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Current Landscape and the Potential Role of HIF and Selenium in Clear Cell Renal Cell Carcinoma Treatment

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Abstract

In the last two decades, the discovery of various pathways involved in renal cell carcinoma (RCC) have led to the development of biologically-driven targeted therapies. Hypoxia inducible factors (HIFs), angiogenic growth factors, von Hippel-Lindau (VHL) gene mutations and oncogenic miRNAs play essential roles in the pathogenesis and drug resistance of clear cell renal cell carcinoma. These insights have led to the development of VEGF inhibitors, mTOR inhibitors and immunotherapeutic agents which have significantly improved outcomes of patients with advanced RCC. HIF inhibitors will be a valuable asset in the growing therapeutic armamentarium of RCC. Various histone deacetylase (HDAC)inhibitors, including selenium and agents such as PT2385 and PT2977, are being explored in various clinical trials as potential HIF inhibitors to ameliorate the outcomes of RCC patients. In this article, we will review the current treatment options and highlight the potential role of selenium in the modulation of drug resistance biomarkers expressed in ccRCC tumors.

Keywords: clear cell renal cell carcinoma; hypoxia inducible factors (HIFs); selenium; PD-L1; miRNA; VEGF; mTOR inhibitors

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common malignancy in the kidney. Over 65,000 new kidney cancer cases and 14,000 deaths were estimated in the United States in 2018 (1). RCC is the most lethal genitourinary cancer given its disease course is largely asymptomatic and incidentally found in more than half of new cases (2, 3). Established modifiable risk factors for RCC include obesity, smoking, and hypertension (4). Other studies link alcohol use, type 2 diabetes, and occupational or environmental exposures to increased risk of RCC (5).

RCC is categorized into three major histological subtypes: ccRCC, comprising 70% of cases, papillary and chromophobe RCC, which together comprise 25% of cases, and tumors of the medullary and collecting systems, which comprise 5% of cases (6, 7). These subtypes arise from distinct genetics and therefore are treated differently (8).

Localized RCC is often managed surgically with partial or radical nephrectomy, with tumor ablation or active surveillance for small tumors. Systemic therapy is primarily reserved for metastatic RCC. Current evidence for adjuvant systemic therapy after complete resection of the tumor has shown no survival benefit (9). For stage IV disease, cytoreductive nephrectomy in addition to systemic therapy has not shown improvement in overall survival (9, 10). In the last two decades, there has been significant improvement in our knowledge of renal cell carcinogenesis that has, in turn, led to the development of biologically-driven targeted therapies.

Role of HIF in Renal Cell Carcinogenesis

Adaptation to a hypoxic environment is a key attribute of cancer cells. This is mediated via transcription factors called hypoxia inducible factors (HIF). These factors are heterodimers

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with an α-subunit (HIF1α, HIF2α or HIF3α) and a β-subunit (HIF1β) (11). Previously, HIF1α was considered to be a predominant oncogenic driver but recent evidence shows HIF2 α as a key player in renal cancer progression (12). In addition to hypoxia, there are additional oncogenic signaling pathways (e.g., PI3K, RAS) that are known to regulate HIF activation. Once activated, HIF transcription factors translocate in the nucleus and bind to the hypoxia response elements which leads to transcription of several target genes involved in angiogenesis (VEGF), oxygen transport and metabolism (erythropoietin), glycolysis (LDH), glucose transport (GLUT1), cell proliferation and migration, which eventually leads to carcinogenesis (**Figure 1**) (13, 14). VEGF plays a vital role in the tumor angiogenesis and is a key target of anti-cancer therapeutic agents. Regulation of HIF-1 pathway is vital for cellular homeostasis.

Regulation of HIF Pathway by VHL Gene

Von Hippel-Lindau (VHL) is a tumor suppressor gene located on the short arm of chromosome 3 that is commonly mutated in both hereditary and sporadic renal cell carcinoma. The VHL gene encodes two isoforms of VHL proteins (pVHL) that play a crucial role in cellular oxygen sensing. In normoxic conditions, the pVHL ubiquitin ligase complex binds to the hydroxylated HIF1α and HIF2α, which subsequently undergoes ubiquitination and proteasomal degradation. However, in cellular hypoxic conditions, pVHL cannot bind to HIF1α and HIF2α, as these transcriptional factors are not hydroxylated by prolyl hydroxylases, which is an oxygen-dependent process, leading to their accumulation and activation of downstream pathways (15, 16). A wide range of intragenic mutations, deletions (complete or partial) and splicing defects have been identified that derange normal function of the VHL gene (17).

