Current Oncology Reports (2019) 21: 6
https://doi.org/10.1007/s11912-019-0752-z

EVOLVING THERAPIES (RM BUKOWSKI, SECTION EDITOR)

HIF Inhibitors: Status of Current Clinical Development

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Published online: 22 January 2019

Abstract

Purpose of Review In this review, the importance of the hypoxia inducible factor (HIF) pathway in tumorigenesis and cancer treatment outcomes will be discussed. The outcomes of phase II and III clinical trials of direct HIF inhibitors in the treatment of cancer will be reviewed.

Recent Findings The HIF signaling pathway is activated by tumor-induced hypoxia or by inactivating mutations of the VHL gene. HIF is a transcription factor which regulates the expression of genes involved in adjusting mechanisms to hypoxia such as angiogenesis or apoptosis as well as tumor growth, invasion, and metastasis. The HIF pathway has a key role in development of resistance to different treatment modalities and higher expression of the HIF molecule is associated with poor prognosis.

Summary Clinical studies of the HIF inhibitors in patients with advanced/refractory cancers suggest benefit and warrant further studies of the HIF inhibitors either as a single agent or in combination with other therapeutic agents.

Keywords HIF · HIF inhibitor · Hypoxia inducible factor · Renal cell carcinoma · VEGF · VHL

Introduction

Hypoxia plays a crucial role in the development and progression of cancer. Tumor cells induce hypoxia by various mechanisms such as high metabolism rate and consumption of oxygen, causing dysfunction of endothelium or disruption of oxygen delivery from mass effect on vessels. The subsequent tumor-induced hypoxia leads to activation of the hypoxia inducible factor (HIF) signaling pathway, which enhances tumor growth and invasion.

The HIF protein was first identified during a study on the erythropoietin gene in 1991. It was found that a gene next to the erythropoietin gene encodes a protein which activates transcription of several genes involved in response to hypoxia-related stress [1].

HIF is a transcription factor with two subunits, HIF1-alpha (or its analogs HIF2-alpha and HIF3-alpha) and HIF1-beta (1, 2). HIF1-alpha is in the cytoplasm and its level increases in hypoxic situations [2, 3]. HIF1-beta (also known as ARNT) is in the nucleus and binds to the HIF1-alpha to activate angiogenic mechanisms which help the cells to adjust to hypoxia [2, 3].

The HIF Pathway

The HIF-1 alpha molecule is persistently synthesized in cells and, depending on oxygen concentration, gets degraded or accumulates in the cell. In the presence of oxygen, prolyl hydroxylase enzyme adds a hydroxyl to proline residue in the HIF1-alpha molecule. The Von Hippel-Lindau (VHL) protein attaches to the hyroxylated proline residue and ubiquinizes HIF-1 alpha. The ubiquitylation of HIF1-alpha makes it identifiable by a protease enzyme and leads to its destruction [4] (Fig. 1).

The HIF signaling pathway gets activated by hypoxia [4] or by inactivating mutations of the VHL gene [5]. In these conditions, hydroxylation, ubiquitylation, and degradation of the HIF-1 alpha does not occur which subsequently leads to accumulation of the HIF1-alpha in cell cytoplasm. Then, HIF1-alpha enters the cell nucleus and dimerizes with the HIF1-beta molecule to form the HIF1 complex [4]. This complex acts as a transcriptional factor for hundreds of genes including genes involved in angiogenesis such as vascular endothelial growth factor

