AB521 is a Novel and Potent Clinical-stage HIF-2α Inhibitor for the Treatment of Renal Cell Carcinoma

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Abstract

Background. The transcription factor Hypoxia-inducible Factor (HIF)-2α is an oncogenic driver in clear cell renal cell carcinoma (ccRCC). HIF-2α protein levels are regulated post-translationally in an oxygen-dependent manner. Hypoxic or pseudohypoxic conditions promote HIF-2α stabilization and transcription of pro-tumorigenic genes. Inhibition of HIF-2α has significant potential to mitigate tumor growth, particularly in cancers like ccRCC that have a high prevalence of molecular alterations associated with pseudohypoxia, namely mutations or epigenetic silencing of the Von Hippel-Lindau (VHL) protein. Herein we report findings associated with bioinformatic gene signature analyses and characterize AB521, a novel small molecule HIF-2α inhibitor.

Methods. Published and novel gene signatures were applied to publicly available datasets. Potency and specificity of AB521 was evaluated using biochemical and cell-based assays. Efficacy, pharmacokinetics, and pharmacodynamics associated with AB521 were evaluated in preclinical species and a healthy volunteer study is on-going.

Results. Evaluated signatures related to hypoxia and HIF-2α were associated with therapeutic response to HIF-2α inhibition in preclinical models. Binding assays and crystal structure elucidation demonstrated that AB521 avidly binds the HIF-2α PAS-B domain. AB521 potently inhibited HIF-2α-dependent reporter transcription and VEGF protein secretion in 786-O cells. AB521 also selectively inhibited HIF-2α-, but not HIF-1α-, regulated gene expression in Hep3B hepatocellular carcinoma cells. *In vivo*, AB521 significantly regressed mouse ccRCC xenograft tumors and decreased on-target pharmacodynamic markers in a dose-dependent manner. Additionally, AB521 enhanced anti-tumor activity of a VEGF-targeting tyrosine kinase inhibitor. AB521 had a favorable pharmacokinetics (PK) *in vitro* and *in vivo*, resulting in a profile suitable for once-daily oral dosing in humans. Recent healthy volunteer data confirms both the predicted PK profile as well as modulation of erythropoietin (EPO) in a dose dependent manner.

Conclusions. Transcriptional data can be further applied to understand the therapeutic potential of HIF-2α inhibition. AB521 is a novel and selective HIF-2α inhibitor with profound anti-tumor activity, and clinical evaluation of this molecule is underway.

HIF-2α Biology and Therapeutic Rationale

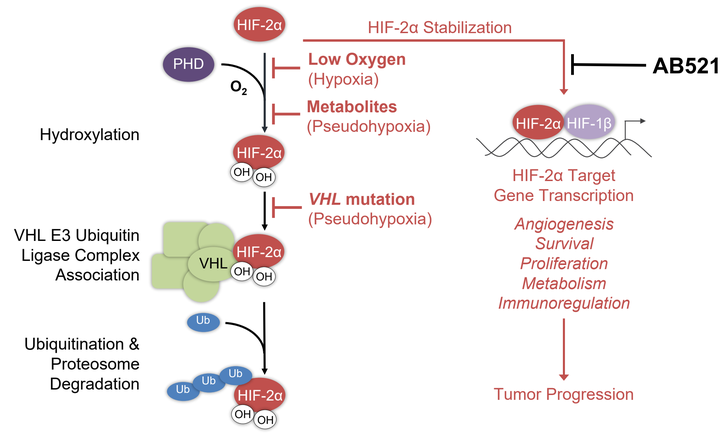


Figure 1. HIF-2α drives physiological changes to adapt to low oxygen that are hijacked by the tumor. HIF-2α is a transcription factor comprised of two proteins: a stably-expressed β subunit (**HIF-1β/ARNT**) and an oxygen-sensitive α subunit (**HIF-2α**). In hypoxic or pseudohypoxic conditions, HIF-2α is stabilized and drives transcriptional programs that promote tumor progression. **AB521** is a novel, potent, and selective allosteric small molecule inhibitor that can prevent HIF-2α-dependent gene transcription and block tumor progression.