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In addition to the VHL gene, multitudinous genetic and enzymatic derangements have been identified which predispose to various histologies of renal cell carcinoma. These include FLCN (folliculin; chromophobe RCC/oncocytoma in Birt-Hogg-Dubé syndrome), MET (papillary type 1 RCC), fumarate hydratase (FH; papillary type 2 RCC in hereditary leiomyomatosis and renal cell cancer syndrome), SDHB/SDHD/SDHC/SDHA (succinate dehydrogenase subunit-related RCC), chromosome 3 translocations associated clear cell RCC, PTEN (papillary RCC) and BAP1 (clear cell RCC) (18).

This interdependency on biological pathways by cancer cells has laid the foundation for development of several targeted therapeutic agents for the treatment of advanced renal cell carcinoma.

Angiogenesis (VEGF Pathway) Inhibitors

Current first-line therapy for stage IV, unresectable, or relapsed disease of clear cell histology includes the oral VEGF tyrosine kinase inhibitors (TKIs) sunitinib and pazopanib (9). Additionally, for intermediate to poor risk groups based on the international metastatic renal cell carcinoma database consortium (IMDC) criteria (19), either the combination of ipilimumab and nivolumab or cabozantinib are options.

Sunitinib is a multi-kinase inhibitor targeting several tyrosine kinase receptors, including platelet-derived growth factor receptors (PDGFR-α and β), VEGF receptors (VEGFR-1, -2, and -3), stem cell factor receptor (c-KIT), FMS-like tyrosine kinase (FLT-3), colony-stimulating factor (SSF-1R), and neurotropic factor receptor (RET) (9). In the landmark phase 3, multicenter clinical trial by Motzer et al., sunitinib was compared with interferon-α in patients with previously untreated metastatic renal-cell carcinoma (20). Progression-free survival (PFS) in the

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sunitinib arm was 11 months and in the interferon-α arm the PFS was 5 months. The overall survival (OS) with sunitinib was 26 months.

Pazopanib is another oral angiogenesis inhibitor targeting VEGFR-1, -2 and -3, PDGFR-α and -β, and c-KIT. In a phase III, open-label study of pazopanib in patients with no prior treatment or one prior cytokine-based treatment, PFS was prolonged significantly with pazopanib versus placebo. For the treatment naïve group, PFS was 11.1 months compared to 2.8 months for pazopanib and placebo, respectively (21). In a phase 3 non-inferiority trial, pazopanib was compared to sunitinib in patients with advanced renal cell carcinoma. The study was positive for non-inferiority with a progression-free survival of 8.4 and 9.5 months for pazopanib and sunitinib, respectively (22). In addition, the median OS with pazopanib was 28.3 months and 29.1 months for sunitinib. In subgroup analysis for patients with favorable-risk disease, the median OS for pazopanib and sunitinib was found to be 52.5 and 43.6 months, respectively (23). Both of these medications had similar rates of adverse events that led to dose reduction and had no differences in grades 3/4 adverse events. Symptoms associated with discomfort such as fatigue, hand-foot syndrome and mouth sores occurred more frequently with sunitinib while pazopanib was associated with elevations in liver-function tests, weight loss, and changes in hair color. The study also showed lower monthly use of medical resources with pazopanib than with sunitinib (22).

Cabozantinib is a small molecule inhibitor of tyrosine kinases, which include VEGF receptors, MET and AXL (9). Cabozantinib was compared to sunitinib in a phase 2 study of intermediate to poor IMDC risk, treatment naïve patients with metastatic RCC (24). In this study, PFS was 8.6 months versus 5.3 months for cabozantinib and sunitinib, respectively, and median OS was found to be 34.5 months and 26.6 months, respectively. Based on these results,

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cabozantinib has been approved by the FDA as a first-line agent. Cabozantinib has also been studied in a phase III trial (METEOR trial) of patients with disease progression after previous TKI therapy (25). The study compared second-line therapy with cabozantinib versus everolimus. The results showed median PFS of 7.4 months as compared to 3.8 months for cabozantinib and everolimus, respectively. Thus, in addition to first-line therapy, cabozantinib is a viable option as a second-line therapy for patients with disease progression on other TKI therapy.