This article is part of the Topical Collection on *Evolving Therapies*

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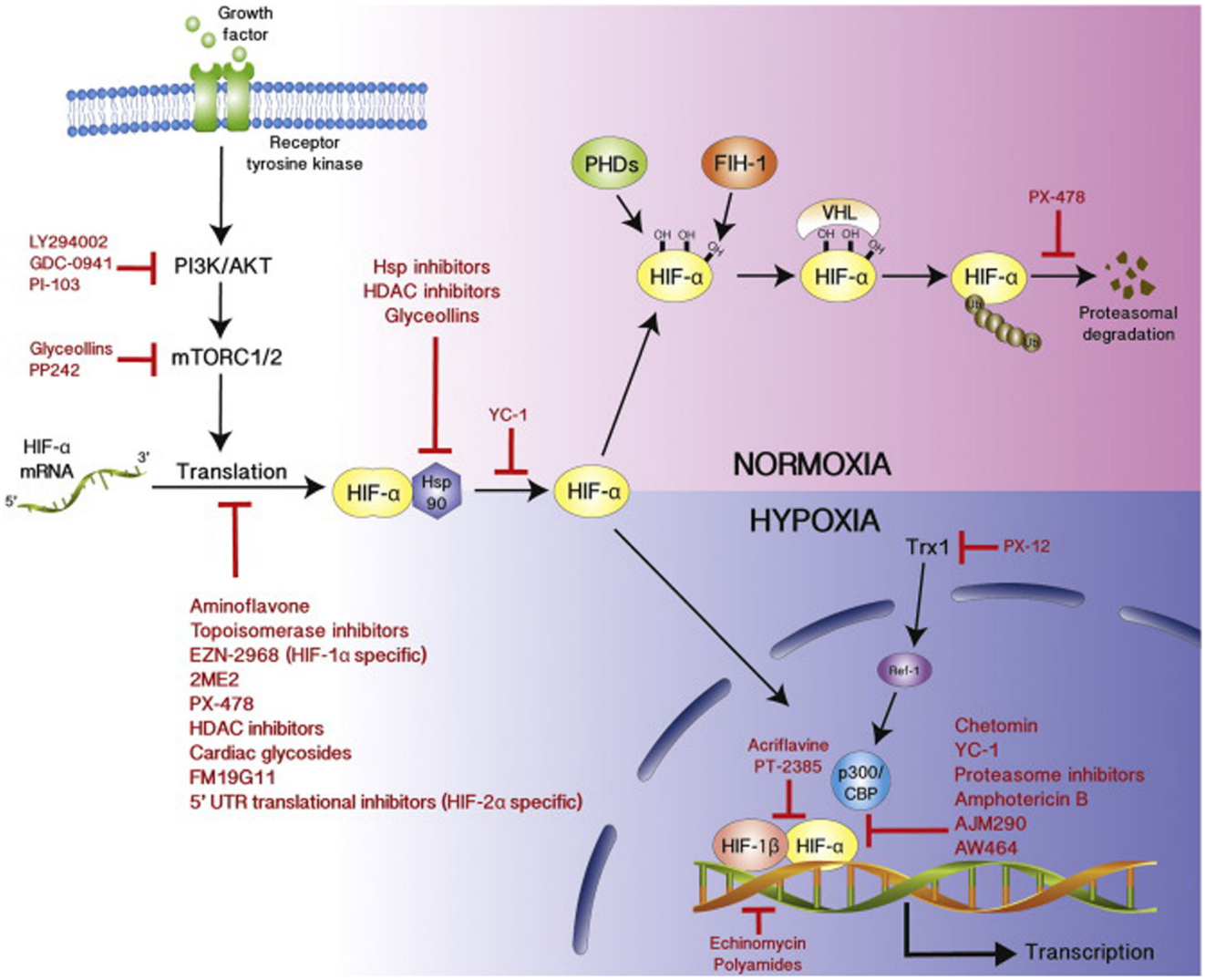

Fig. 1 Mechanism of action of the HIF inhibitors, blocking different steps of HIF signaling pathway. Reprinted from Wigerup C, Pahlman S, Bexell D. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. Pharmacol Ther. 2016;164:152–69, ©2016, with permission from Elsevier. PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; AKT: Protein kinase B; mTOR: Mammalian target of rapamycin; HIF: Hypoxia inducible factor; HSP90: Heat shock protein 90; PHD: Prolyl hydroxylases; FIH-1: Factor inhibiting HIF-1; VHL: Von Hippel-Lindau; P300: E1A binding protein; CBP: CREB-binding protein; Ref-1:human AP-endonuclease; Trx1: Thioredoxin

(VEGF), platelet-derived growth factor (PDGF), and angiopoeitin-1 (ANGPT1) [6]. The HIF1 complex also stimulates transcription of enzymes involved in anaerobic metabolism such as aldolase A and pyruvate kinase, which are essential for cell survival in hypoxic environment [7].

HIF Alpha Isoforms

The HIF alpha isoforms (HIF-1 alpha, HIF-2 alpha, HIF-3 alpha) have different functions and distinct transcriptional activities, although there are some overlap, e.g., both HIF-1 alpha and HIF-2 alpha regulate expression of VEGF-A, glucose transporter 1, and erythropoietin gene [8].

HIF-1 alpha expression is upregulated in acute onset hypoxia whereas HIF-2 alpha and HIF-3 alpha are overexpressed in chronic hypoxia. When acute hypoxia occurs (e.g., due to rapid growth of tumor), the HIF-1 alpha transcriptional program activates adjusting mechanisms such as angiogenesis, apoptosis, or cell cycle arrest. Once hypoxia becomes chronic, there will be a switch in expression of the HIF alpha isoforms and HIF-2 alpha becomes the main driver. Transcriptional activity of HIF-2 alpha enhances tumor adaption, growth, invasion, and metastasis [8].