In vitro Profile of AB521

	Assay	AB521	MK-6482[a]
Cellular	HIF-2α 786-O Luc Reporter IC_{50} (nM)	8.2 ± 2.5 (n = 24)	16.9 ± 10.1 (n = 8)
	Control 786-O Luc Reporter IC_{50} (nM)	> 10,000 (n = 6)	> 10,000 (n = 7)
	HIF-2α 786-O Luc Reporter IC_{50} (nM) [in 100% Serum]	46.5 ± 14.2 (n = 24)	61.8 ± 6.6 (n = 4)
	786-O VEGF AlphaLISA IC_{50} (nM)	28.9 ± 3.6 (n = 11)	47.7 ± 30.8 (n = 4)
	Hep3B *EPO* Transcript IC_{50} (nM)	35.9 ± 5.0 (n = 3)	39.0 ± 9.7 (n = 3)
Biochemical	HIF-2α TSA ΔT_M (°C)	14.7 ± 0.6 (n = 14)	12.1 ± 0.3 (n = 4)
	HIF-2α MST K_D (nM)	2.4 ± 0.8 (n = 3)	15.4 ± 2.7 (n = 3)
	HIF-2α ITC K_D (nM)	53.6 ± 17.9 n = 3)	58.3 ± 19.3 (n = 3)
	HIF-2α SPA IC_{50} (nM)	16.6 ± 5.0 (n = 8)	22.3 ± 5.6 (n = 5)

Table 1. Characterization of AB521 and MK-6482[a] in Arcus biochemical and cell-based assays. Cellular assays: HIF luciferase (Luc) reporter and VEGF secretion in 786-O ccRCC cells; erythropoietin *(EPO)* transcription in Hep3B hepatocellular carcinoma cells. Binding Assays performed with the HIF-2α PAS-B domain: TSA, Thermal Shift Assay; MST, Microscale Thermophoresis; ITC, Isothermal Calorimetry; SPA, Scintillation Proximity Assay. [a]MK-6482/belzutifan was synthesized at Arcus according to published methods[1].

Characterization of AB521 in Xenograft Models

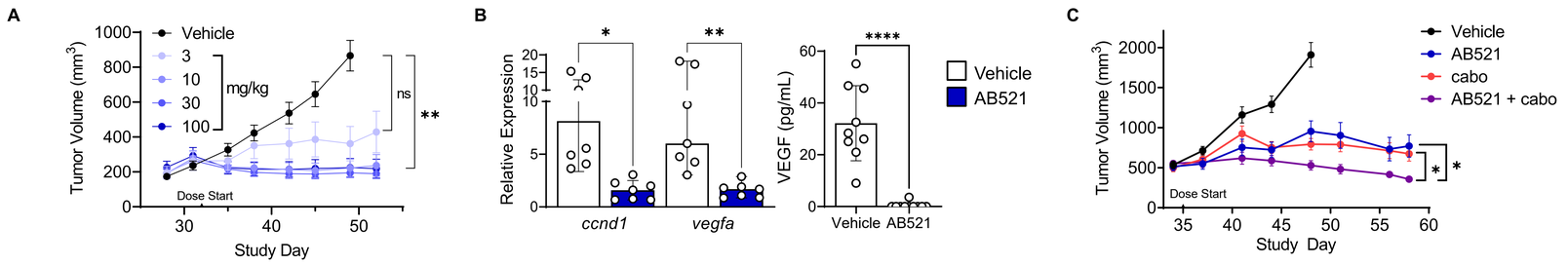
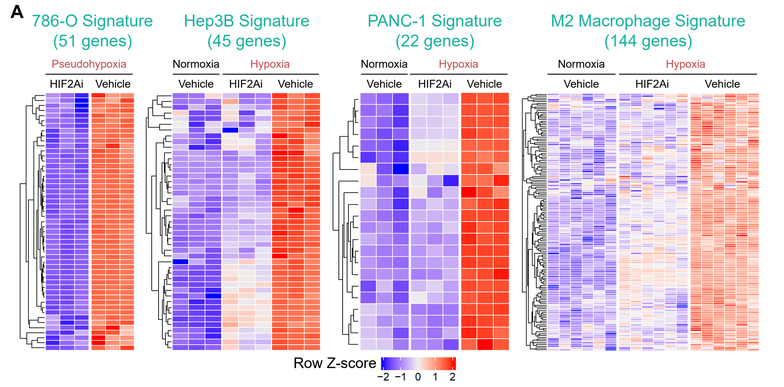