Axitinib is a selective, second-generation tyrosine kinase inhibitor targeting VEGFR-1, -2, and -3 (9). The phase III AXIS trial compared axitinib and sorafenib as second-line therapy following other systemic therapy. PFS was 6.7 for axitinib versus 4.7 months for sorafenib. PFS was favored in both subgroups of patients treated with axitinib whose prior systemic therapy was sunitinib or cytokine therapy. Median OS was 20.1 months with axitinib as compared to 19.2 months with sorafenib, although, this was not statistically significant (26).

Bevacizumab along with interferon (IFN) alfa-2b also has category 1 level of evidence for first-line therapy. It is a recombinant humanized monoclonal antibody that binds to circulating VEGF-A. A double-blind phase III trial (AVOREN) compared bevacizumab plus IFN-alfa-2b versus placebo plus IFN-alfa-2b (27). With the addition of bevacizumab, PFS was significantly increased (10.4 vs. 5.4 months) with a tumor response rate of 30.6% in the bevacizumab group compared to 12.4% in the placebo group. This was achieved without significant increase in adverse events. OS was improved in the bevacizumab group versus the placebo group (23.3 months vs. 21.3 months); however, this was not statistically significant.

Mechanistic Target of Rapamycin (mTOR) Inhibitors

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mTOR proteins are known to regulate cellular metabolism, growth, apoptosis, and angiogenesis through protein expression. Cellular growth factors stimulate the PI3-K/Akt/mTOR pathway and eventually lead to HIF accumulation (28). These discoveries led to evaluation of temsirolimus and everolimus which are both mTOR inhibitors in the management of renal cell carcinoma. Temsirolimus was compared to interferon-alfa in previously untreated patients with poor risk prognostic risk factors per the MSKCC prognostic model (29). The group receiving temsirolimus alone demonstrated significant improvement in median OS compared to IFN-α alone (10.9 vs. 7.3 months, respectively). Similarly, PFS was shown to have improved from 3.1 months with IFN-α to 5.5 months with temsirolimus. Based on these study results, temsirolimus was the first FDA-approved mTOR inhibitor for patients with advanced renal cell carcinoma (30). Currently, temsirolimus is the only mTOR inhibitor that is FDA-approved as a monotherapy.

Everolimus in combination with lenvatinib, a TKI, is utilized in patients who progress on prior therapy. In a phase II clinical trial, lenvatinib plus everolimus was compared to single-agent everolimus in previously-treated mRCC patients (31). The combination therapy showed increased median OS of 25.5 months as compared to 15.4 months for the monotherapy. Similarly, median PFS improved to 14.6 months in the combination group as compared to 5.5 months for the everolimus-only group (31).

Immunotherapy

Until late 2005, medical treatment options for RCC involved cytokine-based immunotherapy with the use of high-dose interleukin-2 (IL-2) and IFN-α. Though high-dose IL-2 is associated with significant toxicity, long-term durable response rates were seen in a small

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fraction of patients. High dose IL-2 therapy is utilized in highly selected patients with excellent performance status and normal organ function (32). IFN-α as a monotherapy has fallen out of favor as a phase III multinational trial between sunitinib and IFN-α demonstrated a strong trend toward a median overall survival advantage of sunitinib over IFN-α (33).

Checkmate-214, a randomized phase III clinical trial, evaluated the combination of two immune checkpoint inhibitors, nivolumab and ipilimumab, in comparison to sunitinib in treatment-naïve patients with metastatic clear cell RCC. In patients with IMDC intermediate and poor risk, PFS was found to be 11.6 months and 8.4 months for nivolumab/ipilimumab combination and sunitinib, respectively. However, discontinuation due to adverse events was 24% in the combination group as compared to 12% in the sunitinib group. The median OS with sunitinib was 26 months whereas the median OS was not reached with the combination therapy (34). Nivolumab was also shown to be effective as second-line therapy. In a phase III trial that studied patients previously treated with at least one line of therapy excluding mTOR inhibitors, nivolumab demonstrated an increase in OS of 5.4 months in comparison to everolimus monotherapy (25 vs. 19.6 months, respectively). Median PFS, however, was not statistically significant, with 4.6 months for nivolumab and 4.4 months for everolimus (35).

Strategies to Inhibit HIF Pathway: A Plausible Therapeutic Avenue

VEGF inhibitors target one of the myriad oncogenic pathways that are activated by HIF. Hence, the cancer eventually develops resistance and progresses despite initial good response to various oral TKIs. Inhibiting the HIF pathway and subsequently its translational activity is an attractive treatment modality as this blocks the activation of all downstream genes. mTOR inhibitors inhibit HIF activation, but the responses are limited as noted above. Recently, further

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strategies were explored targeting the HIF pathway in combination with VEGF inhibitors with variable success.

