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Neurological applications of belzutifan in von Hippel-Lindau disease

Yue Zhang, Catherine C. Nguyen, Nigel T. Zhang, Nicolas S. Fink†, Jordan D. John†, Omkar G. Venkatesh†, Jonathan D. Roe, Steven C. Hoffman, Maciej S. Lesniak, Jean-Paul Wolinsky, Craig Horbinski, Brittany M. Szymaniak, Robin A. Buerki, Jeffrey A. Sosman, Niraj K. Shenoy, and Rimas V. Lukas

Northwestern University, Feinberg School of Medicine, 420 E Superior St. Chicago, IL 60611 USA (Y.Z., N.T.Z., N.S.F., J.D.J., O.G.V., J.D.R., S.C.H.); Loyola University, Chicago, IL, 60660, USA (C.C.N.); Department of Neurology, Northwestern University, Chicago, Illinois 60611, USA (R.A.B., R.V.L.); Lou & Jean Malnati Brain Tumor Institute, Chicago, Illinois 60611, USA (M.S.L., J-P.W., C.H., R.A.B., R.V.L.); Department of Neurological Surgery, Northwestern University, Chicago, Illinois 60611, USA (M.S.L., J-P.W., C.H.); Department of Pathology, Northwestern University, Chicago, Illinois 60611, USA (C.H.); Department of Urology, Northwestern University, Chicago, Illinois 60611, USA (B.M.S.); Department of Internal Medicine, Division of Hematology and Oncology, Northwestern University, Chicago, Illinois 60611, USA (J.A.S., N.K.S.)

†These authors contributed equally to this work.

Corresponding Authors: Rimas V. Lukas, MD, Department of Neurology, Northwestern University, 710 N. Lake Shore Drive, Abbott Hall 1114, Chicago, IL 60611, USA (rimas.lukas@nm.org); Niraj K. Shenoy, MD, PhD, 676 N. St Clair Street, Arkes Pavilion Ste 850, Chicago, IL 60611, USA (niraj.shenoy@nm.org).

Abstract

Von Hippel-Lindau (VHL) disease is a tumor predisposition syndrome caused by mutations in the *VHL* gene that presents with visceral neoplasms and growths, including clear cell renal cell carcinoma, and central nervous system manifestations, such as hemangioblastomas of the brain and spine. The pathophysiology involves dysregulation of oxygen sensing caused by the inability to degrade HIFα, leading to the overactivation of hypoxic pathways. Hemangioblastomas are the most common tumors in patients with VHL and cause significant morbidity. Until recently, there were no systemic therapies available for patients that could effectively reduce the size of these lesions. Belzutifan, the first approved HIF-2α inhibitor, has demonstrated benefit in VHL-associated tumors, with a 30% response rate in hemangioblastomas and ~30%–50% reduction in their sizes over the course of treatment. Anemia is the most prominent adverse effect, affecting 76%–90% of participants and sometimes requiring dose reduction or transfusion. Other significant adverse events include hypoxia and fatigue. Overall, belzutifan is well tolerated; however, long-term data on dosing regimens, safety, and fertility are not yet available. Belzutifan holds promise for the treatment of neurological manifestations of VHL and its utility may influence the clinical management paradigms for this patient population.

Keywords

belzutifan | clear cell renal cell carcinoma | hemangioblastoma | HIF-2α | VHL

Von Hippel-Lindau (VHL) disease is a tumor predisposition syndrome caused by germline inactivation of the *VHL* tumor-suppressor gene. Central nervous system (CNS) manifestations of VHL disease include hemangioblastomas that most commonly affect the cerebellum, spinal cord, and cauda equina. Recently, a small molecule inhibitor of hypoxia-inducible factor-2 alpha (HIF-2α), belzutifan (Welireg), was approved by the Food and Drug Administration for the treatment of VHL disease-associated renal cell carcinoma, CNS hemangioblastomas, and pancreatic neuroendocrine tumors. Approval was based on a nonrandomized phase II clinical trial that demonstrated that belzutifan caused regression of these

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tumors with mild toxicity.[1] Here, we discuss the history and clinicopathologic features of VHL disease, mechanism of action of belzutifan, clinical considerations of treatment, potential mechanisms of resistance, and the impact of belzutifan's approval on the neuro-oncologic management of VHL disease.

Overview of VHL

Historical Perspective

The first case description of a VHL disease manifestation, retinal hemangioblastomas, was in 1894, when British geneticist Edward Treacher Collins reported "peculiar vascular growths" in the retinas of 2 siblings.[2] The German ophthalmologist Eugen von Hippel presented his first case of retinal vascular tumors at the Heidelberg Congress in 1895, and further described familial retinal hemangioblastomas in 1904.[3] The Swedish neuropathologist Arvid Lindau later discovered and reported in 1926 that these familial retinal hemangioblastomas were associated with cerebellar and spinal cord hemangioblastomas, and that these patients also developed kidney cancer and pancreatic cysts.[4] The gene underlying the VHL disease, *VHL*, was finally identified in 1993 at the National Cancer Institute, Maryland, USA[5] (Figure 1). The subsequent 3 decades of research yielded a significant understanding of VHL disease and its associated molecular mechanisms.

Epidemiology

VHL disease is an autosomal dominant disorder caused by germline pathogenic variants in the *VHL* tumor-suppressor gene found on chromosome 3p25–p26.[6,7] VHL disease has an estimated incidence of 1 in 35 500 live births and penetrance of over 90% by the age of 65 years, with men and women being affected equally.[8] It is estimated that 20% of cases are due to *de novo* germline mutation.[9] Patients with VHL disease typically present in the 3rd and 4th decades of life with neural and visceral neoplasms, including CNS hemangioblastomas, retinal capillary hemangioblastomas (retinal angiomas), clear cell renal cell carcinoma (ccRCC), pheochromocytoma, pancreatic islet cell tumor, endolymphatic sac tumors (ELSTs), and reproductive tract papillary cystadenomas.[10,11] Other benign tumors and cysts occur in the kidney, pancreas, and liver.[12] CNS hemangioblastomas occur in up to 80% of patients with VHL disease throughout their lifetime, with up to 40% of cases having hemangioblastoma as their presenting feature.[13] These typically occur in multiple locations at various sites in the CNS.[14] Retinal capillary hemangioblastomas are another common presenting feature, with a mean age of detection of approximately 25 years.[15,16] The presence of a single retinal capillary hemangioblastoma may warrant further VHL testing, especially at a younger age, while 2 confirms the diagnosis.[17] RCC develops in approximately 70% of patients with VHL disease with an average age at diagnosis of 40 years.[11,13,18] Multiple tumors affecting both kidneys are typical.[19] The average life expectancy of patients with VHL disease is between 40 and 52 years, with CNS hemangioblastomas as the most common cause of mortality.[11,20–22]