Figure 2. AB521 alone or in combination with the tyrosine kinase inhibitor (TKI) cabozantinib (cabo), showed enhanced anti-tumor activity in the A498 ccRCC xenograft model. (A and B) Vehicle or AB521 administered once daily by oral gavage (PO QD) when tumors were ~300 mm^3. **(A)** Group averages over time. **(B)** Pharmacodynamic changes in intratumoral gene expression (*ccnd1* and *vegfa*) and plasma protein levels (VEGF) 24 hours after vehicle or 100 mg/kg AB521 treatment. **(C)** Vehicle, 100 mg/kg AB521, 30 mg/kg cabozantinib (cabo), or a combination of the two agents administered PO QD when tumors were ~500 mm^3. Shown are group averages over time. Pharmacokinetic parameters in preclinical species, including mice, has been disclosed previously[2]. ns, not significant.

Application of HIF-2α-Specific Gene Signatures



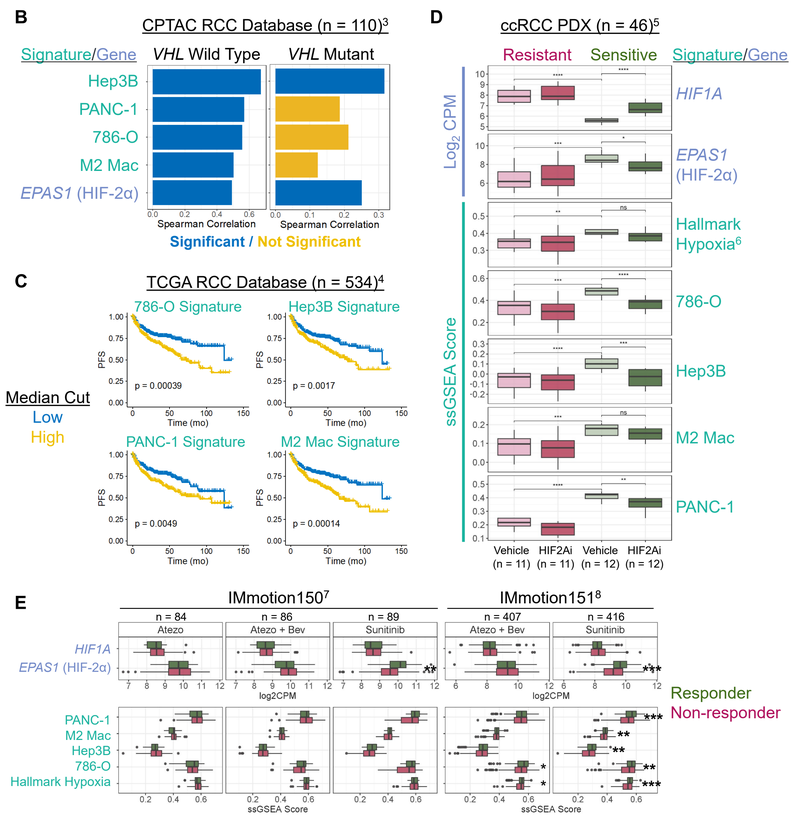


Figure 3. Arcus HIF-2α-dependent gene signatures are associated with responses in patient-derived xenograft (PDX) models and prognosis in sunitinib-treated ccRCC patients. (A) Hypoxia-driven genes downregulated by treatment with a HIF-2α inhibitor (HIF2Ai) *in vitro* were used to derive cell type-specific HIF-2α signatures. Mac, macrophage; HIF2Ai, PT2385 synthesized at Arcus according to published methods[9]. **(B)** Arcus-derived signatures correlate with HIF-2α protein better than *EPAS1* RNA in *VHL* wild type ccRCC tumors[3]. **(C)** High expression of HIF-2α signatures are associated with poorer progression-free survival (PFS) in RCC[4]. **(D)** Expression of *EPAS1* and HIF-2α gene signatures are higher in HIF2Ai sensitive PDXs[5] compared to resistant PDXs (comparing vehicle groups) and downregulated upon treatment with HIF2Ai in sensitive PDXs. **(E)** Expression of *EPAS1* and HIF-2α gene signatures are higher in sunitinib-treated ccRCC responders[7,8]. CPM, counts per million. ssGSEA, single sample gene set enrichment analysis.

