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AB521 potently and selectively inhibits pro-tumorigenic gene transcription by Hypoxia-Inducible Factor (HIF)-2α *in vitro* and *in vivo*

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#P206

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I have the following financial relationships to disclose:

Stockholder in: Arcus Biosciences (RCUS)

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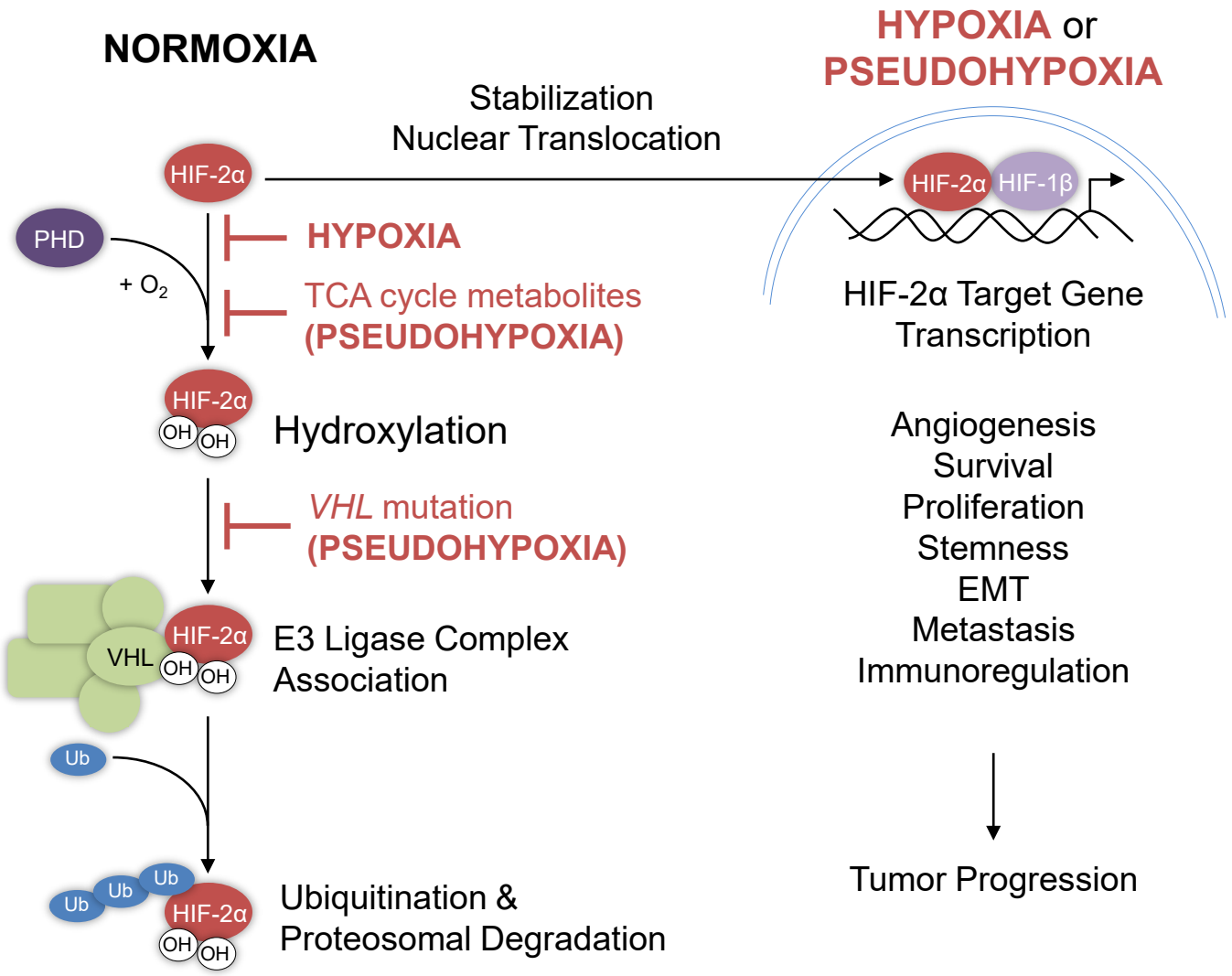
I will not discuss off label use and/or investigational use in my presentation.