Pathophysiology

Adaptation to hypoxic stress is critical for aerobic organisms' survival. Hypoxia-inducible factors (HIFs) play a central role in this stress response (Figure 2). The HIF transcription factors are heterodimers consisting of a hypoxia-inducible α subunit (HIF-1α, HIF-2α, or HIF-3α: The 3 isoforms of HIFα) and a constitutively expressed HIF-1β subunit. An oxygen-rich environment, along with α-ketoglutarate and iron, allows prolyl hydroxylases (PHD) to hydroxylate specific proline sites (Pro564 and Pro402) on the oxygen-dependent degradation domain of HIFα. This then allows the VHL protein (pVHL), the substrate recognition unit of an E3 ubiquitin ligase complex (also composed of Elongin B, Elongin C, Cul2, and Rbx1), to recognize the prolyl-hydroxylated HIFα and facilitate its polyubiquitination. This leads to proteasomal degradation of HIFα and a decrease in active HIFα concentration. In an oxygen-deprived environment, lack of the specific prolyl hydroxylation mentioned above results in HIFα stabilization (as it escapes pVHL recognition) and its subsequent translocation to the nucleus, where it heterodimerizes with HIF-1β and enhances the transcription of hypoxia-related genes, including vascular endothelial growth factor (VEGF), erythropoietin (EPO), glucose transporter (GLUT)-1, etc..[23–30]

In VHL disease, patients inherit a defective allele from 1 of their parents, and the other wild-type allele is either mutated, deleted, or epigenetically silenced. The biallelic inactivation of *VHL* results in impaired recognition and ubiquitination of prolyl-hydroxylated HIFα. The neurological and visceral lesions of VHL disease primarily result from the dysregulation and accumulation of 2 isoforms of HIFα, HIF-1α, and HIF-2α, leading to constitutive activation of hypoxic pathways even during normoxia, a phenomenon called pseudo-hypoxia.[25]This chronic pseudo-hypoxic state underlies the highly vascularized tumors of VHL disease, which are characterized by excessive neoangiogenesis.

In sporadic (non-hereditary) ccRCC, biallelic inactivation of the *VHL* gene is a pathognomonic feature, and the primary/truncal molecular event in its carcinogenesis. Most commonly, 1 allele is mutated, and the other allele is lost (as a part of chromosome 3p deletion). Much of our understanding of the biological consequences of biallelic *VHL* inactivation comes from pVHL-defective ccRCC.[31,32] HIF-2α has been established as the major tumor-promoting isoform of HIFα in pVHL-defective ccRCC.[33–35] While HIF-1α has been shown to have a tumor-promoting role in several other malignancies,[36,37] its cumulative role in pVHL-defective ccRCC is extensively debated, and continues to be an area of active investigation.[35,38–41] Both isoforms have overlapping as well as distinct functions/ target genes mediating the hypoxic response, which varies with cellular contexts.[42–45]

VHL disease-associated hemangioblastomas are also characterized by upregulation of HIF-1α, HIF-2α, and HIF

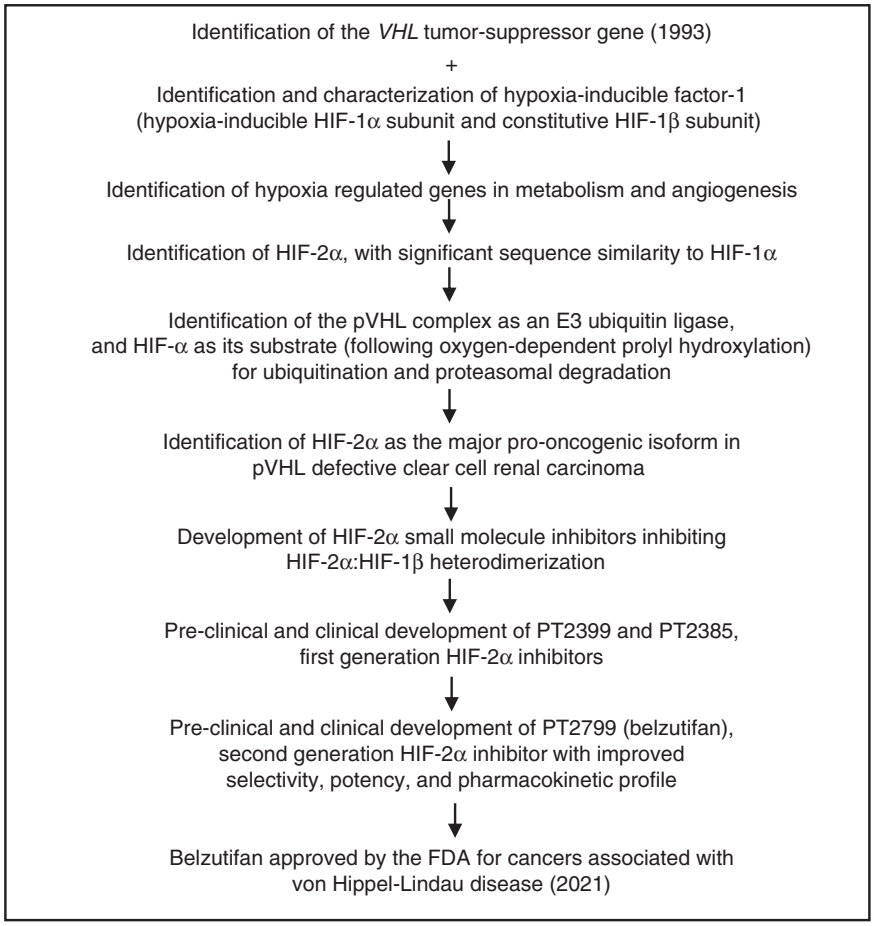

Figure 1. Schema depicting progressive scientific advances from the identification of the Von Hippel-Lindau *(VHL)* gene to the approval of belzutifan for VHL-associated malignancies. Identification and characterization of the *VHL* tumor-suppressor gene and concurrent discovery of hypoxia-regulated genes led to the creation of mechanism-based novel therapeutics, culminating in the development and approval of belzutifan.