The HIF-1 alpha and the HIF-2 alpha subunits have contrasting activities in VHL-associated renal cell carcinoma. An increase in expression of one isoform suppresses the expression of the other isoform in these cells. Moreover, HIF-1 alpha has pro-apoptotic features in *VHL*-associated renal cell carcinoma,

whereas HIF-2 alpha enhances transcription of genes promoting tumorigenesis such as VEGF and cyclin D1 [9].

Immunosuppressive Effect of HIF

VEGF has immunomodulatory activities and increases in VEGF level via activation of the HIF pathway enhance the immunosuppressive microenvironment. Hypoxia and HIF production affect the function of immunosuppressive myeloid-derived suppressor cells (MDSC) in tumor microenvironment [10]. A recent study in hepatocellular carcinoma cells revealed that HIF overexpression due to tumor-induced hypoxia leads to increase in an ectoenzyme in cancer cells which enhances the accumulation of MDSC in tumor microenvironment and facilitates escape of cancer cells from recognition by immune system [11]. High levels of the HIF-1 alpha were also associated with higher immunosuppressive regulatory T cell (Treg) count in peripheral blood [12, 13], which has been shown to be associated with higher grade and stage in patients with non-small-cell lung cancer [13].

HIF and Tumor Survival

Transcriptional activities of the HIF-1 alpha and the HIF-2 has been shown to increase tumor survival in solid tumors through different mechanisms [14, 15]. Induction of the HIF pathway is associated with tumor aggressiveness, invasion, and metastasis through different mechanisms such as genetic alterations in tumor cells [16], promoting epithelial-mesenchymal transition by regulation of E-cadherin and matrix metalloproteinases [17], and hypoxia-induced autophagy [18]. In a study of cultured breast cancer cells under normoxic, hypoxic, and anaerobic conditions, the rate of cell proliferation as well as invasion and migration activities of cancer cells were significantly higher in hypoxic and anaerobic environments and this was associated with upregulation of HIF-1alpha mRNA in these groups [19].

HIF and Resistance to Treatment

Hypoxia and activation of the HIF pathway have a key role in development of resistance to different treatment modalities in cancer cells.

Resistance to Chemotherapy

The main mechanism of chemoresistance by HIF is activation of ATP-binding cassette (ABC) transporter protein which acts as a drug efflux pump and decreases the concentration of chemotherapy agents inside cancer cells in hypoxic tumor microenvironment. Other HIF-related mechanisms of resistance to chemotherapy are a decrease in cell metabolism and inhibition of senescence and apoptosis.

HIF-1 alpha expression was measured in cisplatin-resistant and cisplatin-sensitive bladder cancer cell lines and was found to be significantly higher in cisplatin-resistant group. HIF-1 alpha level was correlated with MDR1 gene expression which encodes multidrug efflux pump P-glycoprotein (P-gp) [20].

In another study using A549 (lung adenocarcinoma cell lines), cell lines were examined in normoxic and hypoxic conditions. Hypoxia-induced upregulation of HIF-1 led to increase in glucose transporter 1 (GLUT1) and carbonic anhydrase IX (CAIX), which are necessary for glycolysis and neutralization of acidosis under hypoxic conditions. The study showed that expression of the HIF1 and CAIX in hypoxia increases chemoresistance in cell lines, which is reversible by inhibition of HIF-1 and CAIX in normoxic conditions [21].

Combination of the HIF1 alpha inhibitor PX-478 and gemcitabine was compared with gemcitabine alone in mice implanted with pancreatic ductal adenocarcinoma cells. Combination therapy had higher anti-tumor activity and tumor shrinkage than gemcitabine alone [22]. In a study of the effect of the HIF-2 inhibition on resistance to cisplatin in lung adenocarcinoma cell lines (A549), small interfering RNA (siRNA) was used to suppress the expression of the HIF-2 gene. The 50% inhibitory concentration (IC50) of cisplatin was significantly lower in the siRNA group, which suggests reversibility of cisplatin resistance in these cells after inhibition of HIF-2 expression [23].

Resistance to Radiation Therapy

Radiation therapy causes damage to cancer cells via production of reactive oxygen species (ROS) which damages the cell DNA. However, ROS induces HIF-1 expression, which increases VEGF levels [24]. VEGF protects endothelial cells from radiation and increases the blood and oxygen supply to tumor cells via angiogenesis which increases cell survival and resistance to radiation therapy [25].