HIF-2α Drives Expression of Pro-Tumorigenic Genes in Hypoxic or Pseudohypoxic Conditions



➢ *Background*

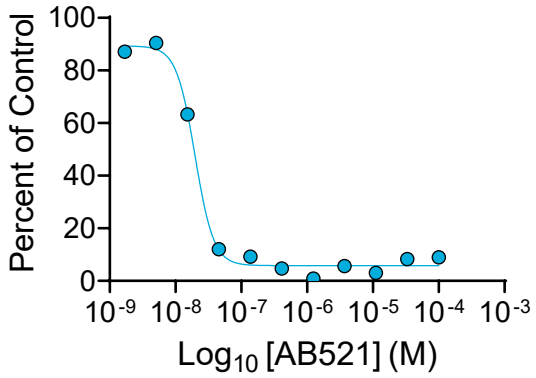
- HIF is a heterodimeric transcription factor
- HIF-2α activity can drive tumor progression in hypoxia or pseudohypoxia
- Inhibition of heterodimer formation with an allosteric small molecule is an effective strategy to mitigate tumor growth, particularly in patients with VHL disease or clear cell renal cell carcinoma (ccRCC)

➢ *Objective*

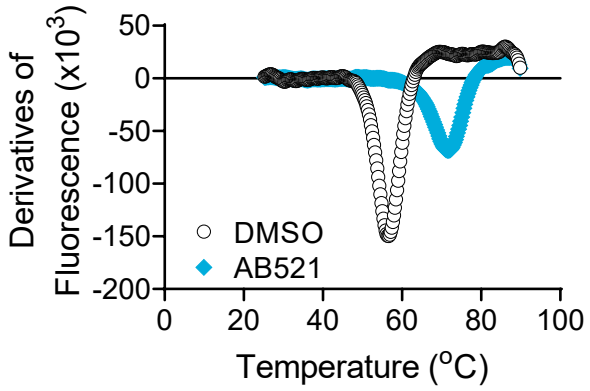
- Applying a pharmacophore mapping and structure-based design approach, we identified a novel and potent small molecule HIF-2α inhibitor, AB521
- Herein we characterize AB521 in a suite of biophysical, biochemical, cellular, pharmacological, and pharmacokinetic assays