target genes such as VEGF and GLUT1.[46–49] In endothelial cells, HIF-1α primarily transcribes genes largely involved in metabolic processes in response to hypoxia, while HIF-2α primarily transcribes genes largely involved in angiogenesis, extracellular matrix organization, and endothelial cell migration.[50]

Clinical Manifestations

The clinical presentation of VHL disease is dependent on tumor burden and location. Hemangioblastomas in VHL disease tend to arise in the posterior fossa and spinal cord.[51,52] Most of the intracranial tumors are in the cerebellum, although it is not uncommon to observe brainstem involvement.[53] Intracranial tumors of VHL disease can cause symptoms related to increased intracranial pressure either due to direct mass effect and cerebral edema or from obstruction of cerebrospinal fluid (CSF) outflow through the 4th ventricle. These symptoms include positional headaches, nausea, vomiting, diplopia, and somnolence. They can also cause symptoms related directly to the structures affected, leading to dysmetria from cerebellar lesions as well as cranial neuropathies, motor, and sensory symptoms due to brainstem lesions.[6] Spinal lesions may be asymptomatic or produce CNS symptoms consistent with the level of the spinal cord that is involved.[35] As the lesions are more commonly located on the dorsal surface of the cord, posterior column symptoms, such as impaired proprioception and vibratory sensation, may be expected. Although rare, patients who develop ELSTs of the middle ear may present with hearing loss.[54] Retinal capillary hemangioblastomas may be asymptomatic or present with a complete visual loss depending on the extent of local tissue involvement.[35] Retinal inflammation and even detachment may occur, producing the common findings of floaters and flashes.

Hemangioblastomas often have radiographic appearances that resemble metastatic RCC.[55,56] RCC metastases would be less likely to be restricted to the posterior fossa, less likely to be as cystic, and relatively unlikely to metastasize if the primary renal lesion is <3 cm in diameter.[57–59] Spinal angiograms with DynaCT can be diagnostic for spinal hemangioblastomas.[60] The tumors tend

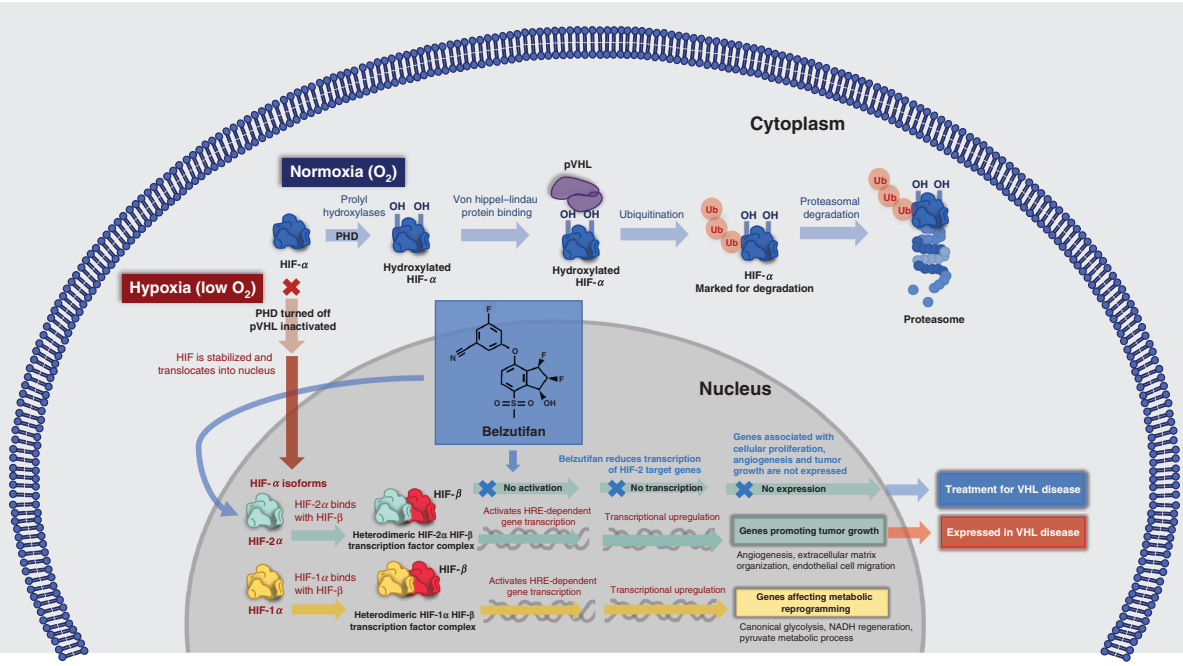

Figure 2. Mechanism of action of belzutifan. The physiologic response to hypoxia relies on the VHL (Von Hippel-Lindau)/HIF (hypoxia-inducible factor)/PHD (prolyl-hydroxylase) axis. The HIF transcription factors are heterodimers consisting of a hypoxia-inducible α subunit (HIF-1α and HIF-2α being the most well-studied isoforms) and a constitutively expressed HIF-1β subunit. Under normal oxygen conditions, specific proline residues of HIF-α are hydroxylated by PHDs. VHL is the substrate recognition unit of an E3 ubiquitin ligase complex that recognizes the prolyl-hydroxylated HIF-α and facilitates its polyubiquitination. The ubiquitinated HIF-α is degraded by the proteasome. Under hypoxic conditions, PHDs are inhibited and HIF-α is not hydroxylated, preventing downstream steps that mark the protein for degradation. HIF-α enters the nucleus and heterodimerizes with HIF-β, forming the HIF transcription factor. Von Hippel-Lindau disease is caused by biallelic inactivation of the VHL gene (one defective allele inherited from a parent and the wild-type allele is inactivated via mutation/deletion/silencing), leading to constitutive HIF transcription factor activation. In endothelial cells, HIF-1α primarily transcribes genes involved in metabolic processes that respond to hypoxia, while HIF-2α primarily transcribes genes involved in angiogenesis, extracellular matrix organization, and endothelial cell migration. In VHL-defective clear cell renal cancer, HIF-2α has been shown to be the major pro-oncogenic isoform of HIFα. Belzutifan is a selective HIF-2α inhibitor that disassembles HIF-2α/HIF-1β complexes.

to be sub-pial and have characteristic blood supplies and drainage. However, MRI is likely the most common means of imaging these tumors.