HIF regulation, either by blocking its production, antagonizing it effects or by enhancing its degradation, has provided multiple opportunities to expand the current therapeutic armamentarium of renal cell carcinoma. In a small study of mRCC, HIF expression was predictive of increased response to sunitinib treatment (36). In this study, 26 of 49 patients had high HIF1α and HIF2α expression on the tumor cells (based on immunoblot analysis). These patients had a higher rate of complete response or partial response when compared to patients with low or absent HIF1α/HIF2α expression.

Oncogenic miRNA-155 and miRNA-210

Non-coding miRNAs are small molecules involved in post-transcriptional regulation of genes that are often associated with increased angiogenesis and drug resistance. MiRNA-155 and -210 are the most widely investigated miRNAs that have been demonstrated to target the VHL tumor suppressor gene and may offer an alternative mechanism for inactivation of VHL along with stable expression of HIFs [34-38]. Because these miRNAs and HIFs are co-expressed in normoxic cells, induced by hypoxia and are regulator of VEGF and immune therapeutic targets, they offer the potential for the development of novel drug combinations for the treatment of ccRCC patients and others with similar selenium target expression.

Base-line Transcription and Translation in ccRCC Cell Lines

We have determined base-line expression of three genes and two miRNAs in four clear cell renal cell carcinoma cell lines: 786-O, RC2, RCC4 and RCC4 cells transfected with wild-type von

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Hippel-Lindau (VHL). The three genes were HIF-1α, HIF-2α and PD-L1, and the two miRNAs were hsa-miR-155 and hsa-miR-210. The results in **Figure 2A** indicate that of the four cell lines, 786-O cells with the lowest normalized transcription levels (ΔCt transcription of displayed the highest miRNA transcription. Results shown in **Figure 2** indicate that there is robust mRNA. However, translation of these messages into protein, as shown in **Figure 2C**, reveals a very different pattern. For example, in the 786-O cell line both HIF-1α and HIF-2α are equally expressed at the RNA level but reveals very different protein levels. Similarly, the RC2 cell line has consistently lower levels of RNA transcripts than 786-O but higher levels of protein for HIF-2α and PD-L1. These inconsistencies suggest that there may be post-transcriptional targeting of these mRNAs by the miRNAs or another, as yet unknown, post-transcriptional process at work. In either case, these results will require further mechanistic studies before they can be explained.

There is evidence to suggest complex interactions among these loci. For example, Yee et al. (2017) show that miR-155 is induced by IFNγ and, in turn suppresses PD-L1. They further show that PD-L1 does contain two functional miR-155 binding sites. While this alone is not sufficient to suggest a mechanism, it does support the idea that such interactions do exist in ccRCC.

Role of Selenium in HIF Pathway

Selenium is an essential micronutrient; in the human body it is involved with the regulation of cell metabolism, DNA and RNA as well as protein synthesis, and is at the active site of several enzymes of the antioxidant network. Inorganic forms of selenium, such as selenide and selenite, are converted by plants into organic forms, such as selenomethione (SLM) and Se-methylselenocysteine (MSC), that are retained in the human body. Studies have shown that

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serum levels of selenium vary from person to person. Dietary intake is influenced by the soil levels of selenium of plants we ingest. Epidemiologic studies have suggested that dietary selenium intake is a protective factor in developing some forms of cancer such as colorectal, prostate, lung, and bladder cancer (37-40).

SLM and MSC are forms of selenium that are currently being investigated as possible anti-tumor agents. In their natural form, these agents have a relatively low toxicity profile. They are converted via β-lyase to the active form methylselenol (MSA). HIF1α appears to be a target of selenium. In pre-clinical studies of head and neck squamous cell carcinoma cells that express HIF1α, it was found that in the setting of hypoxia where HIF1α expression is increased, the cytotoxicity of SN38, the active metabolite of irinotecan, was enhanced with the addition of MSA (41). This is possibly due to the inhibition of HIF1α by MSA and demonstrates the potential for reversal of chemoresistance by MSA. Moreover, selenium has been found to target β-catenin, and increases drug cytotoxicity through the reduction of β-catenin's drug resistant effects (42). Selenium compounds may also improve efficaciousness of other anti-tumor agents through reduction in treatment-induced toxicities, allowing for higher tolerated doses. In one study of A253 and HT 29 xenografts, coadministration of MSC with irinotecan at two to three times the maximum tolerated dose of irinotecan led to response without intolerable toxicity (43).

