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Abstract #252

AB521, a Clinical-Stage, Potent, and Selective Hypoxia-Inducible Factor (HIF)-2α Inhibitor, for the Treatment of Renal Cell Carcinoma

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OVERVIEW

- Preclinical and clinical evidence suggests that HIF-2α inhibition is a valid approach to destroy tumor cells, particularly in clear cell renal carcinoma (ccRCC) and tumors associated with mutant pVHL[1,2].
- Our group has discovered AB521, a novel specific small-molecule HIF-2α inhibitor which is undergoing evaluation in a Phase 1 clinical study in healthy volunteers.
- Herein we present the key properties of AB521, which potently and selectively blocks the heterodimerization of HIF-2α with HIF-1β, resulting in profound antitumor activity in preclinical models. We also project the effective human dose, based on emerging data from the ongoing Phase 1.

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[3].
- The master transcriptional regulators of hypoxia-induced genes are the Hypoxia-Inducible Factor (HIF) proteins[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].

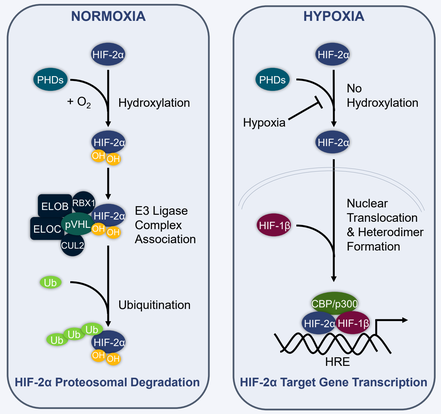


Figure 1. Overview of HIF-2α regulation. In normoxia (left), proline residues present in the oxygen-dependent degradation domain (ODDD) of HIF-2α are hydroxylated by prolyl hydroxylases (PHDs), allowing for recognition by the von Hippel-Lindau (pVHL) E3-ubiquitin ligase complex and subsequent ubiquitination and proteasomal degradation. Upon exposure to low oxygen conditions (hypoxia, right) or in the case of *vhl* mutation or silencing (pseudohypoxia), HIF-2α subunits accumulate and dimerize with HIF-1β/ARNT, resulting in transcription of various gene sets, some of which are pro-tumorigenic, downstream of hypoxia-response element (HRE) DNA binding sites. Adapted from Yu *et al.*[5].

PRECLINICAL CHARACTERIZATION OF AB521 AND PREDICTED HUMAN PHARMACOKINETICS

Fundamentals of Targeting the HIF-2α/ARNT Complex

The potency of AB521 was evaluated using a suite of biochemical and cell-based assays. Binding assays and protein co-crystal structure elucidation have demonstrated that AB521 avidly binds the HIF-2α PAS-B lipophilic cavity with low nanomolar affinity and potently inhibits HIF-2α-dependent transcription in a HIF-2α-specific luciferase reporter transcription in the presence of human serum and VEGF protein secretion in 786-O cells.

Extensive *in vitro* Characterization 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)
Biochemical	HIF-2α TSA $T_M\Delta$ (°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 in biochemical and cell-based assays. Reporter Assays: 786-O renal adenocarcinoma cells (mutant for VHL and HIF-1α) stably expressing HIF or control CMV luciferase (Luc) reporter constructs were treated with compound for 20 hours. Binding Assays: TSA = Thermal Shift Assay; MST = Microscale Thermophoresis; ITC = Isothermal Calorimetry; SPA = Scintillation Proximity Assay. Performed with the HIF-2α PAS-B domain. [a]MK-6482 was prepared according to reference 6. All data in table 1 was generated at Arcus.

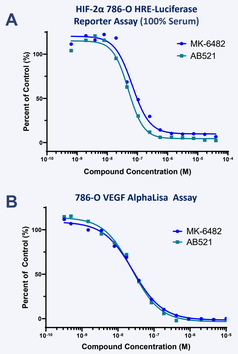


Figure 2. Comparison of AB521 and belzuitifan (MK-6482) in HIF-2α luciferase (100% human serum) (**A**) and VEGF secretion (**B**) assays performed side-by-side.