Screening

Hemangioblastomas are the most common tumor type in VHL disease and are the cause of death in approximately half of the patients with VHL disease due to neurological complications, such as hydrocephalus, herniation, brainstem compression, and intracranial hemorrhage.[21] Screening (detecting new hemangioblastomas) and surveillance (identifying changes to a patient's hemangioblastomas), coupled with history and examination, can reduce morbidity and mortality. MRI of the entire craniospinal axis should be performed every 2 years beginning at age 11 years and prior to planned pregnancy for surveillance of patients with VHL disease. Asymptomatic screening can be stopped after age 65 if they have never developed a hemangioblastoma. Annual age-appropriate history and comprehensive neurological exam are used to screen for symptoms in lieu of MRI for the 1st decade of life, and screening may be done whenever new clinical signs or symptoms arise.[61]

Therapeutic Management

Intracranial hemangioblastomas can be the fastest-growing benign intracranial tumors, exhibiting saltatory growth at an average of 2.38 cm^3/year in untreated patients with VHL disease.[62] Symptoms or progression of hemangioblastomas in VHL disease typically guide management. Hemangioblastomas with a diameter larger than 5.6 mm in the cerebellum, 4.0 mm in the brainstem, and 4.5 mm in the spinal cord are fairly sensitive and specific markers for clinical symptoms.[63] The presence of symptoms typically supersedes the absolute tumor size when considering surgical intervention, as symptoms are significantly predictive of growth.[62] Therapeutic planning must also consider the patients' non-CNS manifestations of VHL disease and their potential impacts on neurosurgical intervention. These include the operative risks of an occult pheochromocytoma,

as well as potential renal impairment related to renal cell carcinoma, renal cysts, and nephrectomies.[64,65]

Complete microsurgical resection of CNS hemangioblastomas is typically curative of the tumor and peritumoral cysts.[66] The potential multiplicity of tumors and their saltatory growth patterns make surgery impractical in many situations. Tumor location, including brainstem involvement, makes surgery for VHL disease-associated hemangioblastomas often high-risk.[67] This can be further compounded by a prior history of multiple surgeries and their associated scarring and gliosis. Surgery has a postoperative mortality rate of 2%–6% with a neurological morbidity rate of 7%–20% but tends to have a total resection rate of 94%–100% and provides improved or stable outcomes in 89%–97% of cases.[67]

Hemangioblastomas are well-circumscribed and spherical, making stereotactic radiosurgery an option for difficult-to-resect tumors, allowing multiple intracranial lesions to be treated in a minimally invasive manner.[68] Tumors larger than 3 cm or accompanied by peritumoral cysts are not favorable candidates.[69] While moderate-term (5–10 years) control is quite good with stereotactic radiosurgery, long-term (15 years) control is more limited.[70–72] Due to limited control over longer periods of follow-up, stereotactic radiosurgery is typically reserved for patients with an unfavorable risk profile for surgery, a history of multiple surgeries, or where definite surgical resection is not possible.[70–72]

Systemic Therapy

Clinical trials have historically investigated multi-targeted tyrosine kinase inhibitors (TKI) to treat the sequelae of VHL disease (Figure 1, Table 1). These included VEGF inhibitors like pegaptanib[73] and semaxanib, as well as the VEGF-targeting antibodies ranibizumab[74] and bevacizumab; multi-target TKI, including against VEGF and platelet-derived growth factor receptor (PDGFR) like vatalanib, sunitinib,[75–77] vandetanib, dovitinib,[78] and pazopanip[79]; heat-shock protein (HSP)-90 inhibitors like tanespimycin; histone deacetylase (HDAC) inhibitors like vorinostat; and HIF-2α inhibitors.[1,80,81] The most efficacious approved therapies among these have been pazopanib and belzutifan.

The phase 2 clinical trial of pazopanib in VHL by Jonasch *et al.* in 2018 investigated treatment with pazopanib 800 mg PO daily for 24 weeks.[79] While non-CNS response rates were favorable, those for the CNS were limited. The reported organ-specific response rate was 52% for renal cell carcinoma and 53% for pancreatic lesions, but only 4% for hemangioblastomas. There were no complete responses, and the hemangioblastomas shrunk on average by 13%.[79] In contrast, the phase 2 clinical trial of belzutifan in VHL disease by Jonasch et al. in 2021 reported that treatment with belzutifan (discussed in detail below) resulted in a response of 30% of hemangioblastomas.[1] The hemangioblastomas decreased by 30%–50%, and 6% of patients had complete responses.[1] While not directly compared, belzutifan appears the superior choice in pharmacologic treatment for hemangioblastomas.

Mechanism of Action of Belzutifan

Belzutifan, formerly MK-6482, is a small molecule inhibitor that acts as a selective HIF-2α inhibitor.[80] By binding to HIF-2α, it limits binding with HIF-1β (Figure 2).[82] Inhibiting this interaction with HIF-2 prevents transcription of various downstream genes. The HIFα subunits are both involved in the biological response to hypoxia. In endothelial cells, HIF-1α primarily transcribes genes involved in metabolic processes that respond to hypoxia, while HIF-2α primarily transcribes genes involved in angiogenesis, extracellular matrix organization, and endothelial cell migration. Data indicate that the HIF-1α isoform is more susceptible to non-VHL/PHD mechanisms of degradation than HIF-2α, such as via HIF asparaginyl hydroxylase (previously called factor inhibiting HIF).[83,84] The antitumor response with selective HIF-2 antagonists in preclinical models of VHL-defective ccRCC demonstrated HIF-2a's dominant pro-oncogenic role in the absence of functional VHL. The impressive clinical response with belzutifan in malignancies associated with VHL disease confirmed HIF-2's role as the major oncogenic isoform of HIF-α in VHL disease.

