UT Southwestern Medical Center

Kidney Cancer Program

Targeting HIF2α with siRNA: from preclinical models to the clinic

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Abstract

Hypoxia-inducible factor 2 alpha (HIF2α) is arguably the most important driver of kidney cancer. HIF2α is constitutively activated following von Hippel-Lindau (VHL) gene inactivation, the signature event of the most common type of kidney cancer, clear cell renal cell carcinoma (ccRCC). HIF2α functions as a heterodimeric transcription factor and regulates a program of gene expression that promotes cell proliferation, stemness, and angiogenesis. Using a highly specific inhibitor designed to target a structural vulnerability in HIF2α (PT2399), we previously showed that approximately 50% of ccRCCs are dependent on HIF2α. However, prolonged drug exposure results in resistance and the acquisition of gatekeeper mutations, which we reported first in patient-derived xenografts (PDXs) and subsequently in humans. Using the same PDX platform that previously validated PT2399, we show that HIF2α can be effectively inhibited using a tumor-directed siRNA (siHIF2). Referring herein to both first- and second-generation (ARO-HIF2) siRNA drugs, siHIF2 is specifically taken up by human ccRCC tumors transplanted in mice, where it depletes HIF2α inhibiting target gene expression and tumor growth. As determined by orthogonal RNA-seq studies integrating both PT2399 and siHIF2 in PDXs, which provide unprecedented detail on the HIF2α effector transcriptome in ccRCC, siHIF2 is highly specific. siHIF2 has activity against both wild-type and drug (PT2385)-resistant mutant HIF2α. Preliminary results from a phase I trial of ARO-HIF2 (NCT04169711) were reported at ASCO GU (Brugarolas et al., 2022). 26 heavily pretreated ccRCC patients (pts) progressing on prior anti-VEGF and checkpoint inhibitor therapy were enrolled into 3 escalating dose cohorts. Five serious AEs were reported as possibly drug related (myocarditis, demyelinating neuropathy [2], hypoxia and hypoxemic respiratory failure). 9/26 pts had stable disease at week 8 and there were 2 partial responses. Among patients with evaluable biopsy samples, 9/14 showed reductions in HIF2α protein by immunohistochemistry. To our knowledge, this is the first example of functional inactivation of an oncoprotein with a ccRCC-directed siRNA in humans.

Introduction

VHL loss is regarded as the signature event of ccRCC. When VHL is inactivated, HIFα subunits accumulate, bind their HIF1β partner, translocate to the nucleus and activate gene expression. Among the targets, VEGF, a secreted ligand, binds its cognate receptor, VEGFR2, in endothelial cells promoting angiogenesis. The VEGF/VEGFR2 axis is of such importance that it is the target of 7 FDA-approved drugs for ccRCC. However, as a more proximal and broader effector, HIF2α would be a more attractive candidate for drug targeting.

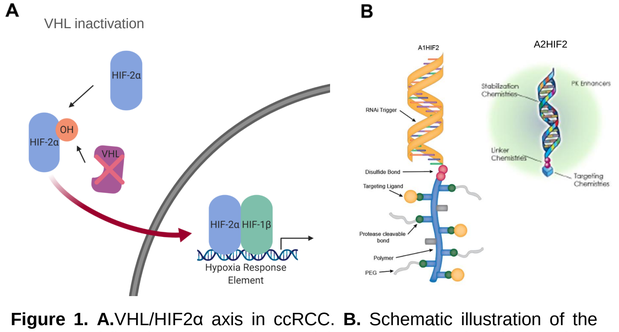


Figure 1. A.VHL/HIF2α axis in ccRCC. **B.** Schematic illustration of the structure of first (A1HIF2) and second (A2HIF2) generation drugs, which share the same RNAi trigger for HIF2α.

Results

Tumor growth inhibition by siHIF2 in ccRCC tumografts

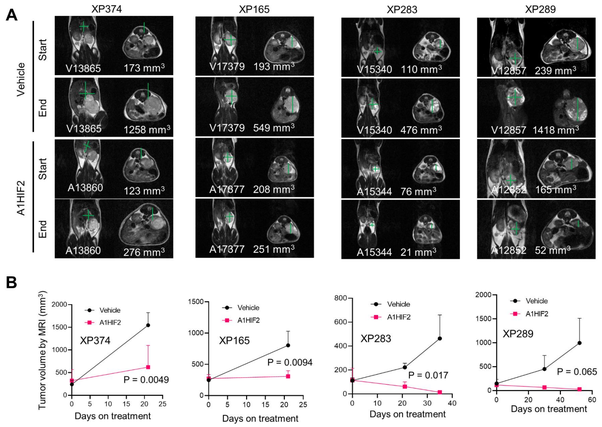


Figure 2. A. Representative MRI images at baseline and after administration of A1HIF2 with corresponding tumor volume quantitation (n=3 per arm). **B.** Tumor volume by MRI (n=3 per arm) at baseline and at the end of treatment.

