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Poster 3007

Selective Inhibition of Hypoxia-Inducible Factor (HIF)-2α for the Treatment of Cancer

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OVERVIEW

- Preclinical and clinical evidence suggests that targeting HIF-2α is a valid approach to inhibit tumor cells, particularly in clear cell renal carcinoma (ccRCC)[1,2].
- Arcus Biosciences is developing novel HIF-2α-specific small molecule inhibitors and investigating the biology of HIF-2α in various cancer and non-cancer cell subsets.
- Here we describe pharmacological properties associated with a novel, potent, and selective HIF-2α inhibitor and findings related to the understanding of HIF-2α biology in human immune and stromal cells and development of a HIF-2α-specific transcriptional signature.

HIF-2α BIOLOGY & REGULATION

- The solid tumor microenvironment (TME) can be hypoxic and cancer cells require induction of genes associated with metabolism, proliferation, and angiogenesis to survive and metastasize in a hypoxic environment[3].
- The master transcriptional regulator of hypoxia-induced genes is the Hypoxia-Inducible Factor (HIF)[4].
- HIF consists of an oxygen-regulated alpha monomer, of which there are three isoforms (HIF-1α, HIF-2α, and HIF-3α)[4].
- Alpha monomers heterodimerize with a constitutively-expressed beta monomer (HIF-1β/ARNT) using Per-ARNT-SIM (PAS) protein-protein interaction domains[4].
- Disruption of HIF-α/HIF-1β heterodimer formation is an effective means to inhibition of HIF-2α-dependent gene transcription[4].

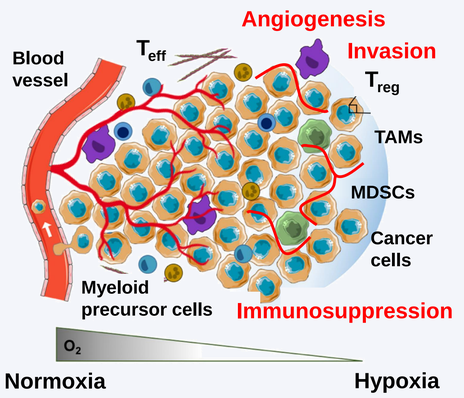

Figure 1. Effects of hypoxia on the tumor microenvironment. Hypoxia is an important feature of the tumor microenvironment (TME). It influences the interactions between cancer, stromal and immune cells, representing a critical step in the tumorigenic process. During tumor development, cells within the TME often have limited access to nutrients and oxygen, which creates a hypoxic environment that promotes a number of events including angiogenesis, cancer cell survival and progression as well as immunosuppression[5]. In a hypoxic TME, tumor vasculature becomes dysfunctional, and infiltrating myeloid precursor cells differentiate into more suppressive cell types such as MDSCs and tumor-associated macrophages, further contributing to cancer progression[5]. Figure adapted from Feng *et al.*[6].

RESULTS

Arcus is Optimizing Several Chemical Series of HIF-2α Inhibitors

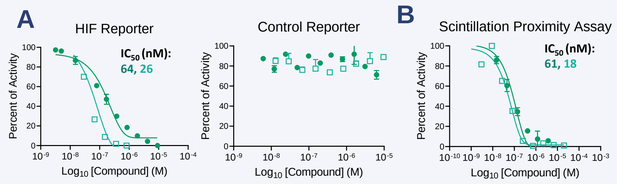

Figure 2. Representative SAR assay examples for Arcus Series 1 and Series 2 HIF-2α antagonist compounds. (A) HIF and Control Cellular Reporter Assay. 786-O renal adenocarcinoma cells (mutant for VHL and HIF-1α) stably expressing HIF or control CMV luciferase reporter constructs (Qiagen) were treated with Arcus compounds for 20 hours (h) at 37°C 5% CO_2. Data normalization for **(A)** was performed using DMSO vehicle controls and IC_{50} quantitation was performed using four parameter curve fitting. **(B) Scintillation Proximity Assay.** 50 nM of biotinylated human HIF-2α PAS-B (240-350) was preincubated at room temperature with Arcus compounds in 2% DMSO for 60 minutes. Afterwards, 3 µg of streptavidin PVT SPA beads were added and incubated for an additional 45 minutes followed by 25 nM of ^{3}H-tracer and a final 60 minute incubation before measuring for luminescence.