Clinical Outcomes With Belzutifan

The international phase 2 nonrandomized trial by Jonasch *et al.* evaluated 120 mg PO daily of belzutifan in patients with VHL disease and non-metastatic RCC not requiring immediate surgery.[1] The full eligibility criteria are presented in Figure 3. This trial was able to demonstrate radiographic responses, as determined by independent review, in hemangioblastoma as well as in renal cell carcinoma (the trial's primary endpoint) and pancreatic neuroendocrine tumors as measured by RECIST criteria.[1] RECIST is a system utilizing the diameters of the measurable lesions, which for the relatively spherical shape of most hemangioblastomas would be a reasonable means of assessment. Among the cohort of 61 patients, there were 60 total hemangioblastomas among 50 patients: 27 in the cerebellum, 23 in the spine, and 10 in other locations such as the brainstem or frontal lobe. This represented a median of 1.5 hemangioblastomas per patient, a relatively low tumor burden relative to the average of 2 by age 50 years, when measured by absolute number.[85] Retinal capillary hemangioblastomas affected 16 eyes among 12 patients. Patients ranged in age from 19 to 66 years (median 41 years). There was a near-equal number of male (52%) and female participants.

The response rate for hemangioblastomas (Figure 4), encompassing both solid and cystic components of the lesions was 30%, with 6% experiencing complete responses. This is less than the 49% response rate observed in RCC or 91% in pancreatic neuroendocrine tumors. The median time to response for CNS hemangioblastomas was 3.2 months (range 2.5–16.4 months), which was shorter than what was observed in non-CNS tumors, such as 8.2 months for renal cell carcinoma (range 2.7–19.1 months), 8.4 months for pancreatic lesions (range 2.5–16.4 months), and 5.5 months for pancreatic neuroendocrine tumors (range 2.3–16.6 months). The

Table 1. Pan-VHL and Hemangioblastoma Clinical Trials

Principal Investigator	ClinicalTrial ID	Year	Patients	Phase	Agent	Target	PFS (Mos.)	RR (PR+CR) (%)
Emily Chew	NCT00056199	2003–2005	5	1	Pegaptanib	VEGF	N/A	20% (rHBL*)
Novartis	NCT00052013	2003–2006	11	2	Vatalanib	RTK	N/A	0%
Adrian L. Harris	N/A	2004	6	1,2	Semaxanib	VEGF	N/A	0%
Emily Chew	NCT00089765	2004–2007	5	1	Ranibizumab	VEGF	N/A	20% (rHBL*)
William M. Linehan	NCT00088374	2004–2009	9	2	Tanespimycin	HSP-90	N/A	0%
Eric Jonasch	NCT00330564	2006–2011	15	2	Sunitinib	RTK	N/A	33% (RCC), 0% (HBL)
Catherine Meyerle	NCT00673816	2008–2011	3	1,2	Sunitinib	RTK	N/A	0%
William M. Linehan	NCT00566995	2008–2015	37	2	Vandetanib	RTK	(11.0 to 22.1)	8% (RCC)
J. Marc Pipas	NCT01015300	2009–2012	1	1	Bevacizumab	VEGF	N/A	0%
Stephane Richard	NCT01168440	2010–2011	5	2	Sunitinib	RTK	N/A	0%
Eric Jonasch	NCT01266070	2012–2015	6	2	Dovitinib	RTK	N/A	0%
Eric Jonasch	NCT01436227	2012–2021	31	2	Pazopanib	RTK	N/A	42% (ORR), 52% (RCC), 53% (pNETs), 4% (HBL)
Kevin D. Courtney	NCT03108066	2014–2016	51	1	PT2385	HIF-2α	53% at 52 Weeks	14% (RCC)
Prashant Chittiboina	NCT02108002	2014–2018	7	0	Vorinostat	HDAC	N/A	0%
Henry E. Wiley	NCT02859441	2016–2020	3	1,2	E10030 and Ranibizumab	PDGF, VEGF	N/A	0%
Eric Jonasch	NCT03401788	2016–2020	95	1	Belzutifan	HIF-2α	14.5 Months	25% (RCC)
Kevin D. Courtney	NCT03108066	2017–2022	4	2	PT2385	HIF-2α	N/A	N/A
Eric Jonasch	NCT03401788	2018–2026	61	2	Belzutifan	HIF-2α	98% at 52 Weeks	47% (ORR), 80% (pNETs), 32% (HBL), 69% (rHBL*)

The clinical trials with the "condition or disease" search terms "Von Hippel-Lindau" and "Hemangioblastoma" were searched on clinicaltrials.gov. This obtained 38 pan-VHL and 14 hemangioblastoma trials. Trials that included a pharmacologic agent were selected for the table. A PubMed search with the filter "Clinical Trials" using the terms "Von Hippel-Lindau" and "hemangioblastoma" returned 59 and 26 papers, respectively. Similarly, only pharmacologic agents were included in the table. The principal investigator was obtained from the corresponding clinicaltrials.gov entry or from the corresponding publication. The response rate was calculated as the sum of partial response and complete response. Retinal hemangioblastomas do not have formal RR; these represent the percentage of patients who experienced improvements in vision following treatment. These searches were performed on June 1, 2022. PFS: Progression-free survival. RR: Response rate. PR: Partial response. CR: Complete response. rHBL: Retinal hemangioblastoma. RCC: Renal cell carcinoma. HBL: Hemangioblastoma. pNETs: Pancreatic neuroendocrine tumors. ORR: Overall response rate. VEGF: vascular endothelial growth factor. RTK: receptor tyrosine kinase; can target multiple kinases including VEGF. HSP-90: heat-shock protein 90. HDAC: histone deacetylase. HIF-2α: hypoxia-inducible factor-2 alpha.

median change in the size of cerebellar hemangioblastomas was –30%; of spinal hemangioblastomas, –51%, and of other hemangioblastomas, –35%. All cases of ocular hemangioblastomas were graded to have improvement. While data continues to mature, responses appear durable thus far.

