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Case Report

Successful Targeting of Somatic *VHL* Alterations With Belzutifan in Two Cases

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ABSTRACT

Clear cell renal cell carcinoma (RCC) is commonly associated with alterations in the *VHL* tumor suppressor gene, resulting in upregulation of hypoxia-inducible factor pathways. Immune checkpoint inhibitors and vascular endothelial growth factor inhibitors are the mainstays of systemic treatment for metastatic RCC; however, most patients encounter disease progression after the initial response. The phase 3 clinical trial LITESPARK-005–belzutifan (HIF-2α inhibitor) demonstrated improvement in progression-free survival compared with everolimus in heavily pretreated patients unselected for somatic/germline *VHL* alterations (an objective response rate of 23% and a median time on therapy of 7.6 months in the belzutifan cohort), resulting in U.S. FDA approval for patients with advanced RCC. Herein, we present two cases of refractory metastatic RCC (including one with brain metastases) with somatic *VHL* mutations who received belzutifan after discussion in the institutional Molecular Tumor Board. Both patients had an excellent clinical response (partial remissions ongoing at >12 and >20 months). Future studies should assess the merits of biomarker selection for belzutifan treatment.

Keywords: renal cell carcinoma, HIF, HIF-2α, belzutifan, VHL alterations

INTRODUCTION

Renal cell carcinoma (RCC) accounts for approximately 2% of cancer diagnoses and mortality.[1] Clear cell RCC (ccRCC) is the most common subtype, representing 75–80% of RCCs.[2] Immune checkpoint inhibitors (ICIs) (anti-PD-1/PD-L1/CTLA-4) or vascular endothelial growth factor (VEGF) axis inhibitors alone or combined with ICI are frontline treatments for advanced ccRCC.[3–5] The most advanced ccRCCs progress even after initial response.

In many cases of ccRCC, the von Hippel-Lindau tumor suppressor gene (*VHL*) is inactivated or mutated. Belzutifan, a novel hypoxia-inducible factor 2α (HIF2α) inhibitor, is Food and Drug Administration approved for patients with germline *VHL* disease-associated RCC and nonrenal cell neoplasms.[6] Early-phase clinical trials have demonstrated

Table 1. Clinical characteristics of two patients with renal cell cancer and *VHL* somatic mutations receiving belzutifan therapy

	Case 1	Case 2
Initial presentation and disease stage	Incidental 6.9-cm left renal mass, T3aN0M0	Left flank pain, imaging with a 7.8-cm mass in the upper pole of left kidney, T3bN0M0
Year of diagnosis	2015	2016
Nephrectomy	Yes	Yes
Approximate time from diagnosis of localized RCC to evidence of first metastatic disease	2.4 years	1 year
Number of lines of systemic treatment in the metastatic setting before the initiation of belzutifan	1	4 (plus gamma knife for brain metastases)
Disease burden at the time of initiation of belzutifan	Right upper lobe lung nodule, multiple perihepatic and peritoneal lesions, rectal metastasis	Multiple liver lesions, right adrenal nodule, soft tissue metastases, right renal mass and left urinary bladder wall lesion with complete response. Brain lesions with decrease in enhancement.
Genomic profiling with tissue sample	*BAP1, VHL*	*PBRM1, PTEN, SETD2, VHL, FANCA*
Liquid biopsy (yes/no)[#]	Yes	Yes
Germline testing	Negative	Negative
Homologous recombination status	Low 1 (Tempus)	Not done
Duration of treatment with belzutifan	12+ months (ongoing)	20+ months (ongoing)
Combination regimen (yes/no)	No	Yes, initially belzutifan + everolimus (for 3 mo) and later changed to belzutifan plus olaparib
Response to the treatment	Partial response (maximum change = 67% regression)	Partial response (maximum change = 63% regression)
Comment		Patient received multiple session of gamma knife to brain lesions. Serial brain imaging with decreased enhancement in brain lesions
Toxicities	Grade 2 anemia, fatigue, dyspnea, recovered with lowered dose of belzutifan from 120 mg daily to 80 mg daily. Currently on belzutifan 80 mg daily, tolerating well with stable Hb ~11 g/dL	Grade 3 anemia, fatigue, dyspnea and pericardial effusion (nonmalignant) while on belzutifan 80 mg daily and everolimus 5 mg daily. Currently on belzutifan 80 mg PO daily plus olaparib 150 mg PO daily without any toxicities
Current status	Continued antitumor response, no new metastatic disease	Continued antitumor response including in brain, no new metastatic disease