AB521 Binds the HIF-2α PAS-B Domain With High Affinity

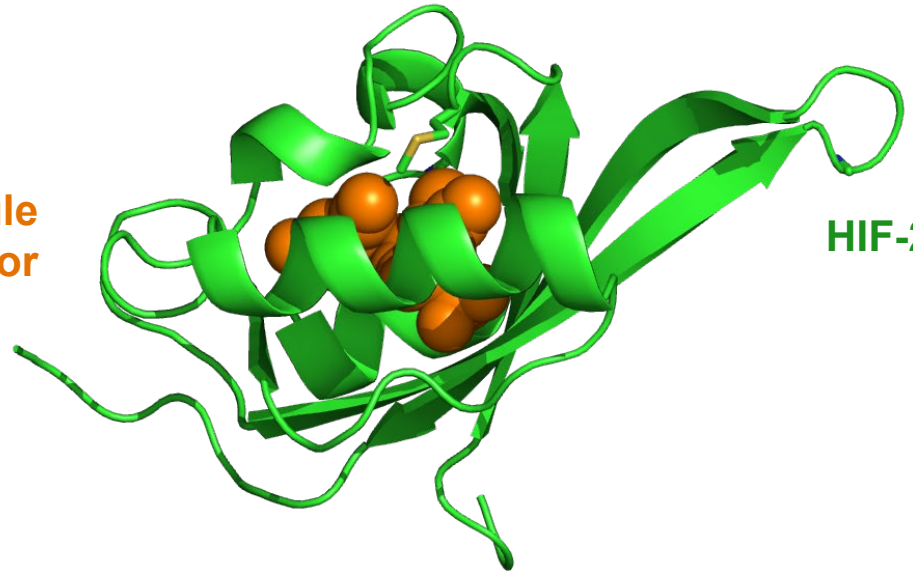
Scintillation Proximity Assay



Thermal Shift Assay



Small Molecule Series Predecessor



HIF-2α PAS-B

HIF-2α PAS-B Binding Assay (Units, Metric)	AB521	MK-6482[1]
Scintillation Proximity Assay[2] (nM, IC_{50} ± SD)	16.6 ± 5.0 (n=8)	22.3 ± 5.6 (n=5)
Thermal Shift Assay[2] (°C, ΔT_m ± SD)	14.7 ± 0.6 (n=14)	12.1 ± 0.3 (n=4)
Microscale Thermophoresis (nM, K_D ± SD)	2.4 ± 0.8 (n=3)	15.4 ± 2.7 (n=3)

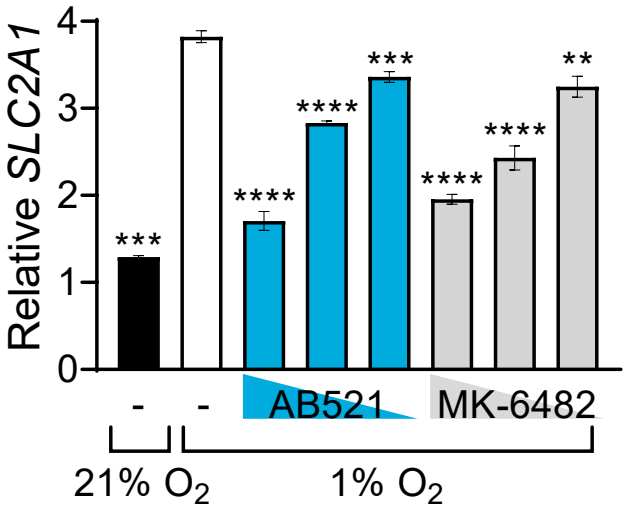
nM, nanomolar; IC_{50}, half-maximal inhibitory concentration; SD, Standard Deviation; n, number of assay replicates; °C, degrees Celsius; ΔT_m, change in melting point; K_D, equilibrium dissociation constant

[1]MK-6482 (PT2977, belzutifan, Welireg) synthesized by Arcus using methodology published in Xu *et al.* DOI: 10.1021/acs.jmedchem.9b00719

[2]Representative assay runs shown on left

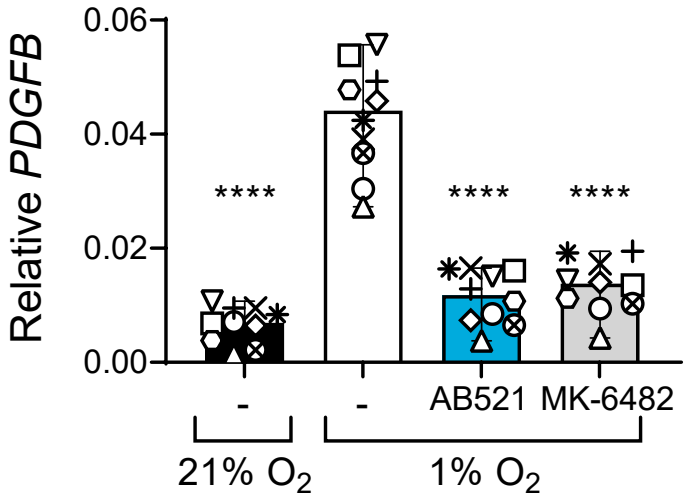
AB521 Selectively Inhibits Endogenous HIF-2α Dependent Transcription in Human Cells