Preclinical Pharmacokinetic Properties of AB521

AB521 exhibits a favorable pharmacokinetic profile characterized by moderate-to-low clearance in preclinical species and is stable to human hepatocytes. Furthermore, AB521 exhibits good bioavailability with minimal potential for direct or time-depended CYP inhibition.

	Hepatocytes		*In vivo*		
Species	CL_{int} (μL/min/10^6 cells)	$T_{1/2}$ (h)	CL (L/h/kg)	Vss (L/kg)	$T_{1/2}$ (h)
Mouse	2.7	10.8	1.22	2.2	1.4
Rat	2.8	10.3	0.91	2.3	2.2
Dog	<0.7	>40	0.05	1.1	16
Human	<0.7	>40			

CYP Inhibition and hERG	
Assay	AB521
CYP IC_{50} (μM) 2C19 / 2C8 / 2C9 / 2D6 / 3A4	>100 / >100 / 60.6 / >100 / >100
CYP TDI (% Activity loss, 30 min) 3A4 / 2C8 / 2C9 / 2D6	9.4 / 7.9 / 2.9 / 1.3
hERG (automatic patch clamp)	IC_{50} > 10 μM

Table 2. Summary of experimental PK parameters in mouse, rat, and dog. Rats were dosed 0.25 mg/kg IV in DMAC:Ethanol:Propylene Glycol:Saline (10:10:30:50). Dogs were dosed 0.33 mg/kg IV in DMA/PG/water (1:1:1). PO doses formulated PEG400/VitE TPGS (95:5). AB521 was evaluated *in vitro* for its potential to inhibit major human drug metabolizing enzymes of the cytochrome P450 family.

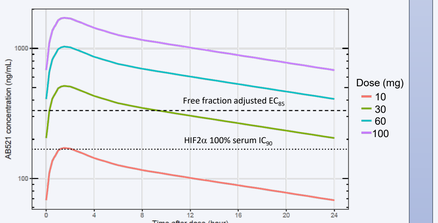
AB521 Human Pharmacokinetics

AB521 is currently under clinical evaluation (ARC-14) in healthy volunteers to assess the tolerability, pharmacokinetics, and pharmacodynamic properties of orally administered AB521. Based on data from the initial single ascending dose cohorts (SAD), we have projected that AB521 will possess a pharmacokinetic profile suitable for once-daily dosing in humans (**Figure 3**, below). Furthermore, modulation of EPO, an established pharmacodynamic marker, has been observed at the 10-mg dose.

AB521 Potency Reference	Value
HIF-2α 100% human serum IC_{90}	383 nM 168 ng/mL
Free fraction adjusted VEGF EC_{85}	756 nM 332 ng/mL

- We project a dose of 45-60 mg once daily (QD) AB521 will match the potency-corrected exposure of 120 mg belzutifan
- We anticipate that we will be able to explore higher doses without the PK saturation seen with belzutifan[6]
- EPO modulation has been observed in the on-going healthy volunteer study

Figure 3. Predicted AB521 PK profile at steady state



Characterization of AB521 in ccRCC Xenograft Models

When administered once-daily orally (PO QD) to mice, AB521 significantly regressed established 786-O and A498 xenograft tumors in a dose-dependent manner (**Figure 4**). AB521, in combination with a TKI (cabozantinib), showed enhanced anti-tumor activity relative to either single agent therapy in the A498 xenograft model (**Figure 5**).

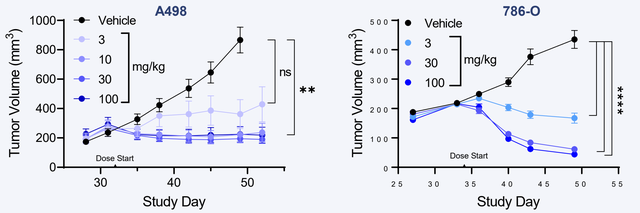


Figure 4. Vehicle [PEG400:Kolliphor HS15 (70:30)] or AB521 administered PO QD at ~220 mm^3 (786-O) or ~300 mm^3 (A498).