Selenium can also affect the tumor microenvironment (TME) and may be able to stabilize the TME to improve drug delivery. MSC has an anti-vascular effect and can increase the antitumor effect of irinotecan through the inhibition of HIF1α which leads to decreased microvessel density, lowered tumor interstitial pressure, and increased pericyte coverage of blood vessels (41).

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Selenium may also be able to act through its effects in the expression of miRNAs. miRNAs are small, noncoding RNAs that function as post-transcriptional modifiers of gene expression and can take the form of oncogenic miRNA that are overexpressed in malignant cells or tumor suppressor miRNAs that are downregulated in tumor cells. In a study of ccRCC xenografts, MSC demonstrated decreased levels of oncogenic miRNAs, such as miRNA-155, -106b, and -210 and upregulated levels of tumor suppressor miRNAs, including miRNA-Let7b, -85, and -328. These specific miRNAs have been found in primary ccRCC tumor biopsies (41).

Selenium has been shown to be associated with a protective effect against drug-induced DNA damage in normal bone marrow cells while at the same time not protecting tumor cells in head and neck cancer cell lines A253 and FaDu through the selective upregulation of the DNA repair gene XPC (44). Additionally, selenium can selectively downregulate Nrf2/Prx1, transcription factors that are involved in protecting normal tissue while at the same time promoting tumor growth, in tumor cells versus normal tissue, and can improve antitumor effects of chemotherapy and radiation in lung cancer A549 and colorectal HT29 while protecting normal tissue (45).

SLM has been FDA-approved for clinical trials. A phase Ib dose-escalation trial in patients with metastatic ccRCC after failure of prior treatment is ongoing (NCT02535533). Preliminary results of 9 evaluable patients demonstrated 2 patients achieving complete response and 3 patients achieving partial response. No dose-limiting toxicities have been noted. The most common side effects included anorexia, fatigue, cough, diarrhea, and proteinuria. There were no grade 4 toxicities or deaths associated with this combination therapy. The phase 2 part of the clinical trial with SLM and axitinib 5 mg BID is planned(46). The multiple avenues in which

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selenium interacts with other chemotherapies, the tumor microenvironment, its interaction with miRNA and transcription factors make it a very favorable target for further research.

Studies on HIF Inhibitors in Advanced Renal Cell Carcinoma

PT2385 is a HIF-2α antagonist that was evaluated in a phase 1, standard 3+3 dose escalation study of heavily pretreated metastatic ccRCC (47). In this study, 51 patients were treated with oral PT2385 twice a day and the recommended phase-2 dose (RP2D) was 800 mg BID. One patient had complete response (2%) and 6 had partial response (12%) and the rest had either stable disease or progression. No dose-limiting toxicities were noted. The most common treatment-related side effects included anemia, peripheral edema, fatigue and nausea. Considering the promising response signals of a single agent in a heavily pretreated patient population, further studies are ongoing with combination PT2385 with nivolumab and cabozantinib, respectively (NCT02293980).

In a multi-institutional, phase I/II clinical trial, vorinostat (histone deacetylase inhibitor) was evaluated in combination with bevacizumab in ccRCC patients (48). HDAC inhibitors modulate the HIF pathway by affecting Hsp90 acetylation and HIF-α nuclear translocation (49, 50). In this study, 33 evaluable patients were treated with vorinostat 200 mg orally twice daily for 2 weeks in combination with bevacizumab 15 mg/kg administered intravenously every 3 weeks. There were no dose-limiting toxicities. Two patients had grade 4 thrombocytopenia. The most common adverse events included fatigue, nausea, pain, anorexia, diarrhea and elevated creatinine. About 10 patients discontinued therapy due to toxicities, but there were no treatment-related deaths. One patient achieved complete response and 5 patients had partial response.

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Currently a phase I/Ib study of pembrolizumab with vorinostat is in progress for patients with advanced renal or urothelial cell carcinoma (NCT02619253).

The safety and efficacy of another HDAC inhibitor, abexinostat, as an epigenetic downregulator of HIF-1α and VEGF expression, was evaluated in combination with pazopanib by Aggarwal and colleagues in a study of advanced solid tumor malignancies (51). The RCC cohort included 22 patients. The dosing schedule of adexinostat was modified due to 5 dose-limiting toxicities that included grade 3 thrombocytopenia ($n = 2$), grade 3 fatigue ($n = 2$) and grade 3 AST/ALT elevation ($n = 1$). There were no treatment-related deaths. The objective response rate in the RCC cohort was 27% including patients who were previously refractory to pazopanib.