HUVEC Endothelial Cells Pro-Angiogenic Transcription



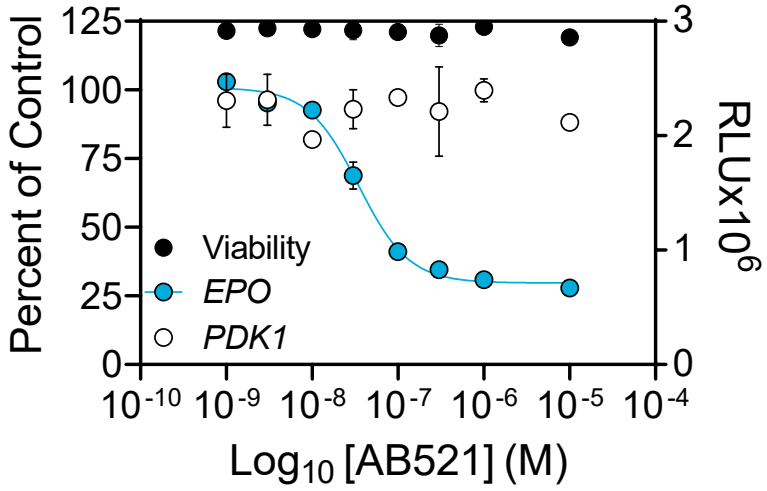
- Representative experiment (n=3); -, DMSO; 1.0, 0.03, 0.01 μM compound; Stats, ANOVA with multiple comparisons test for each group vs DMSO 1% O_2 group
- Similar results seen for *ADM*, *AKAP12*, *DLL4*, and *SLC2A3* genes

CD14⁺ Monocyte-Derived M2-Polarized Macrophages Pro-Tumorigenic Transcription



- Three experiments pooled, each symbol represents an individual donor (n=10); -, DMSO; 1.0 μM compound; Stats, ANOVA with multiple comparisons test for each group vs DMSO 1% O_2 group
- Similar results seen for *ADM* and *SERPINE1* genes

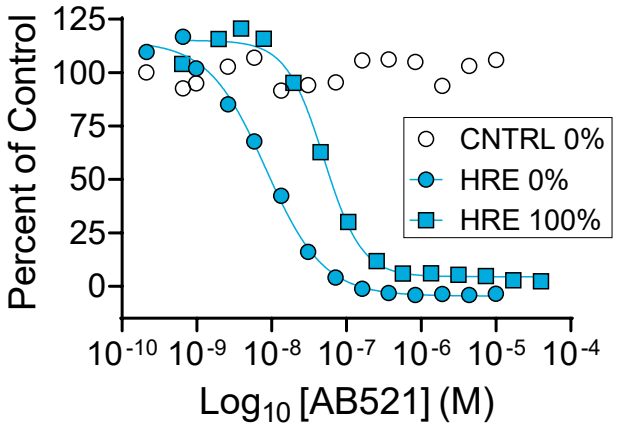
Hep3B Hepatocellular Carcinoma HIF-1α vs HIF-2α Gene Transcription



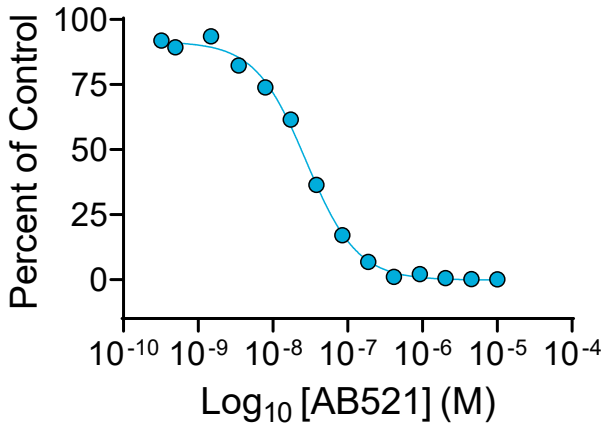
- Representative experiment (n=3)
- Right y-axis, Viability (CellTiter-Glo); RLU, luminescence
- Left y-axis, *EPO* (HIF-2α-specific) & *PDK1* (HIF-1α-specific) transcript
- Similar results seen for *SERPINE1* (HIF-2α-specific) and *PGK1* (HIF-1α-specific) genes

AB521 Inhibits Colony Formation and HIF-2α Activity in *VHL* mutated 786-O ccRCC Cells