HIF2α downregulation and VEGF depletion by siHIF2

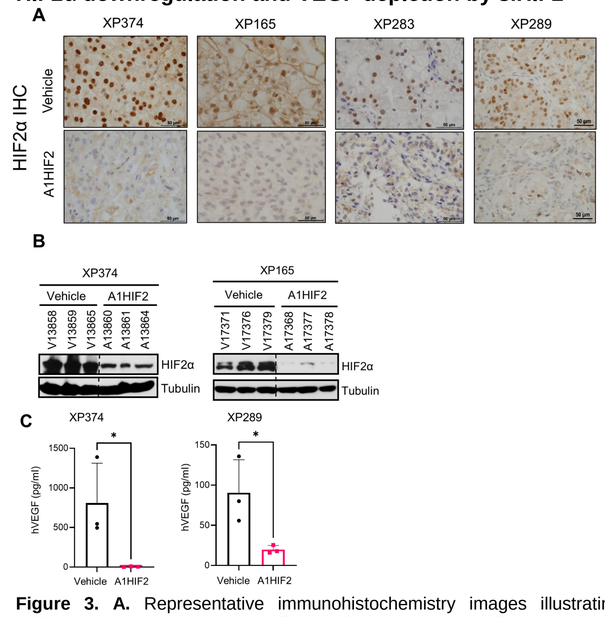


Figure 3. A. Representative immunohistochemistry images illustrating HIF2α protein depletion by A1HIF2 in ccRCC tumorgrafts. **B.** Western blot analyses of HIF2α in trial mice. **C.** hVEGF ELISA showing suppression of circulating tumor-produced VEGF in A1HIF2-treated mice. *, p < 0.05.

Results

Orthogonal studies of siHIF2 and PT2399 in tumorgrafts reveal distinct HIF2 gene expression program

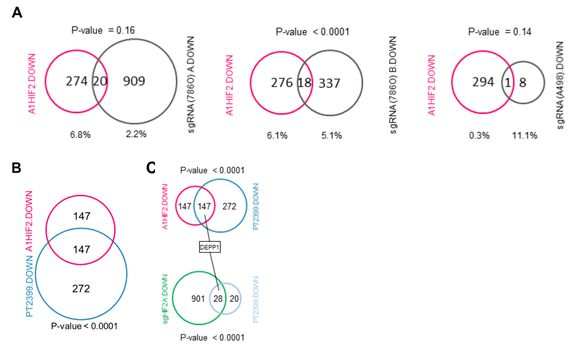


Figure 4. A. Summary of the number of significantly down-regulated genes observed in tumorgraft lines by A1HIF2 vs. HIF2α sgRNA in 786O (GSE72959 and GSE149005) and A498 cells (GSE16622). **B.** Venn diagram showing downregulated genes by RNA-seq for both A1HIF2 (11 vehicle- and 11 A1HIF2-treated tumorgrafts) and previously published PT2399 (12 vehicle- and 12 PT2399-treated tumorgrafts) at FDRq < 0.05 and LogFC < -1. **C.** Venn diagram of genes significantly down-regulated in tumorgrafts vs. 786O cells. DEPP1 is the only overlapping gene.

siHIF2 is active against PT2399 resistant mutant HIF2α

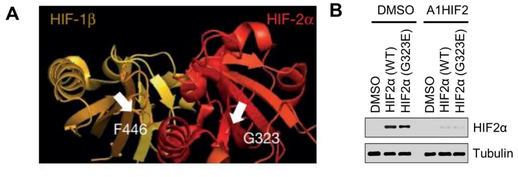


Figure 5. A. Tumorgrafts subjected to prolonged treatment with HIF2α-specific inhibitor (PT2399), develop gatekeeper mutation G323E (Chen *et al.*, Nature, 2016) **B.** siHIF2 is able to deplete G323E mutant HIF2α in HEK293T cells.

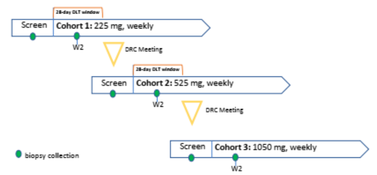
Conclusions

1. Systemic delivery of a tumor-directed HIF2α-specific siRNA resulted in target inactivation and anti-tumor activity in ccRCC tumorgraft models.
2. siHIF2 is highly specific and 50% of genes downregulated by siHIF2 were also downregulated by PT2399. A distinct program was identified with minimal overlap with previously reported studies in cell lines.
3. siHIF2 inhibited not only wild-type HIF2α, but also drug-resistant HIF2α.
4. Initial results from a phase 1 trial of siHIF2 were reported at ASCO GU and showed reasonable tolerability, HIF2α protein depletion, and anti-tumor activity.

Further clinical studies

Initial results of phase I trial of ARO-HIF2 (ASCO GU 2022, Abstract 339)

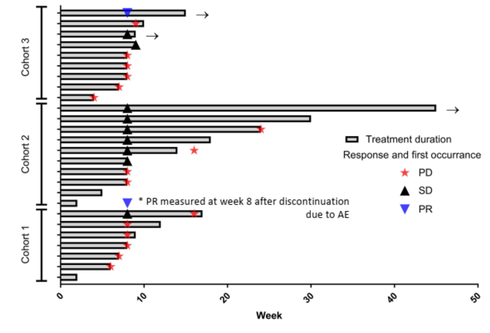
Figure 1: AROHIF21001 Study Design



- ARO-HIF2 was administered IV weekly
- Patients with advanced ccRCC (ECOG ≤ 1), with progressive disease on prior anti-VEGF and checkpoint inhibitor therapy
- Tumor biopsy performed at baseline and at 2 weeks
- Patients were assessed weekly for safety and every 8 weeks for tumor response by RECIST.

- **Assessments**: Safety, pK, changes in tumor HIF2a mRNA by qPCR and HIF2a protein by IHC, VHL mutation status, and RECIST. Minimum sufficient biopsy tumor cell content for qPCR and IHC evaluations were 40% and 5%, respectively.

Figure 2: Summary of Treatment Duration with Tumor Response



References

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