The toxicities of belzutifan gleaned from the phase 1 and phase 2 trials of belzutifan are relatively predictable and fairly well tolerated (Table 2).[1] Overall, the most frequent adverse effect is anemia, affecting the overwhelming majority of patients (76%–90%). Other common side effects include fatigue (66%–71%) and dyspnea (23%–49%). These do not perfectly correlate with the incidence or severity of the anemia. One patient had grade 3 hypoxia, which was transient and resolved with dose interruption for 1 week followed by dose reduction to 80 mg. The cause of hypoxia during belzutifan treatment is not well understood and was not associated with hemoglobin levels. Although a large majority of adverse events were grades 1 or 2, patients with anemia on treatment sometimes required dose delays, dose reductions, and blood transfusions. It is important to note that in the trial, 12 out of 61 patients (~20%) received erythropoietin-stimulating agents, and 3 of these 12 patients also received a blood transfusion. However, the FDA has not approved the use of erythropoietin-stimulating agents with belzutifan. Management of anemia and related symptoms in the real-world setting may therefore be more challenging than the trial data suggest.

In addition, there are concerns regarding the potential teratogenicity of belzutifan.[86] This is of particular importance as belzutifan can decrease concentrations of CYP3A4 substrates, which can lead to the failure of oral contraception. In addition, based on preclinical studies, this agent may decrease fertility in both males and females. Thus, an oncofertility consultation prior to the initiation of treatment may be warranted.

Clinical Experience

Clinical experience with belzutifan is limited and optimizing patient choice and treatment regimen will be an

Inclusion criteria:

1. Has the ability to understand and willingness to sign a written informed consent form before the performance of any study-specific procedures.
1. 18 years of age or older.
2. Has a diagnosis of von Hippel-Lindau disease, based on a germline VHL alteration
3. Has at least 1 measurable solid RCC tumor and no RCC tumor greater than 3.0 cm that requires immediate surgical intervention. The diagnosis of RCC can be radiologic (histologic diagnosis not required). Patients may have VHL disease-associated tumors in other organ systems.
4. Has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1
5. Has organ and marrow function as defined below:
   a. Absolute neutrophil count ≥ 1000/μL, hemoglobin level ≥ 10 g/dL and platelet count ≥ 100 000/μL without transfusion or growth factor support within 2 weeks prior to obtaining the hematology values at screening
   b. Serum creatinine level ≤ 2.0 × upper limit of normal (ULN)
   c. AST and ALT < 2.5 × ULN, total bilirubin < 1.5 × ULN (< 3 × ULN in patients with Gilbert's disease), and alkaline phosphatase ≤ 2.5 × ULN
6. If a female patient of child-bearing potential, or a male patient with a female partner of child-bearing potential (defined as all women physiologically capable of becoming pregnant), must agree to use highly effective methods of contraception during screening, during the period of drug administration and for 90 days after stopping study drug administration. Highly effective contraception methods include the following:
   a. Total abstinence,
   b. Male or female sterilization, or
   c. Use of at least one of the following:
      i. Use of oral, injected or implanted hormonal methods of contraception
      ii. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
      iii. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository
7. Female patients of child-bearing potential must have a negative serum pregnancy test result within 7 days before first administration of study drug
8. Able to swallow oral medications

Exclusion criteria:

1. Has participated in another clinical trial of an investigational drug (or a medical device) within 30 days of study enrollment
2. Has received prior treatment with PT2977 or another HIF-2α inhibitor
3. Has had any systemic anti-cancer therapy (includes anti-VEGF therapy or any systemic investigational anti-cancer agent)
4. Has had radiotherapy within 4 weeks prior to study enrollment
5. Has had surgical procedure for VHL disease or any major surgical procedure completed within 4 weeks prior to study enrollment
6. Has an immediate need for surgical intervention for tumor treatment
7. Has a prior or concomitant non-VHL disease-associated invasive malignancy with the exception of adequately treated basal or squamous cell carcinoma of the skin, cervical carcinoma in situ or any other malignancy from which the patient has remained disease free for more than 2 years
8. Has any history of metastatic disease
9. Has malabsorption due to prior gastrointestinal (GI) surgery or GI disease
10. Has hypersensitivity to the active pharmaceutical ingredient or any component in the formulation
11. Has an active infection requiring systemic treatment
12. Has had any major cardiovascular event within 6 months prior to study drug administration including but not limited to myocardial infarction, unstable angina, cerebrovascular accident, transient ischemic event, pulmonary embolism, clinically significant ventricular arrhythmias (e.g., sustained ventricular tachycardia, ventricular fibrillation, torsades de pointes) or New York Heart Association Class III or IV heart failure
13. Has any other clinically significant cardiac, respiratory, or other medical or psychiatric condition that might interfere with participation in the trial or interfere with the interpretation of trial results, in the opinion of the investigator or medical monitor
14. If a female patient, intends to breast feed a child while on study drug or within 30 days after administration of the last dose of study drug

Figure 3. Inclusion and exclusion criteria for phase II trial. The inclusion and exclusion criteria are displayed, per trial protocol. Notably, the inclusion criteria feature an initial hemoglobin level ≥10 g/dL. Patients who have had metastatic disease, received systemic anticancer therapy at any time, or had surgery/radiotherapy in the past 4 weeks were excluded.

iterative process. Guidance is available for clinicians via the VHL Virtual Tumor Board organized by the VHL Alliance (vhl.org/virtualtumorboard).[61,87] This provides the opportunity for multidisciplinary, multi-institutional expert opinion for clinical cases.

In our clinical practice, we consider the use of belzutifan with VHL disease-associated hemangioblastomas, both symptomatic and asymptomatic, that are progressive. One of the goals of systemic therapy is to delay or obviate the need for surgical intervention. This is a particularly attractive option when there are multiple lesions that cannot be addressed in a single surgical procedure, or for which a surgical procedure would not be indicated as they are relatively small and/or asymptomatic. In our clinical practice, larger progressive symptomatic lesions would still be more likely managed by surgery. In juxtaposition, multiple, progressive, small-to-moderate sized lesions, or lesions in higher-risk locations (i.e., brainstem) would be viewed as appropriate for systemic treatment with belzutifan.