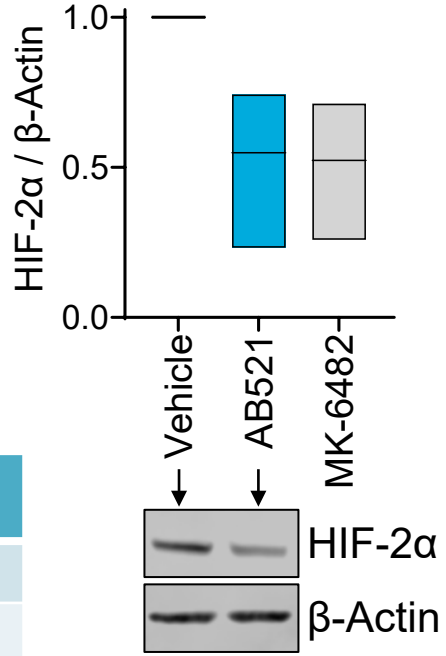
Luciferase Reporter



VEGF Secretion

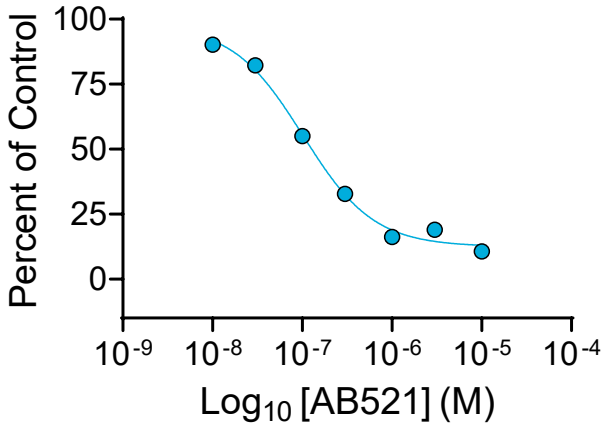


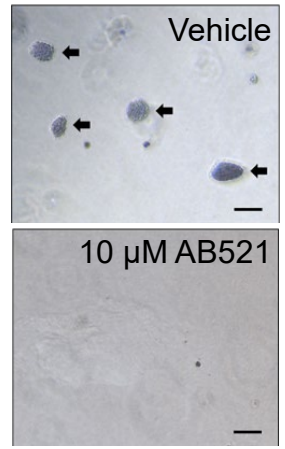
HIF-2α Protein Stability



- 2 μM compound for 16-24 hours
- Floating Bar, median ± range (n=3)
- Bottom, Representative Western blot