Hypoxia Drives a Pro-Angiogenic Gene Expression Profile in Endothelial Cells That is Rescued With HIF-2α Inhibition

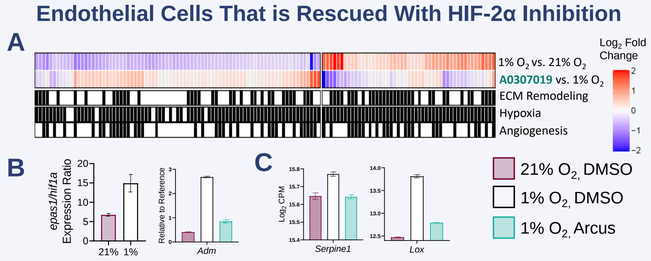

Figure 3. Exposure of HUVECs to hypoxia drives a pro-angiogenic, ECM remodeling gene expression profile that is decreased by HIF-2α inhibition. HUVECs were treated with DMSO or 10 µM HIF-2α inhibitor for 16 h in normoxia or hypoxia. Shown is **(A)** NanoString PanCancer Progression Panel analysis showing differentially expressed genes involved in angiogenesis, hypoxia and ECM remodeling pathways (FDR<0.05). **(B)** Ratio of HIF-2α/HIF-1α and Adm gene expression. Quantitation done by qPCR ($2^{-\Delta Ct}$). **(C)** Modulation of Serpine1 and Lox gene expression with HIF-2α inhibition.

Hypoxia Diminishes T Cell Activation in a HIF-2α Independent Manner

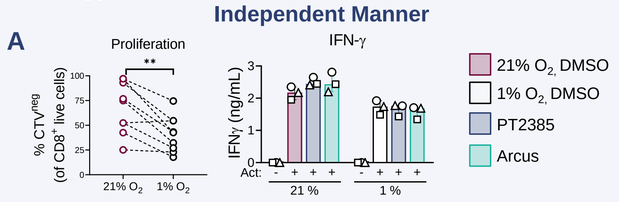

Figure 4. Human T cells are functional, yet less proliferative in hypoxia in a HIF-2α independent manner. Primary human $CD8^+$ T cells isolated from the blood of three to six donors (symbols) were left untreated (-) or activated (+) using a αCD2/αCD3/αCD28 bead cocktail in normoxia (21% O_2) or hypoxia (1% O_2) for three days in the presence of 10 µM HIF-2α antagonist. **(A)** Median ± range of IFNγ secretion as measured by bead array and proliferation capacity as measured by Cell-Trace Violet staining and flow cytometry. *p<0.05. Statistics were calculated using Wilcoxon matched-pairs signed rank test.