Hb = hemoglobin; RCC = renal cell carcinoma.
[#]No significant somatic tumor related alterations detected for both patients in liquid biopsies.

antitumor activity with HIF2α antagonists in unselected patients with advanced ccRCC (response rates, ~14–25%),[7–10] highlighting the importance of HIF2 as a therapeutic target.[11]

Herein, we describe our experience with belzutifan, demonstrating an antitumor response in metastatic ccRCC patients harboring somatic *VHL* mutation. Patients were treated in accordance with the guidelines of the Medical College of Wisconsin (MCW) institutional review board-approved "Profile-related Evidence Determining Individualized Cancer Therapy" (PREDICT) (ClinicalTrials.gov Identifier: NCT05802069) study and any investigational interventions for which they gave consent.

CASE 1

A 65-year-old man was incidentally found to have a 6.9-cm left kidney mass while undergoing diagnostic evaluation for dyspnea in early 2015. He underwent a partial left nephrectomy; pathology revealed a ccRCC (Table 1). Two years later, the patient developed recurrent disease in the nephrectomy bed and underwent left radical nephrectomy. Immediate follow-up (~4 weeks) with computed tomography (CT) of the chest, abdomen, and pelvis (CAP) demonstrated multiple new hepatic and left retroperitoneal nodules. Cabozantinib was started; subsequent CT CAP showed stable liver lesions; however, a new left femoral neck lytic lesion appeared, which was managed with ablation, internal fixation, and radiation. Approximately 7–8 months after initiating cabozantinib, magnetic resonance imaging (MRI) of the lumbar spine showed an L1 vertebral body lesion, which was managed with radiation. His metastatic disease remained relatively stable for approximately 5 years while on cabozantinib; however, CT CAP in late 2022 demonstrated progressive peritoneal/retroperitoneal lesions

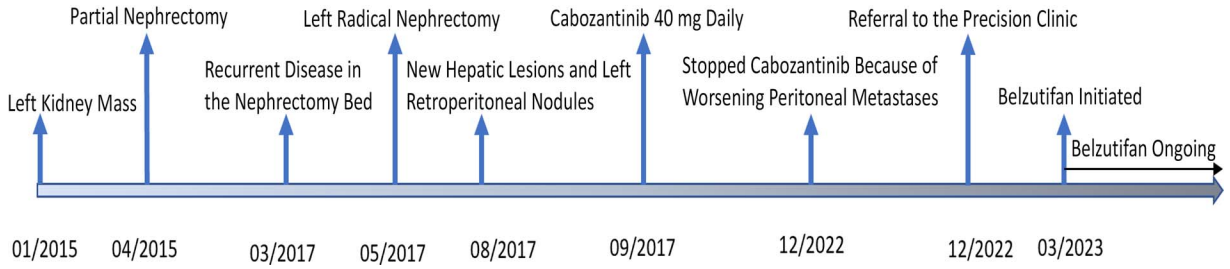

Figure 1. Depicts the timeline and clinical course of case 1.

(biopsy-confirmed metastatic RCC), and cabozantinib was discontinued. Owing to a history of cancer-associated retinopathy, the patient declined ICI treatment.

Tissue next-generation sequencing (NGS) (648 genes, Tempus, USA) was performed (peritoneal nodule) and showed *VHL* p.I151T missense variant-loss of function (variant allele fraction [VAF], 21.1%), *BAP1* p.G420fs frameshift-loss of function (VAF 15.3%), tumor mutational burden, 7.9 mutations/megabase (Mb), and microsatellite stability. The tempus germline panel was negative for pathologic alterations in genes, including *VHL*. The patient was referred to the MCW precision medicine clinic. Based on review and consensus recommendations from the Molecular Tumor Board (MTB), belzutifan 120 mg by mouth (PO) daily was initiated in March 2023 to target the somatic *VHL* mutation (Fig. 1). The patient's hemoglobin dropped from normal to approximately 8.4 gm/dL after 4 months, requiring medication hold for 1 week and resumption of belzutifan at 80 mg PO daily. Response assessment after 2 months showed partial response (PR) (~48% RECIST response) (Fig. 2). Imaging after 5 months showed a 67% decrease in target lesions (Fig. 2). The PR was ongoing at 12+ months and he was tolerating belzutifan 80 mg daily with no further toxicities.