Soft Agar Colony Growth





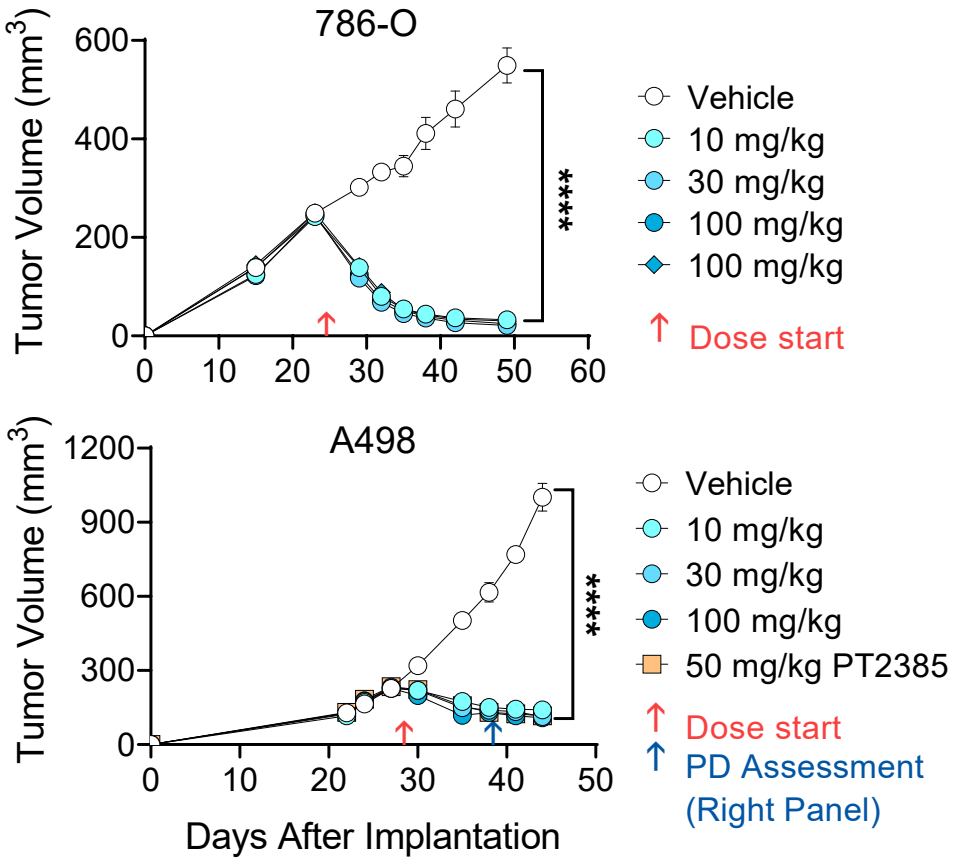
- Arrow, colony
- Bar, 100 μm

Assay[1]	AB521 IC_{50} nM ± SD	MK-6482 IC_{50} nM ± SD
HRE reporter 0%	8.21 ± 2.5 (n=24)	16.9 ± 10.1 (n=8)
HRE reporter 100%	46.5 ± 14.2 (n=24)	61.8 ± 6.6 (n=4)
CNTRL reporter 0%	> 10,000 (n=6)	> 10,000 (n=6)
VEGF secretion	28.9 ± 3.6 (n=11)	47.7 ± 30.8 (n=4)
Soft agar colony growth	51.2 ± 37.2 (n=5)	54.6 ± 45.2 (n=5)

IC_{50}, half-maximal inhibitory concentration; nM, nanomolar; SD, Standard Deviation; n, number of assay replicates; HRE, hypoxia response element reporter; CNTRL, control reporter; 0% or 100%, percentage human serum

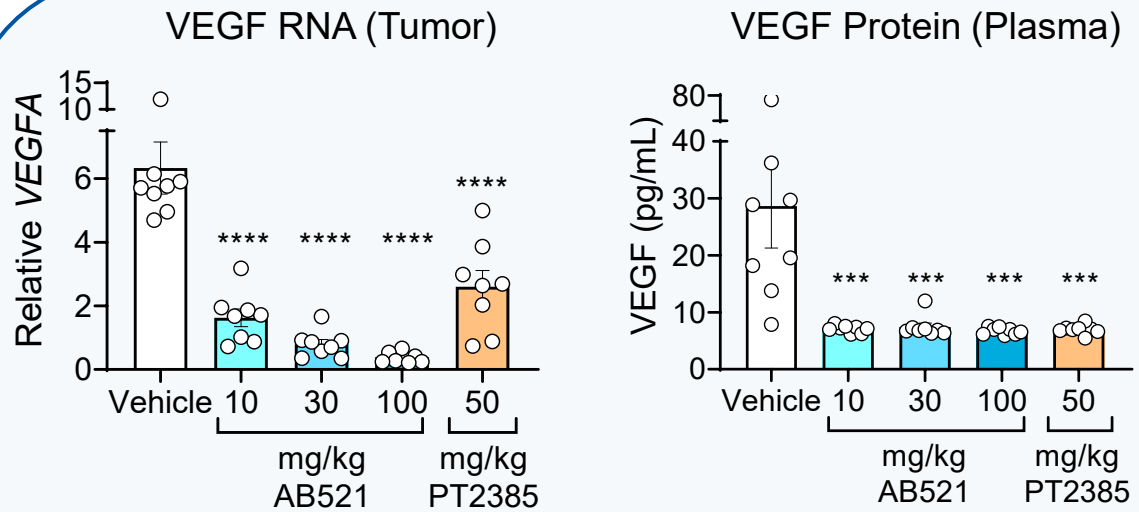
[1]Representative assay curves shown on top