Using the HIF inhibitors with radiation therapy may increase sensitivity to radiation. Nelfinavir is a protease inhibitor being used for treatment of HIV infection. It decreases the expression of HIF-1 alpha and VEGF via downregulation of PI3K/Akt pathway. Nelfinavir was used in combination with radiation therapy in mice bearing A549 tumors (lung adenocarcinoma cell lines). Tumor regrowth assays showed that there was more tumor regrowth delay in the group treated with combination therapy compared with radiation therapy or nelfinavir alone, which suggests that inhibition of HIF/VEGF expression can act as radiosensitizer [26]. In a recent study of colorectal cancer cells, SN-38 which is an active

irinotecan metabolite and can inhibit HIF-1 alpha and VEGF formation, was used. It was found out that SN-38 can act as a radiosensitizer by inducing cell cycle arrest and keeping cells in S and G2/M phases which are more prone to damage by radiation exposure [27].

Resistance to Targeted Therapy

Treatment with VEGFR inhibitors decreases angiogenesis in the tumor microenvironment which decreases delivery of oxygen and metabolites to the tumor. The resultant hypoxia activates the HIF signal which induces transcription of VEGF. Higher production of VEGF can overcome the inhibitory effect of VEGFR inhibitors and results in resistance to VEGFR inhibitors. In an orthotopic mouse model of glioblastoma, it has been shown that HIF-1alpha recruits bone marrow–derived CD45+ myeloid cells via increase in SDF1alpha and VEGF in tumor cells [28]. The CD45+ bone marrow–derived cells migrate to the tumor site and secrete matrix metallopeptidase-9 (gelatinase B), which increases VEGF production, increases the activity of proangiogenic cytokine IL-8, and inactivates platelet factor 4 which is an angiogenesis inhibitor [29].

Another mechanism of resistance to VEGF TKIs is that these medications initially affect the tumor and decrease the size of tumor by decrease in angiogenesis, hypoxia, and apoptosis. Upregulation of the HIF pathway by hypoxia activates the anaerobic metabolism via the expression of anaerobic metabolism–associated genes (glucose transporter 1 and aldolase-A) [30]. Over time, tumor clones with higher HIF expression and ability to adapt to hypoxia get selected and the new clones will be resistant to VEGF TKIs [31].Studies of renal cell carcinoma and hepatacellular carcinoma cell (HCC) lines showed that treatment with VEGF TKIs sunitinib and sorafenib disrupted the balance of the HIF-1 alpha and HIF-2 alpha and upregulated the expression of HIF-2 [32, 33].

The significant role of HIF upregulation in developing resistance to VEGF TKIs in tumor cells suggests that inhibition of the HIF pathway may control tumor growth after resistance to VEGF TKIs occurs. A study of HCC showed that HCC cell lines transfected with HIF-2 alpha siRNA had lower expression of VEGF and HIF-2 alpha and low EGFR activity. Cells transfected with HIF-2 alpha siRNA and control siRNA were treated with sorafenib and results showed a synergistic effect of sorafenib (VEGF TKI) and HIF-2 alpha inhibition in reducing viability of hypoxic cells and inducing apoptosis [33]. In another study, a tumor xenograft model was derived from a patient with VHL-associated RCC expressing both HIF-1 alpha and HIF-2 alpha, with progression of disease on sunitinib (VEGF TKI) and everolimus (mTOR inhibitor). The mice bearing RCC tumors were divided into three groups, a vehicle control group, a group which received sunitinib, and a group which was given PT2385 (HIF-2 inhibitor). Assessment of tumor growth after 4 weeks showed complete inhibition of tumor growth in the group treated with PT2385 which inhibits the HIF-2 molecule, whereas no antitumor activity was seen in the other two groups [34].

HIF and PD-L1 Expression

In two studies of renal cell carcinoma cell lines, PD-L1 expression was significantly higher in cell lines with *VHL* mutation and this higher expression was associated with high levels of HIF-2 in these cells but not with HIF-1 [35, 36]. The HIF-1alpha produced under hypoxic tumor microenvironment of tumor-bearing mice, binds to a hypoxia-response element in the PD-L1 proximal promoter which leads to upregulation of PD-L1 on myeloid-derived suppressor cells and tumor cells [37]. In a study of patients with hepatocellular carcinoma, it was found that patients who had coexpression of PD-L1 and the HIF-1 alpha had higher risk of disease recurrence, metastatic disease and higher mortality, compared with other patients [38].