CASE 2

A 57-year-old man presented with left flank pain in May 2016 (Table 1). Imaging demonstrated a 7.8-cm left kidney mass. He underwent a left nephrectomy in June 2016; pathology revealed a ccRCC with focal rhabdoid features. A surveillance scan (April 2017) showed a right lung lesion, managed with surgical resection (pathology-confirmed metastatic ccRCC). Within a few months, a

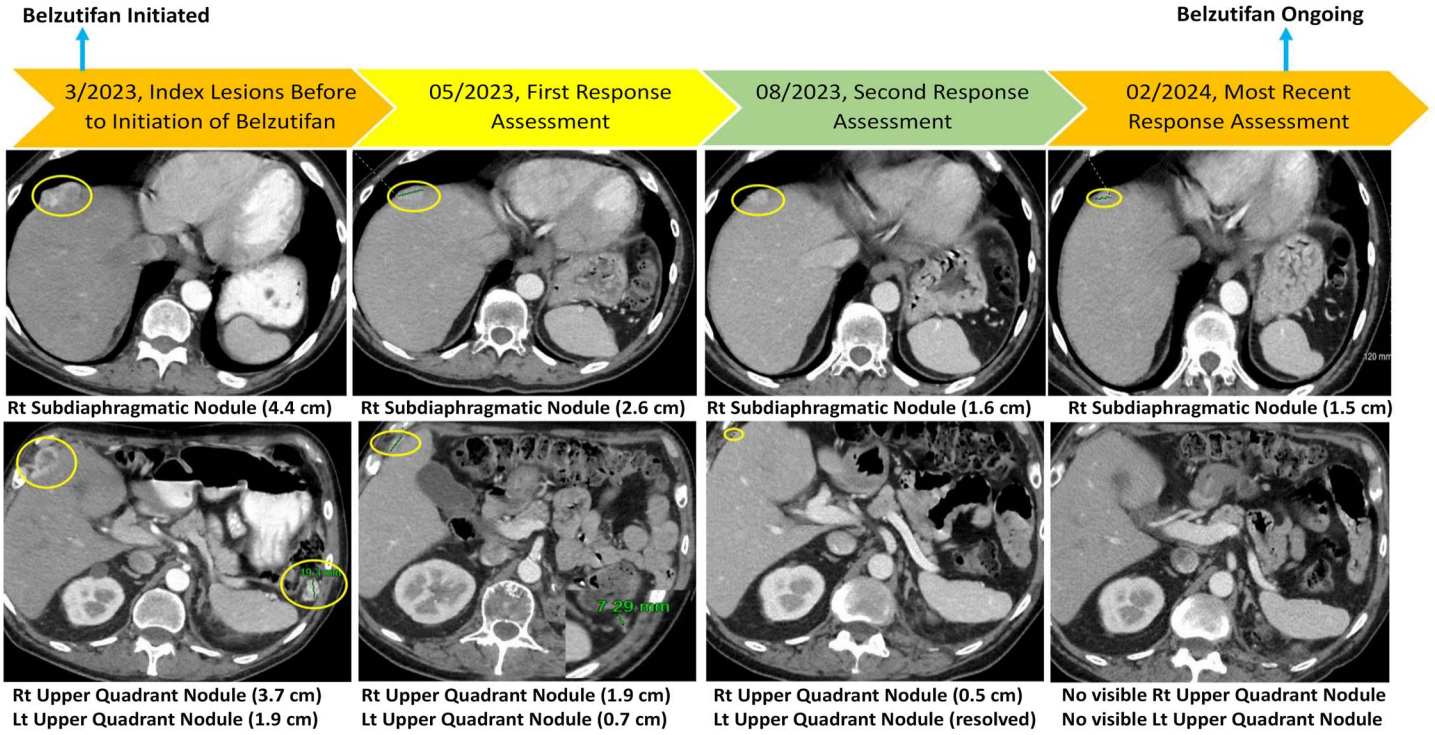

Figure 2. CAT scans showing antitumor response in three index lesions. Yellow circles mark the site of the lesion. Upper panel demonstrates a right (Rt) subdiaphragmatic nodule, which regressed significantly after the initiation of belzutifan. Lower panel shows a Rt upper quadrant lesion and left (Lt) upper quadrant nodule. Complete resolution of the Lt upper quadrant peritoneal lesion in the imaging obtained in August 2023 is shown. Best response is a partial response with 67% decrease in total target lesions compared with baseline.

