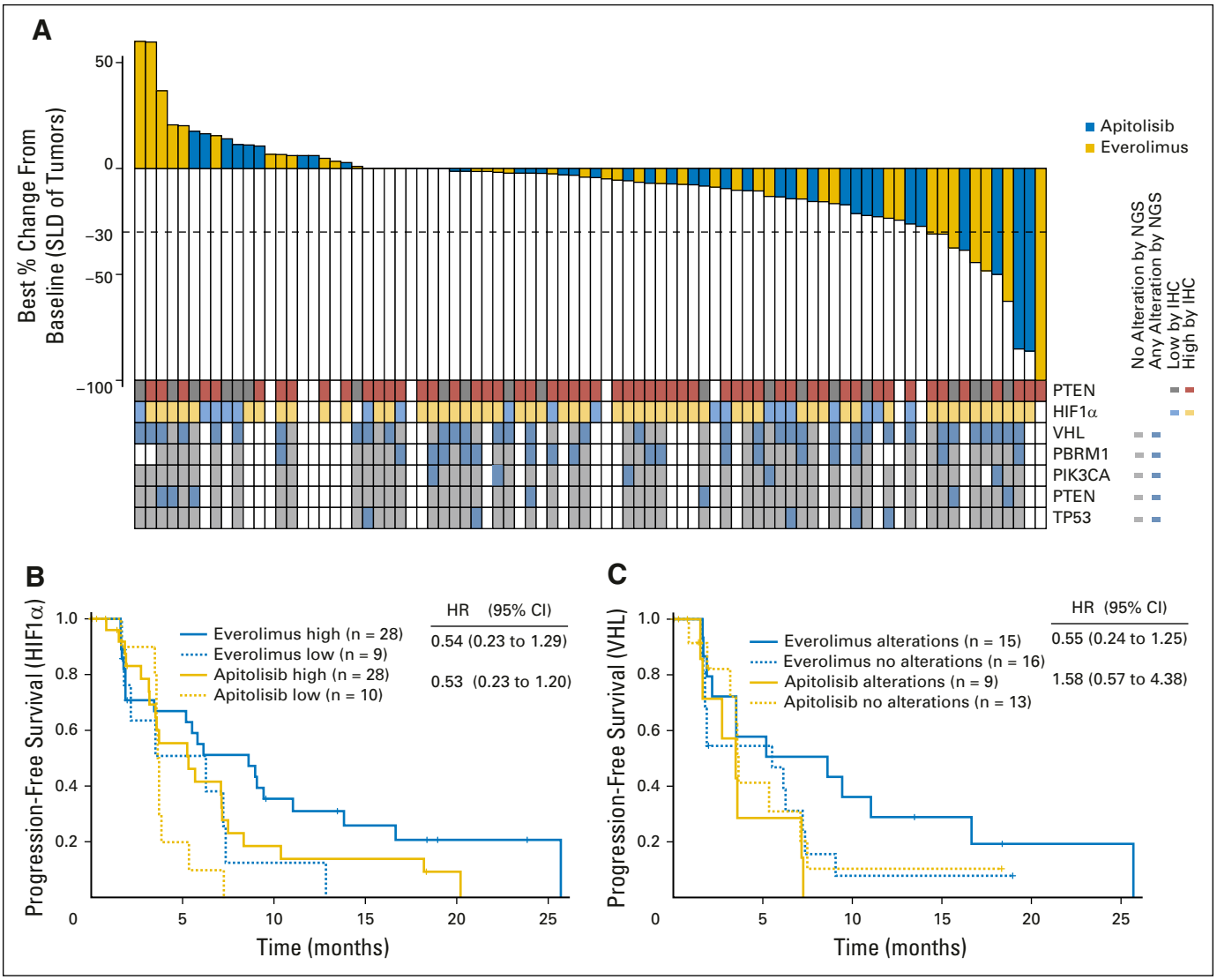


Fig 5. Study PIM4973 (ROVER) tumor response. (A) Waterfall plot with associated biomarkers. Genetic alterations identified were frameshift/nonsense/deletions for *VHL* and *PBRM1*, activating alterations for *PIK3CA*, frameshift/nonsense/deletions/missense for *PTEN*, and missense for TP53. (B) Comparison of progression-free survival between patients with high HIF1α protein levels and those with low levels. (C) Comparison of progression-free survival between patients with *VHL* deleterious mutations and those with no *VHL* deleterious mutations. HIF, hypoxia-inducible factor; HR, hazard ratio; IHC, immunohistochemistry; NGS, next-generation sequencing; SLD, sum of longest diameters of target lesions.

mutation, HIF1α protein, and *STK11* mRNA expression emerged as potential pathway-related biomarkers for everolimus that warrant prospective validation in mRCC. Simultaneous dual inhibition of the PI3K and both mTOR complexes does not seem to be an effective strategy for improving on mTORC1-targeted therapy in RCC.