HIF-α Isoform-Specific Signature Development

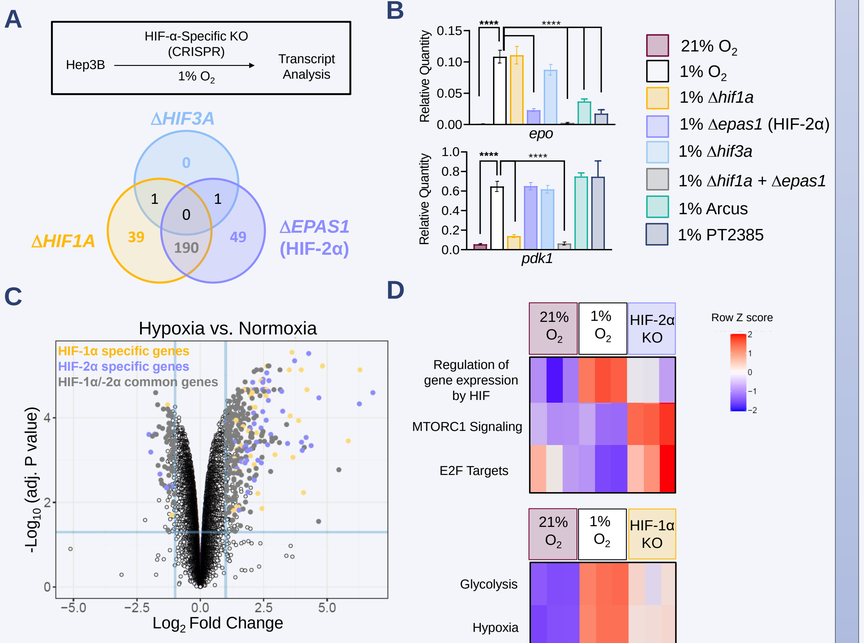

Figure 6. Analysis of HIF-α isoform transcriptional biology. (A) Overview. Shown is the experimental outline for CRISPR- (Δ) based approach to derive HIF-α isoform-specific gene signatures. Experiments performed on non-clonal population of Hep3B cells with KO efficiency of 92%, 87.5% and 74% for HIF-1α, HIF-2α and HIF-3α respectively. Hep3B cells nucleofected with RNP complex alone or with HIF-α isoform-specific guide RNAs were exposed to hypoxia for 16 h. Venn diagram **(A)** illustrating genes that are upregulated in 1% O_2 and rescued in each experimental condition (FDR<0.05, Fold change>2) **(B) Validation.** Shown are the mean ± SEM of *epo* and *pdk1* transcript levels. ****p<0.0001. Statistics calculated using one-way ANOVA with Dunnett's multiple comparisons test vs 1% O_2 control. Gene expression quantitation done by **qPCR** ($2^{-\Delta Ct}$). **(C) Hypoxia induced HIF-1α specific, HIF-2α specific and common genes.** Volcano plot of differentially expressed genes (DEG; FDR<0.05, Fold change>2) that are HIF-1α driven, HIF-2α driven and driven by both in 1% O_2. **(D) Hallmark pathways.** Pathway scores were calculated using ssGSEA[8] against MSigDB Hallmark[9] genesets. **(E) Genes modulated by HIF-3α are hypoxia independent.** DEG in HIF-3α KO (FDR<0.05, Fold change>2) that are independent of hypoxia are involved in oxidative phosphorylation (negatively regulated by HIF-3α) and β-catenin/Wnt signaling (potential direct transcriptional target(s) of HIF-3α).

Hypoxia Drives a Pro-Tumorigenic Gene Expression Profile in Macrophages That is Rescued With HIF-2α Inhibition

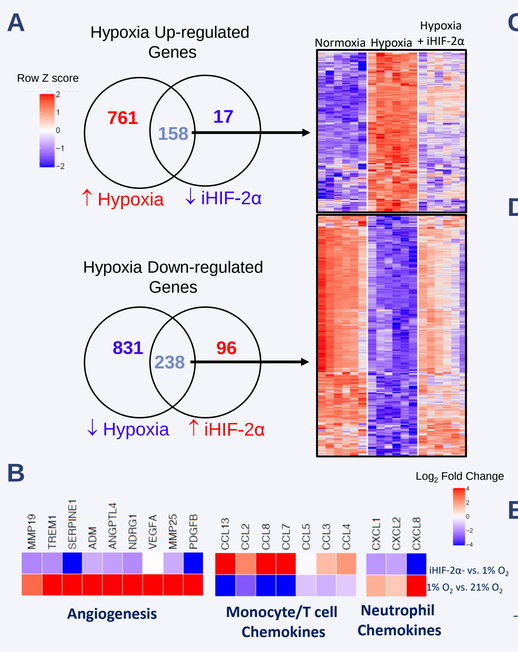

Figure 5. Exposure of M2-polarized or M0 macrophages to hypoxia drives a pro-tumorigenic gene expression profile that is decreased by HIF-2α inhibition. Primary human $CD14^+$ monocytes isolated from the blood of six donors (symbols) were differentiated in normoxia for six days with M-CSF before polarizing with IL-4 for one day in normoxia or hypoxia in the presence of 10 µM HIF-2α inhibitor PT2385. Shown is **(A)** a heatmap of differentially expressed genes that are altered in hypoxia and rescued with HIF-2α inhibition (FDR<0.05, Fold change>2) and **(B)** differentially expressed genes (DEG) involved in angiogenesis and chemoattraction. **(C) HIF-2α inhibitor treated hypoxic M2-polarized macrophages does not rescue M2-mediated $CD8^+$ T cell suppression.** IFNγ secretion as measured by bead array and proliferation capacity as measured by Cell-Trace Violet staining and flow cytometry. Act, activation. **(D) Macrophage chemokine secretion is modulated in hypoxia and rescued with HIF-2α inhibition.** Secretion measured by Luminex Mean ± Range. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05. Statistics were calculated using one-way ANOVA with Dunnett's multiple comparisons test vs 1% O_2 DMSO control for each gene/chemokine. Gene expression quantitation done by qPCR ($2^{-\Delta Ct}$). PT2385 was synthesized by Arcus utilizing methodology described in Wehn *et al.*[7]. **(E) Common hypoxia-induced gene signature.** Venn diagram showing DEG in 1% O_2 vs. 21% O_2 from Hep3B cells and M2 macrophages and a heatmap of the genes common to both cell types (FDR<0.05, Fold change>2).