Bortezomib is a proteasome inhibitor currently approved for the treatment of multiple myeloma and mantle cell lymphoma. It is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome in mammalian cells. By inhibiting proteasomes, it causes protein buildup and then leads to cell cytotoxicity. In preclinical models, Shin and colleagues have shown its role in HIF-1α repression by inhibiting the recruitment of p300 coactivator (52). In a phase II clinical trial of treatment naïve metastatic ccRCC, 17 patients were treated with sorafenib 200 mg orally twice daily in combination with bortezomib 1mg/m^2 intravenously administered on days 1, 4, 8, and 11, then every 21 days (53). The combination was safe but the study was negative as it did not meet the prespecified endpoint of median progression-free survival of 70 weeks. Further studies are not planned with this combination.

The clinical efficacy of bortezomib in combination with bevacizumab was evaluated in 91 patients with treatment-refractory advanced cancers (54). In the RCC cohort, 5 of 20 patients had partial response or stable disease. No treatment-related deaths were noted. Common

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toxicities included thrombocytopenia, fatigue, nausea/vomiting, diarrhea, neuropathy, anemia, neutropenia, and hypertension. **Table 1** summarizes the concluded clinical trials.

Conclusions

Insights into the HIF pathway have led to exploration of various therapeutic agents inhibiting its downstream activity as a potential cancer therapy. Numerous studies are underway evaluating several agents targeting the HIF pathways in combination with immunotherapy or VEGF inhibitors (See **Table 2**.).

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Figure Legends

Figure 1. Inhibitors of the HIF pathway currently being evaluated in clinical trials.
VHL: von Hippel-Lindau; HIF: hypoxia inducible factors; HDAC: Histone deacetylase; VEGF: vascular endothelial growth factor; LDH: lactate dehydrogenase; GLUT1: glucose transporter 1