Clinical Evaluation of AB521: ARC-14

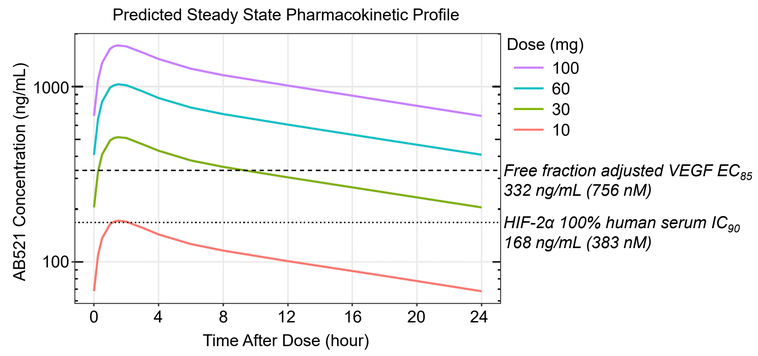


Figure 4. AB521 is currently under clinical evaluation in healthy volunteers (ARC-14) to assess the tolerability, pharmacokinetics, and pharmacodynamic properties of orally administered AB521. Based on data from the initial single ascending dose (SAD) cohorts, the predicted pharmacokinetic profile of AB521 confirms a once-daily dosing in humans. *In vitro* potency reference values are indicated next to the dashed and dotted lines.

Dose Group	Average % Change From Baseline in EPO Levels 24 Hours Post-dose (n = 4-6)
Placebo	5
3 mg AB521	3
10 mg AB521	-41
30 mg AB521	-76

Table 2. Average percent reduction in erythropoietin (EPO) levels observed at 24 hours after a single dose of AB521, relative to pre-dose, in each treatment cohort. Each SAD cohort evaluated was comprised of at least 4 human subjects.

Summary and References

- AB521 has been extensively characterized in a suite of biochemical and cell-based assays and demonstrates a high affinity for the HIF-2α PAS-B domain and potent inhibition of HIF-2α-dependent transcription (**Table 1**).
- In ccRCC xenograft models, AB521 significantly inhibits tumor growth, and enhanced anti-tumor activity was observed when administered in combination with the TKI cabozantinib (**Figure 2**).
- Arcus-derived HIF-2α signatures are correlated with HIF-2α protein expression in *VHL* wild type ccRCC and high scores are associated with poorer PFS. PDX models sensitive to HIF-2α inhibition express higher signature scores and downregulate these signatures upon HIF-2α inhibition. ccRCC responding to sunitinib express higher levels of HIF-2α RNA and hypoxia/HIF-2α signatures (**Figure 3**).
- We project a dose of 45-60 mg once daily AB521 will match the potency-corrected exposure of 120 mg belzutifan and anticipate an ability explore higher doses without the PK saturation observed with belzutifan[1] (**Figure 4**).
- Significant modulation of EPO levels in healthy volunteers following administration of a single dose of AB521 has been observed (**Table 2**). Dose escalation continues in the ARC-14 healthy volunteer study and initiation of clinical trials in cancer patients is expected to begin in the latter half of 2022.

1) Xu *et al.* 2019 J Med Chem; DOI: 10.1021/acs.jmedchem.9b00719
2) Gauthier *et al.* 2021 Mol Cancer Ther; DOI: 10.1158/1535-7163.TARG-21-P206 (https://arcusbio.com/publications/)
3) Clark *et al.* 2019 Cell; DOI: 10.1016/j.cell.2019.10.007
4) The results shown here are in part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga
5) Chen *et al.* 2016 Nat; DOI: 10.1038/nature19796
6) Liberzon *et al.* 2015 Cell Sys; DOI: 10.1016/j.cels.2015.12.004
7) McDermott *et al.* 2018 Nat Med; DOI: 10.1038/s41591-018-0053-3
8) Rini *et al.* 2019 Lancet; DOI: 10.1016/S0140-6736(19)30723-8
9) When *et al.* 2018 J Med Chem; DOI: 10.1021/acs.jmedchem.8b01196