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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Randomized Open-Label Phase II Trial of Apitolisib (GDC-0980), a Novel Inhibitor of the PI3K/Mammalian Target of Rapamycin Pathway, Versus Everolimus in Patients With Metastatic Renal Cell Carcinoma

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or jco.ascopubs.org/site/ifc.

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Acknowledgment

We thank the patients, study investigators, and staff who participated in this study. Editing and writing support was provided by A. Daisy Goodrich (Genentech, South San Francisco, CA) and was funded by Genentech.

Appendix

PI3K/mTOR pathway				RCC/Metabolism				Housekeeping
AKT2	FGFR2	PIK3IP1	TSC2	AMACR	FH	LRRK2	STC2	PPIA
EGFR	FGFR3	PRR5		AQP1	FHIT	MAP2	YPEL2	RPLP0
EIF4EBP1	GSK3B	PTEN		CA3	GAPDH	PAX2		TMEM55B
ERBB2	IRS2	STK11		CA9	HBEGF	PLIN2		VPS33B
ERBB3	MET	TLR2		EPPK1	KRT7	RUNX3		
FBXW7	PIK3CA	TSC1		FABP7	LIMK2	SDS		

Angiogenesis			Cell growth/Differentiation					
ANGPTL4	EPHA7	PDGFB	BRCA1	CDC7	EFNA1	KITLG	RASSF1	TGFB1
CSF1R	EPHB1	PDGFRA	CAV1	CDH1	FLCN	MEOX2	RET	TMEFF2
CXCR4	FLT1	STAT1	CCND2	CDH2	GATA3	MYC	SKP2	VAV3
EPAS1	HIF1A	VCAN	CCNE1	CDKN1B	IGF2R	PBRM1	SMAD3	VIM
EPHA3	KIT	VEGFA	CCNG2	DTL	IGFBP2	PPAP2B	SOSTDC1	WT1
EPHA4	NOS3	VHL	CD9	E2F1	INHBA	PROM1	TGFA	

Fig A1. Composition of renal Fluidigm panel. mTOR, mammalian target of rapamycin;