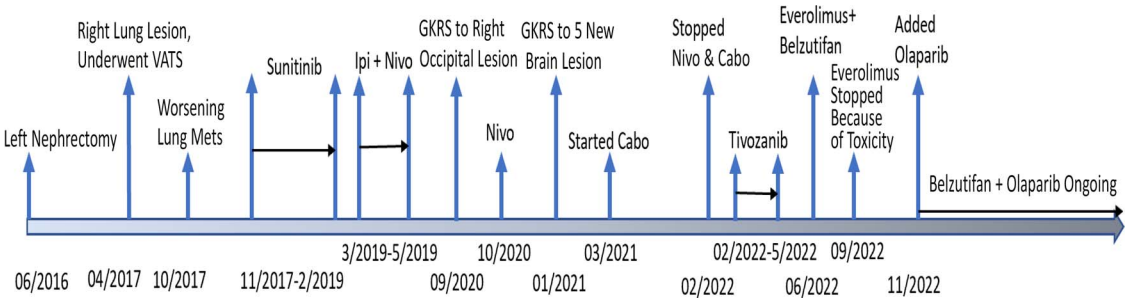

Figure 3. Depicts the clinical course and timeline for Case 2. Cabo: cabozantinib; GKRS: gamma knife radiosurgery; Ipi: ipilimumab; Nivo: nivolumab; VATS: video-assisted thoracoscopic surgery.

CAT scan demonstrated lung metastases. He received single-agent sunitinib with disease control for approximately 1.5 years. In early 2019, because of progressive cancer, the patient received four cycles of nivolumab in combination with ipilimumab, complicated by immune-related colitis. A restaging CT CAP showed significant improvement in pulmonary nodules; the patient was monitored without treatment. Subsequently, an MRI (August 2020) showed a 2.3 × 2 cm right brain occipital lesion, managed with gamma knife radiosurgery, and a CT chest demonstrated enlarging left bronchial/infra hilar lymphadenopathy. Nivolumab was initiated, and cabozantinib was subsequently added. An MRI of the brain (January 2021) showed new brain lesions treated with gamma knife radiosurgery. Owing to disease progression, nivolumab and cabozantinib were discontinued in February 2022. Subsequently, he received tivozanib, but the disease worsened within 2–3 months.

NGS (315 genes, Foundation Medicine, USA) on a lung sample (obtained November 2017) showed *PTEN* F90fs*2, *FANCA* loss exons 19–20, *PBRM1* N198fs*26, *SETD2* Y2296fs*73, *VHL* G114C, microsatellite stability, tumor mutational burden 2 mutations/Mb. Repeat NGS from a bladder lesion (May 2022) and bronchial sample (June 2022) showed similar mutational profiles. Germline testing (Ambry genetics) was negative for *VHL* mutation.

Based on the patient evaluation in the MCW precision medicine clinic and consensus recommendations