Patients with significant side effects may need dose reductions (to 80 mg daily, and rarely, to 40 mg daily). The overall clinical situation, such as symptoms from tumors, side effects from medications, patient tolerance, and median time to response in the malignancy of highest clinical concern, all influence collaborative decisions on dose reduction (between neuro-oncology, medical oncology, neurosurgery, urology, and/or ophthalmology, as relevant to the patient). As the neuro-oncology community's collective experience with systemic therapies for VHL disease-associated hemangioblastomas grows, we presume that clinical practice will evolve.

Potential Mechanisms of Resistance to HIF-2 Antagonists

Both primary and acquired resistance have been observed with HIF-2 antagonists. Evaluation of PT2399, a 1st-generation HIF-2α antagonist, on patient-derived ccRCC xenografts demonstrated that 22% (4–18) were resistant.[88] Interestingly, PT2399 was able to selectively disassemble HIF-2 heterodimers (HIF-2α/HIF-1β) in both resistant and nonresistant xenografts and suppress downstream targets, such as EPO, suggesting that non-HIF-2 mechanisms governing resistance are involved.[88]

Acquired resistance to HIF-2 antagonists was observed in the preclinical studies of PT2399 and the clinical studies of PT2385/MK3475 (phase 1) and PT2977 (belzutifan).[1,88,89] A G323E substitution in *EPAS1* (HIF-2α) occurs in the entrance of the cavity to which the HIF-2 antagonists bind, and the glutamate side chain prevents their access.[88,89] The mutant HIF-2α/HIF-1β complexes are not dissociated by the inhibitors.[88,89] A suppressor mutation occurring in the HIF-1β PAS-B domain was also resistant to dissociation by PT2399.[88] The F446L mutation replaces the residue with more flexible amino acid and is postulated to permit the drug-bound HIF-2α to bind HIF-1β.[88]

Investigations are ongoing to identify targetable mechanisms of primary and acquired resistance and synergy with belzutifan. Early preclinical evidence demonstrates synthetic lethality in *VHL*$^{-/-}$ human cell lines and xenografts with CDK4/6 inhibition.[90] This was observed independently of HIF-2α activity, suggesting an avenue of therapy for both primary and secondary resistance.[90]

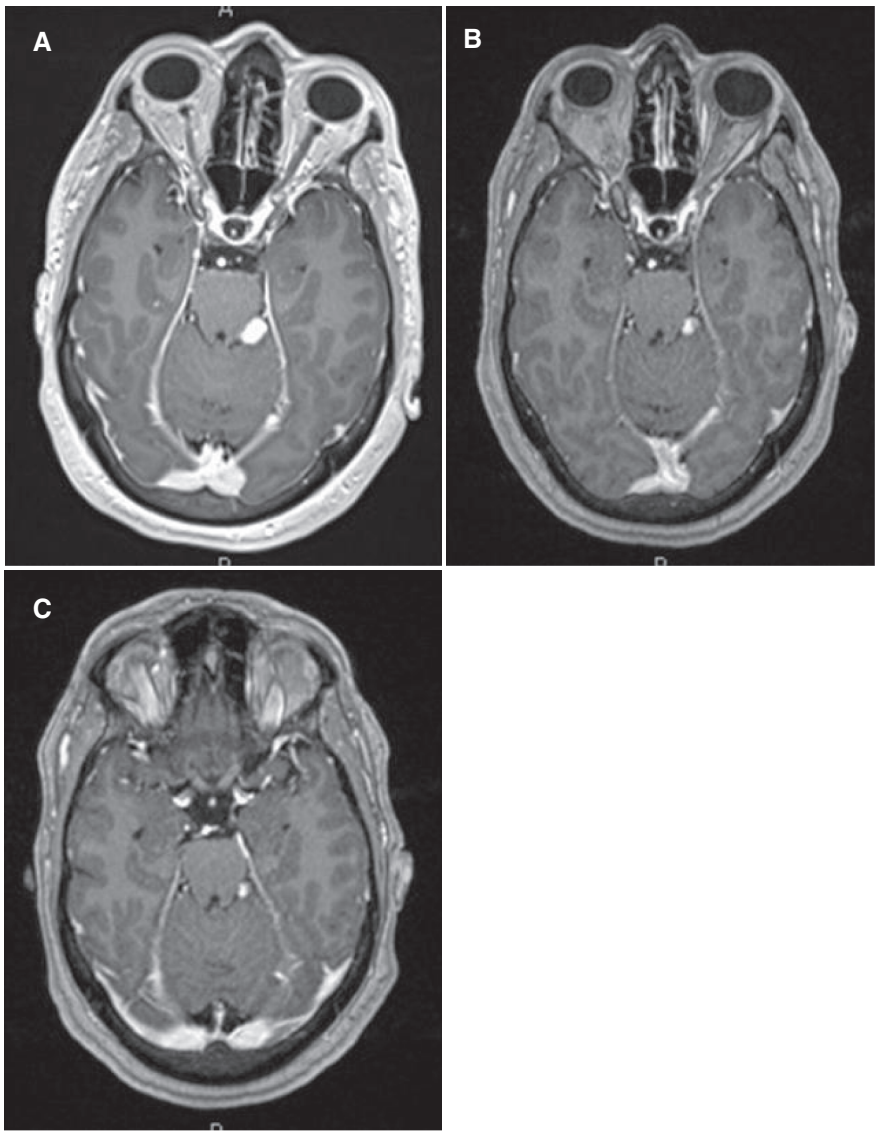

Figure 4. Radiographic response of Central nervous system **(CNS) hemangioblastoma treated with belzutifan.** (**A**) Axial T1 post-contrast MRI demonstrating a left peri-pontine enhancing lesion consistent with a hemangioblastoma on baseline imaging. (**B**) A notable decrease in size is observed 40 days after initiating belzutifan. (**C**) There is a further slight decrease in size observed 7 months after initiating belzutifan.