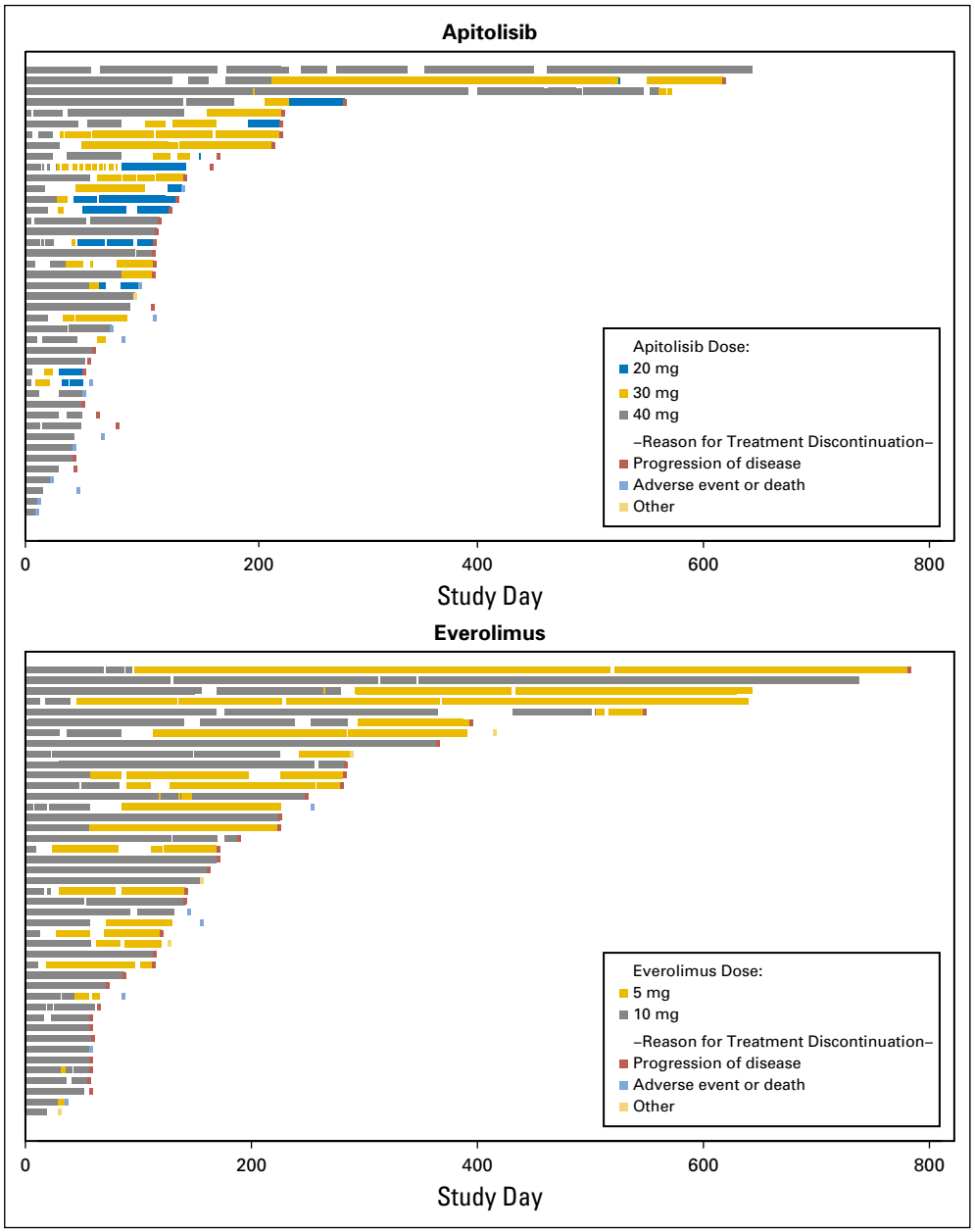

Fig A2. Swimlane plot showing duration of therapy, dose reductions, and reason for discontinuation for individual patients. Each bar represents an individual patient.

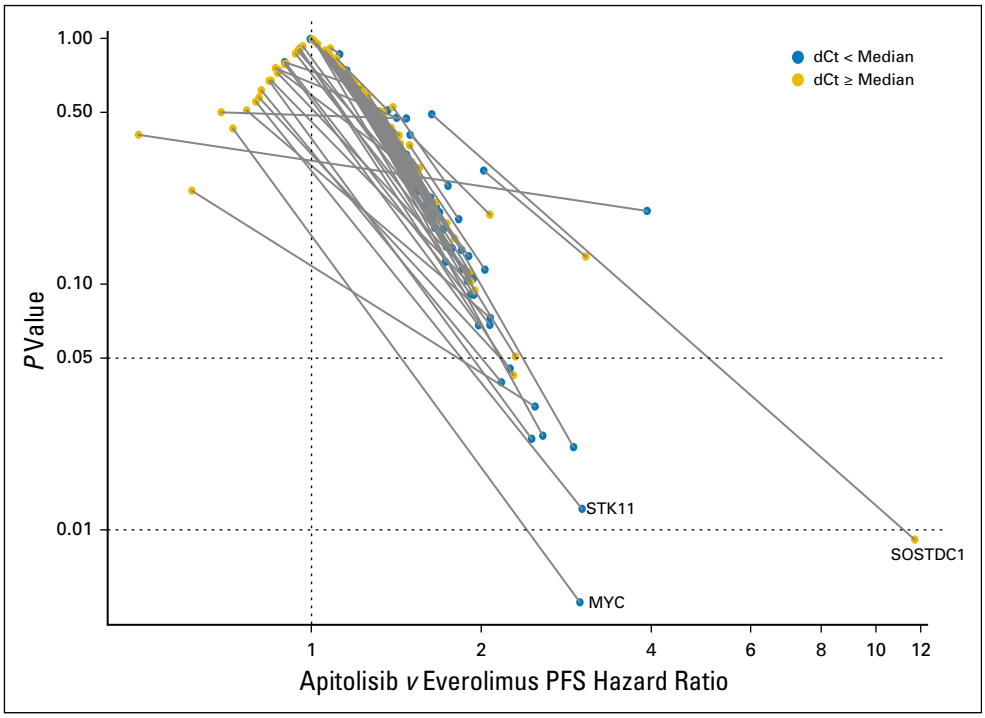

Fig A3. Significance of individual gene expression level (*P* value) is given on the *y*-axis, and hazard ratio is given on the *x*-axis. dCt, Ct value relative to control genes; PFS, progression-free survival; RCC, renal cell carcinoma.