Association of HIF with Prognosis

Association of the HIF1 and the HIF2 expression with patient outcome has been evaluated in several studies. Most of the studies showed that higher expression of HIF1 or HIF2 molecules was associated with poor prognosis and HIF expression can be used as a biomarker for response to treatment. In a study of patients with oral squamous cell carcinoma, HIF-2 alpha overexpression was associated with early recurrence within 2 years of diagnosis [39]. In another study of HIF-2 alpha in patients with NSCLC, expression of HIF-2 alpha was associated with higher tumor size, involvement of lymph nodes, higher tumor stage, and histology subtypes (higher HIF-2 alpha level in squamous cell carcinoma than adenocarcinoma) [40]. Overexpression of HIF-1 alpha was also found to be associated with decreased survival, higher recurrence, and metastasis to lymph nodes [38, 41–43]. In a study of patients with medullary thyroid cancer, 5-year overall survival was significantly lower in patients with positive HIF-1 alpha expression (94% in patients with negative HIF-1 alpha expression versus 65% in those who had HIF-1 alpha expression) [44].

The Role of HIF Inhibitors in Treatment of Cancer

Regarding the significant role of the HIF signaling pathway in tumorigenesis, invasion and metastasis of cancers, and developing resistance to different treatment modalities, the HIF

pathway is a potential target for treatment of cancer. Several HIF inhibitors have been developed and investigated in preclinical and clinical studies [45]. There are two major categories of the HIF inhibitors: direct HIF inhibitors which affect the expression or function of the HIF molecules, and indirect HIF inhibitors which regulate other molecules in upstream or downstream pathways which ultimately affect the HIF signal as one of the targets. One of the well-known and widely used indirect HIF inhibitors is the class of mTOR inhibitors, such as everolimus and temsirolimus, which has been widely studied and found to be effective in treatment of cancer such as metastatic renal cell carcinoma (RCC). In this review, the therapeutic outcomes of direct HIF inhibitors in phase II and III clinical trials will be discussed. Direct HIF inhibitors target HIF expression and or function by various mechanisms, which are: inhibition of mRNA expression, the HIF protein synthesis, dimerization of HIF alpha and beta subunits, DNA binding, and transcriptional activities of HIF.

HIF inhibitors have been studies as a single agent or in combination with other agents, mainly for treatment of advanced or refractory cancers. Select completed phase II/II clinical trials of treatment with HIF inhibitors as single agent are summarized in Table 1. Ongoing phase II clinical trials of the HIF inhibitors are summarized in Table 2.

2ME2 NCD

2ME2 NCD (panzem) is an endogenous metabolite of estradiol which inhibits HIF-alpha protein synthesis, and transcriptional activity.

In a phase II study, 60 patients with multiple myeloma who had refractory or plateau phase disease were treated with panzem. The primary endpoint of the study was objective response rate (ORR) and the secondary endpoint was progression-free survival (PFS). Panzem was initially given at dose of 1000 mg per day, which was increased to 800 mg twice daily after accrual of 39 patients. No objective response was seen. Median time to disease progression was 3.8 months (2.3 months for refractory myeloma and 5.6 months for myeloma in plateau phase) and PFS was 24%, 17%, and 11% at 1, 2, and 3 years, respectively. Treatment was well tolerated in patients, however, plasma levels of panzem indicated that drug levels at given doses were inadequate which may account for lack of objective response to treatment [47].

Higher dose of panzem (1000 mg four times per day) was studied in a phase II trial, in 18 patients with relapsed platinum-refractory ovarian cancer who had a median of 5 prior treatments. Primary endpoint was ORR which was zero, however, 7 patients (31.3%) showed stable disease, and treatment was well tolerated [49].

Panzem was studied in a phase II trial in patients with castration resistant metastatic prostate cancer who had disease progression on one prior taxane-based therapy at a dose of 1500 mg four times per day. The study was planned to accrue 50 patients, however, it was terminated after enrollment of 21 patients due to futility and lack of objective response to treatment and only one patient with > 50% reduction in PSA level [46].

In another phase II study, patients with metastatic renal cell carcinoma were stratified into two treatment arms, depending on whether they were still taking sunitinib or had discontinued it. Patients in treatment arm A received panzem at a dose of 1500 mg three times per day. Patients in treatment arm B received similar dose of panzem plus sunitinib at patient's maximum tolerated dose. The study was terminated after enrollment of 17 patients due to treatment toxicity in both arms (35% of enrolled patients had to discontinue treatment due to toxicity) and lack of objective response to treatment [48].