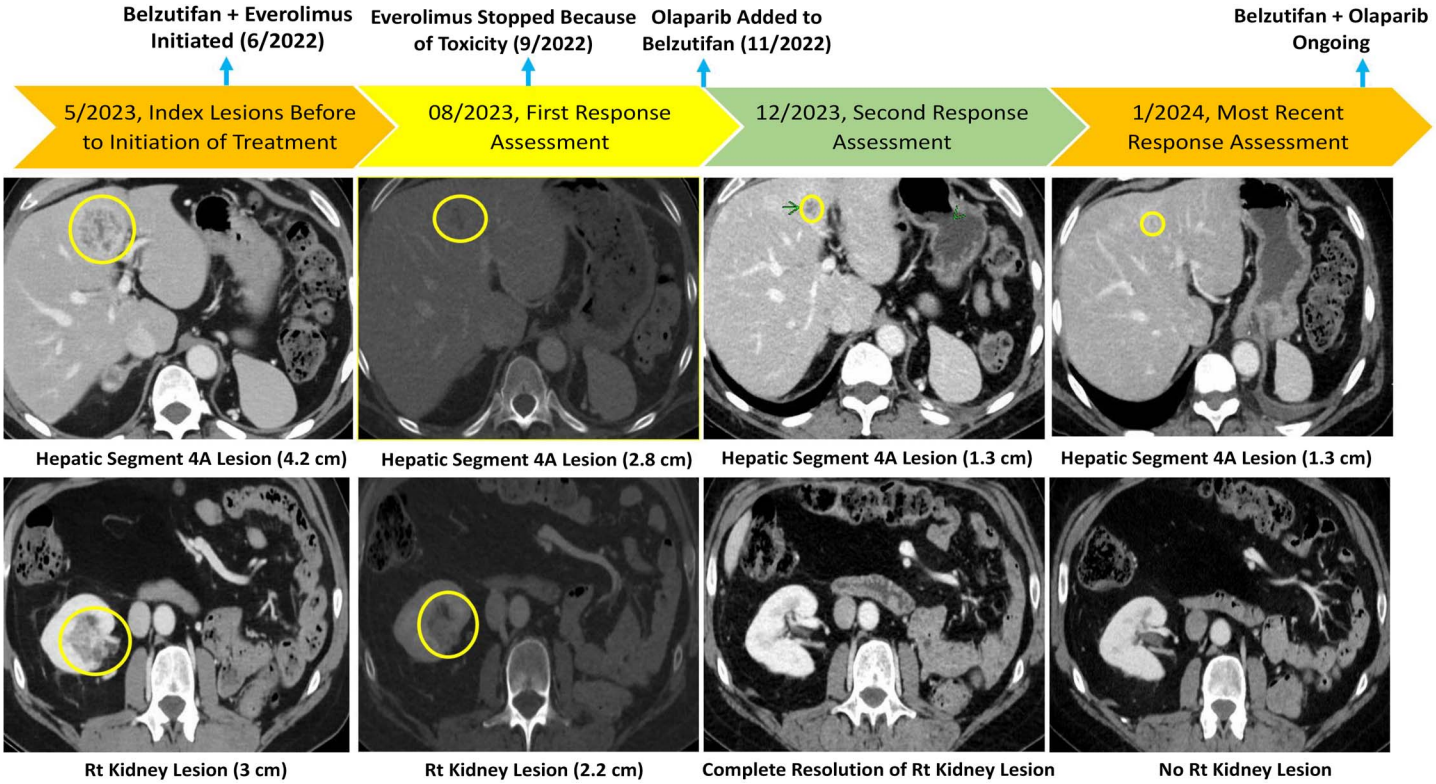

Figure 4. Shows consecutive imaging with antitumor response in two index lesions. Yellow circles mark the site of the lesion. Upper panel demonstrates a hepatic segment 4A lesion, which responded well after initiation of personalized treatment (belzutifan + everolimus followed by belzutifan + olaparib). Lower panel shows a right kidney lesion, which entirely resolved after 6–7 months of systemic treatment. The best response is a partial response with a 63% decrease in sum target lesions.

from the MTB, everolimus (5 mg PO daily; approved dose = 10 mg PO daily) was started to target *PTEN* mutation and abrogate signaling via the PI3K/mTOR pathway. Belzutifan (80 mg PO daily; approved dose=120 mg PO daily) was added to target the *VHL* pathway via HIF-2α inhibition. Within 4–5 weeks, hemoglobin dropped from approximately 11 to approximately 6.5 g/dL. Workup for anemia revealed undetectable haptoglobin and evidence of hemolysis in peripheral blood smear. The patient was on dapsone for pneumocystis jirovecii prophylaxis while on corticosteroids for brain metastases. Other etiologies for hemolysis, such as infections and autoimmune processes, were ruled out. Therefore, acute severe anemia likely resulted from the combined effect of dapsone and belzutifan. Dapsone was discontinued, and anemia improved after holding belzutifan for 2 weeks (hemoglobin, 9–10 g/dL). Belzutifan was resumed at 40 mg daily and subsequently escalated to 80 mg PO daily with good tolerance. A CT CAP after 3 months of treatment showed PR with a decrease in the size of index lesions (right kidney, bladder, and liver). However, the patient developed pericardial effusion (negative for malignancy, drug vs. prior radiation to lymph node near esophagus), and everolimus was discontinued. Within 5–6 weeks, the patient recovered. Subsequently, olaparib (150 mg PO daily) was added to the treatment regimen to target the *FANCA* mutation; belzutifan was continued at 80 mg daily (Fig. 3). With belzutifan-based therapy, the patient attained a PR (63% decrease in target lesions per RECIST) with complete resolution of the right kidney and bladder lesion and a significant decrease in size of the index liver lesion. The patient continued to have a durable antitumor response ongoing at 20+ months (Fig. 4). Serial brain imaging demonstrated decreased intracranial enhancement of multiple metastatic brain lesions. The patient was not experiencing any dose-limiting toxicities.