Future Directions

Several questions regarding belzutifan's efficacy in hemangioblastomas and other tumors remain, including optimal patient selection, the timing of initiating treatment, and the duration of therapy. In the phase 2 trial, patients had a limited CNS tumor burden and may not be representative of patients seen in many VHL clinics. In addition, the racial and ethnic background of the patient population was unclear. As referred to above, the optimal duration of therapy is uncertain. Finally, choosing which patients to treat and when to treat is still an active area of debate, and 1 which will likely evolve as our collective experience and comfort with this new class of drugs increases. In some clinical practices, it is the patients with multiple asymptomatic or minimally symptomatic growing lesions, who are not ideal surgical candidates, who may benefit most from systemic therapy. This, however, is not a direct representation of the patients studied in the phase 2 trial.

In addition, avenues exist for belzutifan's investigation in both HIF pathway aberrant sporadic tumors such as ccRCC as well as more common CNS tumors with overactivity of angiogenic pathways. An understanding of the molecular targets enables the application of belzutifan to other tumor predisposition syndromes with shared pathways. For example, Pacak-Zhuang syndrome is a rare tumor predisposition syndrome caused by gain of function mutation

Table 2. Adverse Effects of Belzutifan in Phase I and II Trials

Adverse Event, No. (%)	Phase I Cohort ($n = 55$)			
MedDRA SOC, CTCAE 5.0	All	Grade 1 or 2	Grade 3	Grade 4
Any	55 (100)	16 (29)	33 (60)	2 (4)
Blood and lymphatic system disorders				
Anemia	42 (76)	27 (49)	15 (27)	0 (0)
Gastrointestinal disorders				
Constipation	12 (22)	12 (22)	0 (0)	0 (0)
Diarrhea	12 (22)	12 (22)	0 (0)	0 (0)
Nausea	20 (36)	19 (35)	1 (2)	0 (0)
Vomiting	16 (29)	16 (29)	0 (0)	0 (0)
General disorders and administration site conditions				
Fatigue	39 (71)	36 (65)	3 (5)	0 (0)
Localized Edema	15 (27)	15 (27)	0 (0)	0 (0)
Investigations				
Creatinine Increased	14 (25)	13 (24)	1 (2)	0 (0)
Hyperkalemia	12 (22)	11 (20)	1 (2)	0 (0)
Metabolism and nutrition disorders				
Dehydration	11 (20)	10 (18)	1 (2)	0 (0)
Musculoskeletal and connective tissue disorders				
Arthralgia	14 (25)	14 (25)	0 (0)	0 (0)
Back pain	12 (22)	11 (20)	1 (2)	0 (0)
Nervous system disorders				
Dizziness	13 (24)	13 (24)	0 (0)	0 (0)
Headache	14 (25)	13 (24)	1 (2)	0 (0)
Respiratory, thoracic, and mediastinal disorders				
Cough	17 (31)	17 (31)	0 (0)	0 (0)
Dyspnea	29 (49)	24 (44)	3 (5)	0 (0)
Hypoxia	17 (31)	8 (15)	9 (16)	0 (0)

Adverse Event, no. (%)	Phase II cohort ($n = 61$)				
MedDRA SOC, CTCAE 5.0	All	Grade 1	Grade 2	Grade 3	Grade 4
Any	61 (100)	N/A	N/A	9 (15)	1 (2)
Blood and lymphatic system disorders					
Anemia	55 (90)	24 (39)	26 (43)	5 (8)	0 (0)
Gastrointestinal disorders					
Nausea	21 (34)	15 (25)	6 (10)	0 (0)	0 (0)
Constipation	12 (20)	10 (16)	2 (3)	0 (0)	0 (0)
General disorders and administration site conditions					
Fatigue	40 (66)	29 (48)	8 (13)	3 (5)	0 (0)
Musculoskeletal and connective tissue disorders					
Arthralgia	12 (20)	10 (16)	2 (3)	0 (0)	0 (0)
Myalgia	12 (20)	9 (15)	2 (3)	1 (2)	0 (0)
Nervous system disorders					
Headache	25 (41)	20 (33)	5 (8)	0 (0)	0 (0)
Dizziness	24 (39)	20 (33)	4 (7)	0 (0)	0 (0)
Respiratory, thoracic, and mediastinal disorders					
Dyspnea	14 (23)	13 (21)	0 (0)	1 (2)	0 (0)

The adverse effects of belzutifan were obtained from the phase I and phase II trials. Adverse events affecting 20% or more of the study participants are listed here. They were categorized according to CTCAE 5.0 MedDRA SOC and converted into CTCAE terms. The adverse events were categorized from grades 1–4 and presented as the number of individuals and percent of the total. There were no grade 5 adverse events. The most common adverse event was anemia, which occurred in 76% of patients in the phase I trial and 90% of the patients in the phase II trial.

in the gene encoding HIF-2α (*EPAS1*).[91] Patients with this syndrome have both polycythemia and the development of metastatic paragangliomas or somatostatinomas. More frequently, cases of isolated polycythemia or paraganglioma are found in *EPAS1* mutations. Initiation of belzutifan in a patient with Pacak-Zhuang syndrome led to the resolution of daily headaches, hypertension, and polycythemia; furthermore, there was a decrease in serum paraganglioma markers chromogranin A and plasma normetanephrine.[91] Value of blocking HIF-2α in tumors such as glioblastoma, where some antiangiogenic therapies such as bevacizumab have demonstrated some benefit where others have not, or in certain meningiomas, may warrant additional study.

Conclusions

Belzutifan is the 1st systemic therapy approved for VHL disease and the 1st approved agent targeting HIF-2α. It has demonstrated impressive response rates in VHL disease-associated malignancies, including CNS hemangioblastomas, with an overall manageable short-term adverse effect profile. The most frequent adverse events include anemia and related sequelae. However, long-term data on safety is lacking and the impact on fertility and teratogenicity is unknown. While belzutifan has demonstrated efficacy in obviating the need for surgery in the short term, the balance between long-term adverse effects and overall risk reduction in patients with VHL disease has yet to be established. As such, whether therapy should be continuous or intermittent over many years is currently not well known and will likely depend on tolerance and degree of clinical benefit in the individual patient. Careful patient selection, the timing of initiation of therapy, close monitoring, and appropriate management of adverse effects will be important to ensure a favorable clinical benefit to adverse effect profile for most patients in the clinical setting.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

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