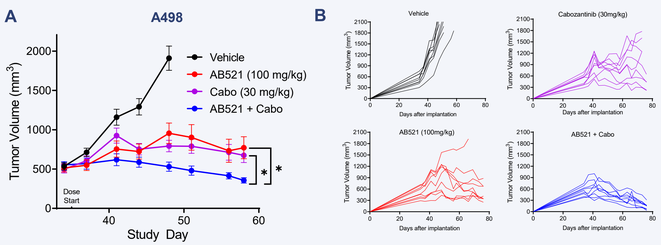


Figure 5. Vehicle [PEG400:Kolliphor HS15 (70:30)], cabozantinib (Cabo), or AB521 administered PO QD when A498 xenograft tumors reached ~500 mm^3. Shown are group averages **(A)** or individual growth **(B)** plots.

HIF ISOFORM SELECTIVITY

AB521 Selectively Inhibits HIF-2α Gene Transcription

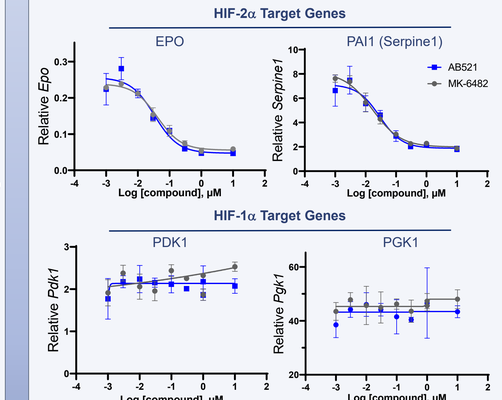


Figure 6. AB521 inhibits HIF-2α-, but not HIF-1α-, mediated transcription of pro-tumorigenic gene sets. Hep3B hepatocellular carcinoma cells (wild-type for VHL and HIF-1α) were treated with 1 nM to 10 μM AB521 or MK-6482 and exposed to hypoxia (1% O_2) for 16 hours. Gene expression levels of HIF-2α target genes (*EPO* and *Serpine1*) and HIF-1α genes (*PDK1* and *PGK1*) were determined by qPCR relative to *HPRT1* ($2^{-\Delta Ct}$) . AB521 inhibited *EPO* and *Serpine1* gene transcription with IC_{50} values of 33 and 28 nM, respectively.

SUMMARY

- AB521 is a novel and potent small molecule inhibitor of HIF-2α which blocks HIF-2α mediated gene transcription under physiologically relevant conditions **(Table 1, Figure 2)**.
- In Hep3B cells, AB521 potently inhibited HIF-2α target gene expression. In contrast, HIF-1α target gene expression was not altered, indicating AB521 is highly selective for HIF-2α **(Figure 6)**.
- AB521 exhibits a favorable pharmacokinetic profile in preclinical models with minimal direct or time-dependent inhibition of major CYP isoforms **(Table 2)**.
- 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 4 & Figure 5)**.
- Emerging healthy volunteer data projects that AB521 will possess a human pharmacokinetic profile suitable for once-daily dosing **(Figure 3)**. Significant modulation of EPO levels in healthy volunteers following administration of AB521 has been observed.

CITATIONS

1) Jonasch *et al.* (2020) ASCO 2020, Abstract #5003.
2) Srinivasan *et al.* (2020) ESMO, Abstract #LBA26.
3) Hockel & Vaupel (2001) JNCI 93, 266-276.
4) Li *et al.* (2019) J Med Chem.
5) Yu *et al.* (2019) Drug Disc Today 00, 1-9.
6) Xu *et al.* (2019) J Med Chem, 62, 6876