In a phase II clinical trial, panzem was used in combination with bevacizumab (monoclonal antibody against VEGF molecule) for treatment of patients with locally unresectable or metastatic carcinoid neuroendocrine tumor. Thirty one patients were enrolled into the study and 12 (39%) of the patients received concurrent octreotide. Reduction in tumor size was reported in 68% of patients and median PFS was 11.3 months, which was slightly lower than that of similar studies which resulted in median PFS of 14.4 months in patients who were treated with bevacizumab or everolimus plus octreotide [50].

17-AAG

17-AAG (tanespimycin) is derived from the antibiotic geldanamycin. It is a potent inhibitor of heat shock protein-90 which increases degradation of the HIF-alpha.

In a phase II trial, 22 patients with relapsed lymphoma (anaplastic large cell lymphoma, classical Hodgkin lymphoma, and mantle cell lymphoma) and at least one prior line of therapy were enrolled into study. Tanespimycin was given at a dose of 220 mg/m^2 on days 1, 4, 8, and 11 every 21 days. Of 18 patients who were evaluable for response, 7 patients (39%) had reduction in their tumor sizes and 2 patients (11%), both with Hodgkin lymphoma achieved a partial response [52].

In another phase II study, 20 patients with metastatic RCC (clear cell and papillary histologies) were treated with tanespimycin at similar dose as prior study (220 mg/m^2 on days 1, 4, 8, and 11 every 21 days); however, no objective response was achieved [51].

Tanespimycin was studied in a phase II trial of patients with castration-resistant metastatic prostate cancer who had rising PSA and had received at least one prior systemic therapy. Tanespimycin was given intravenously at a dose of 300 mg/m^2 weekly for 3 of 4 weeks. The primary endpoint of the study was decreased in PSA level which was not met in any of the 15 enrolled patients [53].

Table 1 Summary of select phase II/III clinical trials of HIF inhibitors in the treatment of cancer

Treatment agent/mechanism of action	Study phase	Disease	Study size	Major treatment outcome
2ME2 NCD (panzem)/inhibits HIFα protein synthesis, and transcriptional activity	II	mCRPC (≤ 1 prior taxane-based tx)	Terminated after 21 pts. due to futility	ORR: 0 (no objective response) [46]
	II	Multiple myeloma (plateau or refractory)	60	ORR: 0 PFS at 1, 2, and 3 years were 24%, 17%, and 11%, respectively [47]
	II	mRCC Arm A: panzem Arm B: panzem + sunitinib	12 (terminated early due to tx intolerance)	ORR: 0 Arm A: SD 57% (4 out of 7) Arm B: SD 60% (3 out of 5) [48]
	II	Recurrent, platinum-resistant epithelial ovarian cancer	18	ORR: 0 (7/18 SD) [49]
	II	Metastatic carcinoid tumor (panzem+ bevacizumab)	31	Reduction in tumor size: 68% PFS: 11.3 months [50]
17-AAG (tanespimycin)/increases HIFα degradation	II	mRCC	20	ORR: 0 (no objective response) [51]
	II	Relapsed lymphoma	18	Tumor reduction: 39% PR: 11% [52]
	II	mCRPC (≥ 1 prior systemic tx)	15	No activity with regard to PSA response [53]
Vorinostat (SAHA)/increases HIFα degradation	II	Recurrent gliobastoma	66	6mo PFS: 17% Median OS: 5.7 mo [54]
	II	Cutaneous/ocular melanoma	32	PR 6% SD 50% [55]
	II	Cutaneous T-cell lymphoma (CTCL) (≥ 2 prior systemic tx)	74	ORR 30% (in stage IIB or higher) = > FDA approved for CTCL [56•]
	II	Follicular lymphoma	39	ORR 49% (CR 10%) median PFS 20 mo [57]
	III	Mesothelioma (≥ 1 prior systemic tx)	661 (1:1 vorinostat vs placebo)	Median OS: 30.7 vs 27.1 wks (HR 0.98, p 0.86) [58•]
PT2385/inhibits HIF-2 dimerization and DNA binding	I/II	mRCC (≥ 1 prior systemic tx)	51	Disease control:66% (CR 2%, PR 12%, SD 52%) [59•]
EZN-2208 (pegylated SN-38)/inhibits HIF1 mRNA expression	II	Advanced colorectal cancer Arm A: EZN-2208 Arm B: EZN-2208 + cetuximab Arm C: irinotecan + cetuximab	292	OS and PFS was equal in arm B and arm C [60]
CRLX101/inhibits HIF-1α expression	I/II	Refractory mRCC (CRLX101 + bevacizumab)	22	Median PFS 9.9 mo PFS > 4 mo in 55% of patients [61]

mCRPC metastatic castration resistant prostate cancer, *ORR* objective response rate, *PFS* progression-free survival, *OS* overall survival, *mRCC* metastatic renal cell carcinoma, *tx* treatment

Vorinostat (SAHA, Zolinza)

Vorinostat is a synthetic hydroxamic acid derivative which inhibits histone deacetylase (HDAC). It suppresses the HIF pathway by increasing the degradation of the HIF-alpha molecule and has been studied in solid tumors and hematologic malignancies which showed encouraging results.