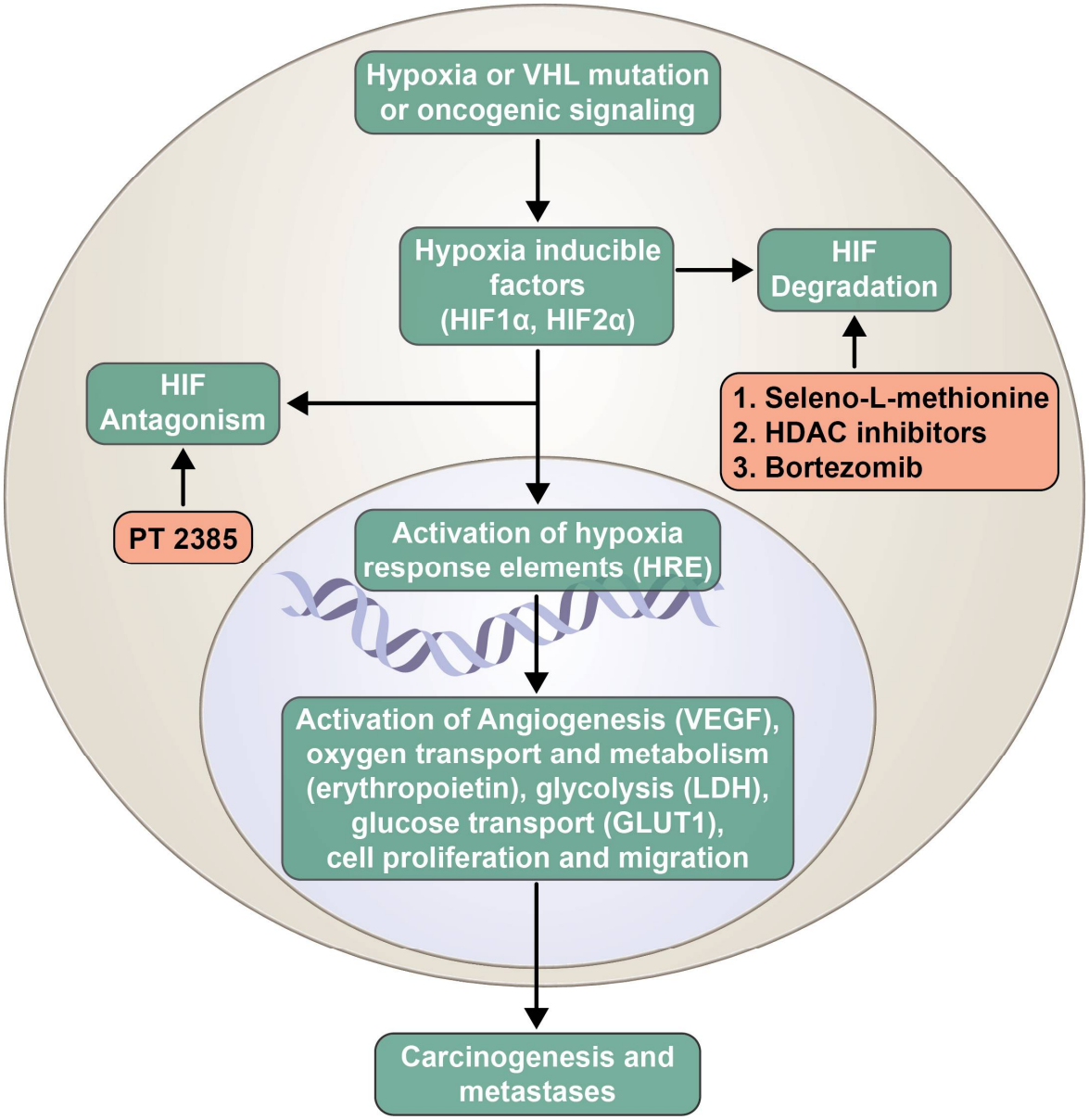

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Figure 2. Base-line levels of constitutively expressed miRNAs (A), mRNAs (B), HIFs and PD-L1 protein(C) in normoxic clear cell renal cell carcinoma cell lines. These cell lines show minimal differences in mRNA levels of PD-L1, HIF-1 and HIF-2 (B), but express differential levels of PD-L1, HIF-1 and HIF-2 protein (C).

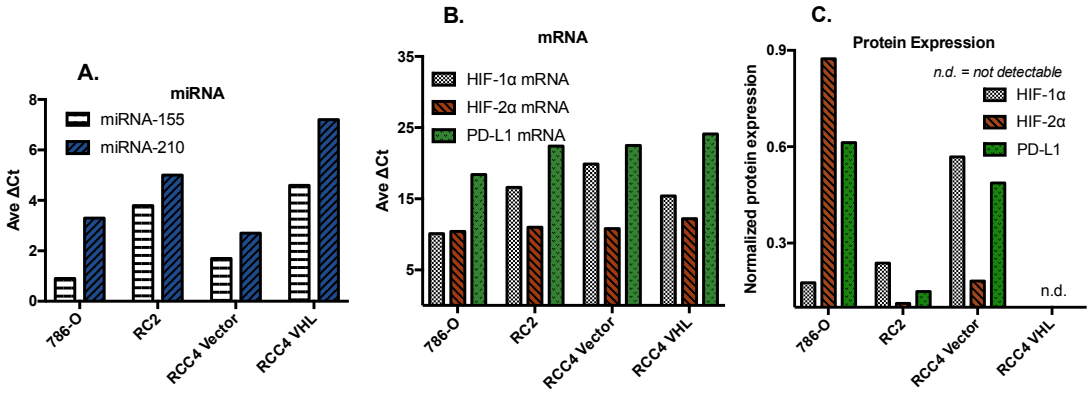

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Table 1. Summary of completed clinical trials exploring HIF inhibitors in metastatic renal cell carcinoma.

Investigational Agent(s)	Phase	N	Trial design	Dose-limiting Toxicities (DLTs)	ORR	PFS, OS
HIF Antagonist						
PT2385	Phase 1	51	PT2385 administered twice daily orally from 100 to 1800 mg followed by RP2D expansion phase	No DLTs reported	CR: 2% PR: 12% SD: 52% PD: 34%	PFS, OS: N/A
HIF Degradation						
Seleno-L-methionine (SLM) + axitinib	Phase 1b	9	SLM administered at 2500, 3000, or 4000 µg twice daily orally for 14 days followed by once daily in combination with axitinib	No DLTs reported	CR: 22% PR: 33% SD: 11% PD: 33%	PFS, OS: N/A
HIF Degradation via Proteasomes						
Vorinostat + bevacizumab	Phase 1/2	36	Vorinostat administered at 200 mg twice daily orally for 14 days in combination with bevacizumab at 15 mg/kg intravenously every 3 weeks	No DLTs reported in phase 1; 2 patients with grade 4 thrombocytopenia and grade 3 thromboembolic events	CR: 2.7% PR: 13.8%	mPFS: 5.7 months mOS: 12.9 months
Abexinostat + pazopanib	Phase 1	RCC cohort: 22 Total: 51	Pazopanib administered once daily on days 1 to 28 and abexinostat orally twice daily on days 1 to 5, 8 to 12, and 15 to 19 or on days 1 to 4, 8 to 11, and 15 to 18	Total cohort: 5 DLTs were reported including fatigue in 2 patients, thrombocytopenia in 2 patients and elevated transaminases in 1 patient	RCC cohort: ORR (CR,PR): 27%	PFS: N/A OS: N/A
Bortezomib + bevacizumab	Phase 1	91	Bevacizumab administered at 2.5-15 mg/kg intravenously on day 1 of each 21 day cycle; bortezomib administered at 0.7-1.3 mg/ m^2 intravenously on days 1, 4, 8, and 11 of each 21 day cycle	One patient with DLT from acute renal failure at highest dose level; 4 patients with partial response, 7 patients with stable disease at 6 months; toxicities included thrombocytopenia in 23% and fatigue in 19% of patients	CR: 0% PR: 4.4% SD: 42% PD: 47%	PFS: N/A OS: N/A