DISCUSSION

Patients with *VHL* germline mutations are susceptible to ccRCCs, paragangliomas, pancreatic neuroendocrine tumors, and hemangioblastomas. Biallelic *VHL* inactivation is generally found in sporadic ccRCC.[2] The VHL protein plays a role in oxygen-dependent proteasomal degradation/substrate ubiquitination, which are HIF1α and HIF2α related.[11] The inactivated *VHL* gene product activates the HIF-1 pathway, creating a favorable tumor microenvironment and facilitating tumor metastasis via activating oncogenic growth factors[12]; aberrant VHL also dysregulates cyclin D-CDK4/6 pathway.[13] VEGF is a proangiogenic factor that is upregulated by activated HIF-1. VEGF inhibitors/tyrosine kinase inhibitors (TKIs) are important in ccRCC management; however, the disease almost universally progresses after the initial response.

Our two patients with somatic *VHL* mutation had an excellent antitumor response with belzutifan. Patient 1 received belzutifan as ICI, which was contraindicated, and the patient did not want to risk his vision because of a history of immune retinopathy. The patient had an ongoing PR at >12 months. He had mild to moderate anemia from belzutifan, which required a dose adjustment, and he was maintained without toxicity on 80 mg PO daily of belzutifan (approved dose = 120 mg PO daily).

Patient 2 had multiple prior lines of treatment and was on the verge of hospice as he exhausted the standard of care. His treatment was determined based on molecular profiling: belzutifan targeting his somatic *VHL* mutation and everolimus targeting the *PTEN* mutation (PI3K/mTOR pathway). Imaging demonstrated antitumor activity with this novel combination; however, the patient developed (nonmalignant) pericardial and pleural effusions while on everolimus 5 mg PO daily and belzutifan 80 mg PO daily. Therefore, everolimus was discontinued, and belzutifan continued with recovery from effusions; subsequently, olaparib (150 mg PO daily) was added to target the *FANCA* mutation (DNA repair pathway). Evidence suggests that personalized treatment based on molecular targets is effective in advanced malignancies.[14] This patient had several genomic alterations, including *VHL*, and based on genomic profiling, a molecularly matched therapy (N-of-1 combination) was carefully selected to achieve the best clinical outcome and minimize the chances of resistance to treatment. He continues belzutifan 80 mg daily, which he tolerates well with an ongoing PR at 20+ months.

The results from the phase-1 trial of PT2385 (first HIF-2α antagonist) demonstrated antitumor activity (response rate ~14%) in heavily pretreated ccRCC.[9] Anemia and fatigue were frequent treatment-related adverse events. A second-generation HIF2α inhibitor (MK-6482/belzutifan) was also evaluated in an early-phase trial[8] and demonstrated an objective response rate (ORR) of 25% in 55 patients with metastatic ccRCC. A combination of belzutifan with cabozantinib (phase 2 trial) demonstrated antitumor activity in advanced ccRCC (ORR, 31% [16/52]).[10] The LITESPARK-005 phase 3 clinical trial (NCT04195750) compared belzutifan with everolimus in previously treated advanced ccRCC (ESMO Congress 2023[15]) unselected for germline or somatic *VHL* alterations. Enrolled patients were heavily pretreated with ICIs and VEGF-TKIs, with approximately 87% of patients having received 2–3 lines of therapy. At the time of analysis, there were no statistically significant differences in the overall survival. However, the trial met its coprimary endpoint, with 22.5% of patients remaining progression-free with belzutifan versus 9% with everolimus (hazard ratio: 0.74, 95% CI: 0.63–0.88). The ORR in the belzutifan cohort was better at 22.7% (3.5% of patients had a complete response) versus 3.5% (no complete responses) in patients who received everolimus. Based on these data, belzutifan is now FDA approved for patients with advanced RCC who progressed after ICI and VGEF-TKIs.

No biomarker selection was undertaken in the studies mentioned above; however, the *VHL* gene is commonly altered in ccRCC and correlates with increased expression of HIFs and VEGF.[11] Our two cases highlight the successful use of belzutifan in two patients with refractory RCC and somatic *VHL* alterations, who achieved durable partial remissions ongoing at >12 and >20 months. The cases illustrate the value of an individualized/N-of-1 MTB and specialized Precision Medicine Clinic approach.[14] Because somatic *VHL* alterations are common in patients with sporadic RCC,[2] further exploration of belzutifan in biomarker-driven (somatic *VHL* altered, epigenetic changes in the *VHL* gene expression) cohorts is warranted.

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