Vorinostat was recently approved by FDA for treatment of patients with cutaneous T cell lymphoma (CTCL), based on results of a phase II single arm clinical trial. A total of 74 patients with stage IB or higher CTCL and at least two prior lines of therapy were enrolled into study (> 80% of patients had stage IIB and higher). The starting dose of treatment was 400 mg per day, and two dose reductions were allowed. The objective response in the skin disease measured by severity-weighted assessment tool (SWAT) was achieved in 30% of patients with stage IIB and higher. Median duration of response was 168 days and median time to disease progression was 148 (169 days in patients with stage IIB and higher) [56•].

In a phase II study of patients with relapsed or refractory non-Hodgkin lymphoma and mantle cell lymphoma and at least two prior systemic therapies, vorinostat was given at a dose of 200 mg twice daily for 14 consecutive days in a 21-day cycle. Of 50 patients enrolled into study, 39 patients had follicular lymphoma (FL). The primary endpoint of study was defined as ORR in patients with FL and secondary endpoint was PFS. Among patients with FL, 49% achieved an objective response (10% complete response and 39% partial response) and median PFS was 20 months [57].

Table 2 Summary of select ongoing clinical trials of HIF inhibitors in cancer

Treatment agent/mechanism of action	Study phase	Disease	Status	Primary endpoint	Clinical trial identifier
PT2385/inhibits HIF-2 dimerization and DNA binding	Phase II	Recurrent glioblastoma	Active, not recruiting	Tumor radiographic response	NCT03216499
	Phase II	VHL-associated RCC (non-metastatic)	Active, not recruiting	ORR	NCT03108066
	Phase I	Advanced ccRCC (Part 1: PT2385 only) (Part 2: PT2385 + nivoumab) (Part 3: PT2385 + cabozantinib)	Active, not recruiting	Maximum tolerated dose	NCT02293980
PT2977/inhibits HIF-2 dimerization and DNA binding	Phase II	VHL-associated RCC (non-metastatic)	Recruiting	ORR	NCT03401788
	Phase II	Advanced ccRCC (PT2977 + cabozantinib)	Not yet recruiting	ORR	NCT03634540
Digoxin/inhibits HIF-1 synthesis	Phase II	Kaposi's sarcoma	Recruiting	Tumor response (at 3 months)	NCT02212639
CRLX101/inhibits HIF-1α expression	Phase II	Recurrent platinum-resistant ovarian, tubal and primary peritoneal cancer (CRLX101 + bevacizumab)	Completed	PFS (at 6 months)	NCT01652079

ORR objective response rate, *NCT* National Clinical Trial (clinicaltrials.gov), *VHL* Von Hippel-Lindau, *RCC* renal cell carcinoma, *ccRCC* clear cell renal carcinoma, *PFS* progression-free survival

Based on encouraging results of a phase I trial of vorinostat in patients with solid tumors including those with advanced malignant pleural mesothelioma [62], a decision was made to study its efficacy in a double-blinded randomized phase III trial (VANTAGE 0-14). In this phase III study, 661 patients with advanced malignant pleural mesothelioma who had one or two prior treatments were randomized (by 1:1 ratio) to receive vorinostat or placebo. Vorinostat at dose of 300 mg (or matching placebo) was given twice daily on days 1, 2, 3, 8, 9, 10, 15, 16, and 17 of each 21-day cycle. The primary endpoint of study was overall survival. There was no statistically significant difference in median overall survival of two treatment arms, which was 30·7 weeks for vorinostat and 27·1 week for placebo (HR 0·98) [58•]. Most common side effects reported in vorinostat arm include grade 1–2 nausea, vomiting, and diarrhea. Fatigue and malaise were the most common grade 3–4 toxicity, reported in 14% of patients in treatment arm and 7% of patients in placebo arm [58•].