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Sorafenib + bortezomib	Phase 2	17	Sorafenib administered orally twice daily in combination with bortezomib 1 mg/m^2 intravenously on days 1, 4, 8, and 11, then every 21 days	N/A	CR: 0% PR: 5.8% SD: 70% PD: 23%	mPFS: 13.7 weeks mOS: 110 weeks

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; ORR: objective response rate; OS: overall survival; N/A: not available; HIF: hypoxia inducible factor; RP2D: recommended phase 2 dose; RCC: renal cell carcinoma

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Table 2. Ongoing clinical trials of HIF inhibitors in metastatic renal cell carcinoma.

Clinicaltrials.gov NCT identification number	Phase	Title	N	Allocation/Treatment	Primary Objective/ Outcome Measures	Status	Expected Completion
NCT03401788	Phase 2	A Phase 2 Study of PT2977 for the Treatment of Von Hippel-Lindau Disease-Associated Renal Cell Carcinoma	50	PT2977 (small molecule inhibitor of HIF2α)	Overall response rate	Recruiting	March 2023
NCT03592472	Phase 3	A Randomized, Double-blind, Placebo-controlled Study of Pazopanib with or without Abexinostat in Patients With Locally Advanced or Metastatic Renal Cell Carcinoma (RENAVIV)	413	Pazopanib + abexinostat vs. pazopanib + placebo	Progression-free survival; overall survival	Recruiting	January 2022
NCT02535533	Phase 1	A Therapeutic Trial for Safety and Preliminary Efficacy of the Combination of Axitinib and Seleniomethionine (SLM) for Adult Patients with Advanced Metastatic Clear Cell Renal Cell Carcinoma	30	SLM administrated orally twice daily for 14 days followed by SLM once daily in combination with axitinib 5 mg twice daily	Safety	Recruiting	September 2020
NCT02974738	Phase 1	A Phase 1, Multiple-Dose, Dose-Escalation and Expansion Trial of PT2977, a HIF-2α Inhibitor, in Patients With Advanced Solid Tumors	125	PT2977	Safety	Recruiting	June 2019
NCT02293980	Phase 1	A Phase 1, Multiple-Dose, Dose-Escalation Trial of PT2385 Tablets, a HIF-2α Inhibitor, in Patients With Advanced Clear Cell Renal Cell Carcinoma	107	Part 1: PT2385 tablets Part 2: PT2385 tablets in combination with nivolumab	Safety, DLT	Active, not recruiting	December 2018

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				Part 3: PT2385 tablets in combination with cabozantinib			
NCT02619253	Phase 1/1b	A Phase 1/1b, Open Label, Dose Finding Study to Evaluate Safety, Pharmacodynamics and Efficacy of Pembrolizumab (MK-3475) in Combination with Vorinostat in Patients with Advanced Renal or Urothelial Cell Carcinoma	42	Pembrolizumab and vorinostat	Safety/DLT	Recruiting	May 2020
NCT03634540	Phase 2	A Phase 2 Trial of PT2977 in Combination with Cabozantinib in Patients with Advanced Clear Cell Renal Cell Carcinoma	118	PT2977 in combination with cabozantinib tablets	Overall response rate (CR,PR)	Not yet recruiting	September 2022

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; OS, overall survival; N/A, not available; HIF, hypoxia inducible factor; RP2D, recommended phase 2 dose; RCC, renal cell carcinoma; DLT, dose limiting toxicity.