Effects of HIF-2α Inhibition on TME in Xenograft Tumor Models

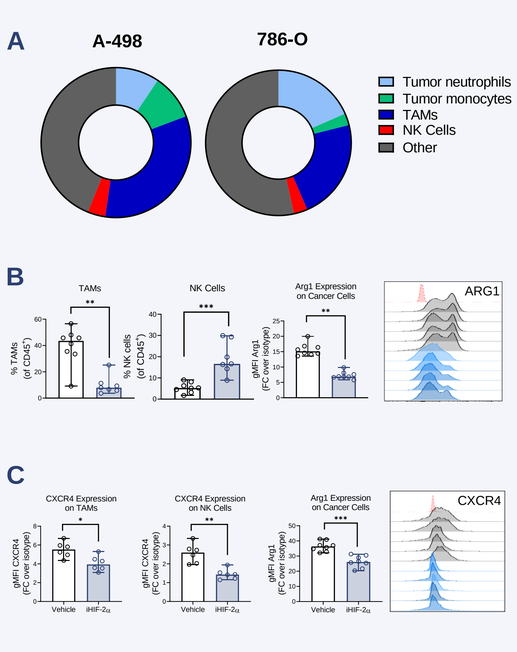

Figure 7 Differential effects of HIF-2α antagonist in two VHL-mutated ccRCC xenograft tumor models. (A) Immune composition of 786-O and A-498 tumors**. (B)** 786-O tumors treated with HIF-2α inhibitor, PT2385 (60 mg/kg) PO QD show decreased infiltrating tumor associated macrophages (TAMs), increased NK cells and decreased Arginase1 (Arg1) expression on cancer cells. **(C)** A-498 tumors treated with HIF-2α inhibitor PT2385 have decreased CXCR4 expression on TAMs and NK cells and decreased Arg1 on cancer cells. *p<0.05 **p<0.01 ***p<0.001. Statistics were calculated using Mann-Whitney test. Red Histograms are isotype control, black histograms are vehicle and blue histograms are PT2385 treated mice.

RESULTS

Evaluation of HIF-2α and HIF-1α Specific Signature Score in TCGA for Potential Tumor Indications

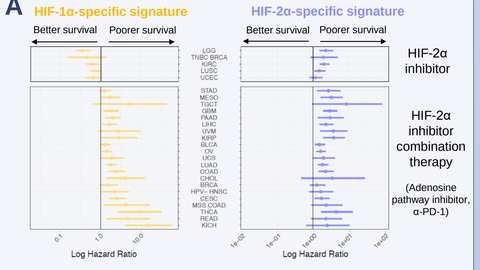

Figure 8. Evaluation of HIF-2α and HIF-1α specific signature in The Cancer Genome Atlas (TCGA)[8]. (A) Hazard ratios for HIF-2α specific and HIF-1α specific signatures were calculated using Cox proportional hazards model against overall survival in cancer patients in TCGA[10]. **(B)** HIF-2α specific and HIF-1α specific scores predict survival in pancreatic adenocarcinoma, colon adenocarcinoma and lung squamous cell carcinoma. HIF-2α scores were binarized using optimal cutoffs for maximally selected rank statistics with at least 20% of patients in one group. Logrank test was used to estimate p-values.

SUMMARY

- Several compound series are undergoing SAR optimization to develop novel HIF-2α inhibitors. Representative compounds from each series show both HIF-2α binding and functional activity in cell-based assays **(Figure 2)**.
- In human cells, HIF-2α inhibition does not significantly impact T cell function **(Figure 4)** but does decrease expression of genes that encode proteins implicated in tumorigenicity in macrophages and endothelial cells **(Figure 3, 5)**.
- A representative inhibitor selectively inhibited HIF-2α target gene expression in Hep3B cells **(Figure 6).**
- A gene signature derived from pharmacological inhibition of HIF-2α as well as deletion of HIF-α isoforms in Hep3B cells was used to identify isoform-dependent gene expression profiles **(Figure 6)**.
- ccRCC xenograft tumors show different immune compositions and likely contribute to differences in phenotype with HIF-2α inhibition **(Figure 7)**.
- HIF-2α and HIF-1α specific profiles derived from Hep3B cells were used to predict survival in various tumor types from TCGA **(Figure 8)**.

CITATIONS

1) Wallace *et al.* (2016) Cancer Res 76, 5491-5500.
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7) Wehn *et al.* (2018) J Med Chem 61, 9691-9721.
8) Barbie et al. (2009) Nature 462.7269
9) Liberzon et al. (2015) Cell Systems 1.6
10) https://www.cancer.gov/tcga