PT2385

PT2385 is a first-in-class HIF-2 alpha antagonist. It is a small molecule which inhibits dimerization of the HIF-2 alpha and its binding to DNA. Safety and efficacy of PT2385 was assessed in a phase I/II clinical trial in a total of 51 patients with metastatic RCC who had at least one prior systemic treatment. The recommended phase 2 dose of PT2385 was 800 mg twice a day and 66% of patients had clinical benefit with treatment; 1 patient achieved complete response (2%), 6 patients had partial response (12%), and 26 patients (52%) had stable disease. PT2385 was well tolerated and no patient had to discontinue treatment due to treatment-related toxicity. Most common adverse events reported in this study were anemia, peripheral edema, and fatigue. Erythropoietin, which is a target of HIF-2 inhibitors, was used as a biomarker of pharmacodynamic response, with higher doses of PT2385 associated with lower erythropoietin levels. In this study, there was remarkable pharmacokinetic variability in patients treated with P2385, which may lead to variation of drug level, altered treatment efficacy, and toxicity profile between patients [59•].

There is an ongoing phase II clinical trial (NCT03108066) investigating the efficacy of PT2385 in patients with VHL-associated RCC who have no evidence of metastatic disease. PT2385 crosses the blood-brain barrier and its efficacy in treatment of gliobastoma is being assessed in a phase II clinical trial (NCT03216499) in patients with recurrent glioblastoma, with primary endpoint of radiographic tumor response.

The safety of the combination of PT2385 with nivolumab (PD-1 inhibitor) and cabozantinib (VEGF TKI) is being investigated in a phase I dose escalation clinical trial (NCT02293980) in patients with metastatic RCC. This study has three parts. In part 1, patients were assigned to sequential dose cohorts of PT2385. In part 2 and part 3, patients were assigned to dose cohorts of the combinations of PT2385 and nivolumab or PT2385 and cabozantinib, respectively. The primary endpoint of study was maximum tolerated dose. This clinical trial enrolled 107 patients and results are pending.

PT2977

PT2977 is a second-generation HIF-2 alpha inhibitor with a mechanism of action similar to PT2385. It is more potent and has a superior pharmacokinetic profile compared to PT2385.

PT2977 is currently being investigated in the frontline setting in a phase II clinical trial (NCT03401788) for treatment of patients with VHL-associated RCC who have no metastatic disease.

Combination of PT2977 (a HIF-2 inhibitor) plus cabozantinib (a VEGF tyrosine kinase inhibitor) is going to be investigated in patients with advanced RCC with clear cell histology. This clinical trial (NCT03634540) was registered recently and is anticipated to enroll patients in the near future.

EZN-2208 (Pegylated SN-38)

EZN-2208 is a pegylated prodrug of antineoplastic drug SN-38 which is an active and potent metabolite of irinotecan. EZN-2208 inhibits the HIF pathway by suppression of the HIF1 mRNA expression. Efficacy of EZN-2208 in patients with advanced colorectal cancer was investigated in a phase 2 clinical trial. This study had three treatment arms: arm A (EZN-2208), arm B (EZN-2208 plus cetuximab), and arm C (irinotecan plus cetuximab). Patients who had KRAS mutation were all assigned to treatment arm A. Patients with wild-type KRAS were randomized (2:1) to treatment arm B or C. No objective response was seen in treatment arm A and PFS was 1.8 months. There were comparable ORR seen in 8 out of 75 patients (10.7%) in arm B and in 5 out of 35 (14.3%) in arm C. There was no statistically significant difference in PFS and OS between treatment arms B and C. Median PFS was 4.9 and 3.7 months in treatment arms B and C, respectively and median OS was 9.8 and 9.1 months in treatment arms B and C, respectively [60].

CRLX101

CRLX101 is another HIF inhibitor which has been used in combination with bevacizumab for treatment of patients with refractory metastatic RCC who had one prior conventional therapy. Twenty two patients were enrolled into study. Median PFS was 9.9 months and 55% of patients achieved PFS of more than 4 months [61].

Recently, a phase II clinical trial of combination of CRLX101 plus bevacizumab in patients with recurrent platinum-resistant ovarian, tubal, and primary peritoneal cancer was completed (NCT01652079). Sixty three patients were enrolled into the clinical trial and study results are pending.

Conclusions

The HIF pathway has a significant biologic role in the pathogenesis of many malignancies, most notably renal cell carcinoma. The pathway has pleiotropic effects on tumor growth and invasion as well as resistance to therapeutic agents. HIF inhibitors, therefore, may have a role to lead to cytotoxic or cytostatic effects on malignant cells, in addition to a role to reverse, reduce, or prevent resistance to treatment. The precise application of HIF inhibitors, however, awaits further biologic insights into which specific tumors have a greater reliance on this pathway, biomarkers to identify such tumors and/or signal effective HIF inhibition, and clinical study across malignancies as monotherapy and in combination. HIF pathway inhibitors are emerging as a viable therapeutic modality in cancer.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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