**OVERVIEW**

- Preclinical and clinical evidence suggests that HIF-2α inhibition is a promising approach for the treatment of solid tumor cells, particularly in vitro cell renal carcinoma (CC786).1

- Inhibitor design challenges:
  - Various by Compound (mutant profile 1
  - **Basal**
  - Various by Compound (mutant profile

**HIF-2α BIOLOGY & REGULATION**

- The solid tumor microenvironment (TME) can be highly hypoxic and cancer cells may induce a series of genetic and epigenetic changes to survive and metastasize.2

- The master transcriptional regulator of hypoxic-induced genes are the Hif-1α and Hif-2α proteins.3

- 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-1α/1β; HIF-2α/1α; HIF-3α/1α).5

- Disruption of HIF-2α/1α heterodimer formation is an effective means to inhibit HIF-2α-dependent gene transcription.6

**INITIAL DESIGN, OPTIMIZATION AND CHARACTERIZATION OF ARUCUS HIF-2α INHIBITORS**

**Fundamentals of Targeting the HIF-2α/ARNT Complex**

Small molecules have been designed to inhibit HIF-2α/ARNT heterodimerization by binding a small, internal cavity in the HIF-2α/ARNT-B domain. The hydrophobic cavity shown in blue (iso-surface below) is a fully accessible with a volume of 389 Å³ and is occupied by 8 water molecules in the apo form. It has been demonstrated that small molecules can enter the cavity and induce a large, conformational change in the HIF-2α-ARNT-B domain, which, in turn, results in HIF-2α/ARNT-B complex degradation.7

**Basal**

**Basis for regulation of protein-protein interaction**:8

- Small molecule binds to HIF-2α-ARNT cavity (calculated)

**Inhibitor design challenges**:9

- Small internal pocket limits ligand size
- Binding affinity may not correlate with functional activity
- HIF-2α can be highly expressed in processes undermining physiological properties (high functionality)

**Extensive Characterization of Initial HIF-2α Initial Lead Series**

- Compound 1
- Compound 2
- Compound 3

**Table 1. Representative Initial lead examples for Arcus Series 1, Series 2, and Series 3 HIF-2α inhibitor compounds.**

**Optimization of Series 1 HIF-2α Inhibitors**

Series 1 inhibitors were optimized to improve potency and pharmacodynamic properties via structure-based design and kinetic-based virtual screening. A selection of optimized advanced compounds are shown in Table 2 which potently inhibit HIF-2α function in numerous assays formats without appreciable off-target activity.

**Table 2.** Potency of series 1 inhibitors. 1HIF-2α reporter and SPA assays performed as described in Table 1. VEGF Secretion Assays: 786 human cancer cells were treated with inhibitors for 48 hours at 0-10 µM; VEGF released with vehicle was quantified by Cytoseq (Perkin Elmer).

**Figure 2.** Extensive Characterization of Initial HIF-2α Initial Lead Series.

**Figure 3.** Optimization of Series 1 HIF-2α Inhibitors.

**Figure 4.** Representative VEGF dose-responses curves of optimized Arcus cell lines with Compound 3 (µM).

**Figure 5.** Representative VEGF dose-responses curves of optimized Arcus cell lines with Compound 3 (µM).

**Figure 6.** Pharmacokinetic Characterization of Compound 3.

**Figure 7.** Pharmacokinetic Characterization of Compound 3.

**Figure 8.** Pharmacokinetic Characterization of Compound 3.

**Figure 9.** Pharmacokinetic Characterization of Compound 3.

**Table 3.** Summary of Hepatocyte viability following exposure to Compound 3.

**Table 4.** Pharmacokinetic properties for optimized Compound 3.

**Table 5.** Percentage of control (mean ± SEM).

**Table 6.** Summary of experiment FI parameters in nL and d.p. Rat were dosed 76.8 mg/kg IV in DMAC/DMSO/sodium phosphate (pH 7.35, 10% and 0.1% PO) in TEG/1% (65S). Dogs were dosed 0.03 mg/kg IV in SAVYSLIDE (11% and mg/kg) as a 1% DMSO.

**SUMMARY**

- Three distinct compound series are undergoing iterative SAR optimization to develop potent and selective HIF-1α inhibitors from each compound series with each show both HIF-1α binding and functional activity.

- Series 1 inhibitors have been optimized via structure-based design and kinetic-based virtual screening to yield compounds with a median 35 hours half-life and high potency (10 nM for 786 cells) that exhibit IC50 values of levels of HIF2α target genes (VEGF and PDGFA) in human tumor cells.

**CITATIONS**