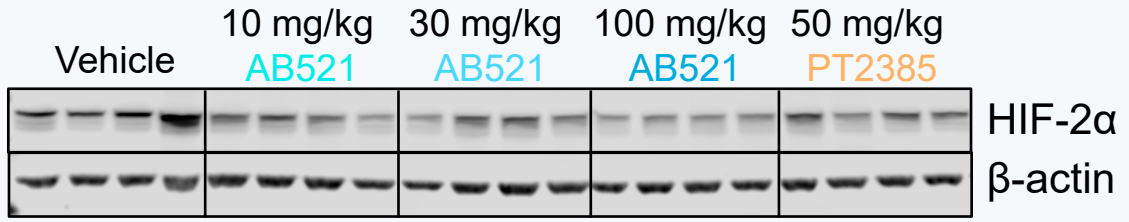
AB521 Inhibits Tumor Growth and HIF-2α Activity in ccRCC Tumor-Bearing Mice



- **Circles**, Vehicle or AB521 given orally twice-daily; **Diamond**, AB521 given orally once-daily; **Square**, PT2385 (synthesized by Arcus using methodology published in Wehn *et al.* DOI: 10.1021/acs.jmedchem.8b01196) given orally once-daily
- Efficacy (n=10) and PD data representative of two independent experiments; Stats, ANOVA with multiple comparisons test for each group vs Vehicle

10 Days After Treatment PD Assessment in A498 Model





- Top, each symbol represents an individual mouse
- Bottom, Western blot; Each lane contains tumor lysate from an individual mouse

AB521 is Expected To Be Suitable For Once-daily Oral Dosing in Humans

Pharmacokinetic Properties of AB521						
Species	Hepatocytes		*in vivo*			
	CL_{int} (µL/min/10^6 cells)	$T_{1/2}$ (h)	CL (L/h/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	F (%)
Mouse[1]	2.7	10.8	1.22	2.2	1.4	ND
Rat[1]	2.8	10.3	**0.91**	2.3	2.2	51
Dog[1]	**<0.7**	>40	**0.05**	1.1	16	75
Human[2]	**<0.7**	>40	*0.012 (projected)*	*0.86 (projected)*	*50 (projected)*	*60 (projected)*

[1]IV dosage: 0.25 mg/kg (mouse and rat), 0.33 mg/kg (dog)
[2]Projected human *in vivo* PK parameters determined by allometry (mouse, rat, and dog)
CL_{int}, intrinsic clearance; $T_{1/2}$, elimination half-life; CL, total body clearance; V_{ss}, steady-state volume of distribution determined by Øie-Tozer method; F, oral bioavailability; µL, microliter; min, minute; h, hour; L, liter; kg, kilogram; ND, not determined

CYP Isoform IC_{50} (µM)[1]				
2C8	2C9	2C19	2D6	3A4
>100	60.6	>100	>100	>100

[1]Inhibition human drug metabolizing enzymes of the cytochrome P450 family (CYP)
IC_{50}, half-maximal inhibitory concentration; µM, micromolar

- AB521 exhibited a favorable *in vitro* profile with low intrinsic clearance in dog and human hepatocytes and moderate-to-low clearance in rat and dog
- AB521 exhibited negligible inhibition against a panel of CYP isoforms and no time-dependent CYP inhibition (not shown)

AB521 is a Novel and Selective HIF-2α Inhibitor That is Ready For Clinical Evaluation

- AB521 binds the HIF-2α PAS-B domain with a high affinity
- AB521 potently inhibited 1) HIF-2α-specific reporter activity under no and high-serum conditions, 2) VEGF protein secretion, 3) colony formation in soft agar, and did not exhibit off-target cytotoxicity in 786-O cells *in vitro*
- AB521 selectively inhibited HIF-2α-, but not HIF-1α-, mediated gene expression in hypoxic Hep3B cells
- AB521 inhibited the transcriptional activity of endogenous HIF-2α in primary human cells, including angiogenic endothelial cells and pro-tumorigenic M2-polarized macrophages
- When delivered orally in mice, AB521 regressed established 786-O and A498 ccRCC xenograft tumors and decreased pharmacodynamic markers associated with HIF-2α in a statistically significant manner
- AB521 has a favorable preclinical PK profile and is projected to be suitable for once-daily oral dosing in humans
- Inhibiting HIF-2α did not impact functionality of activated hypoxic human T cells, and gene signature score correlations across the cancer genome atlas (TCGA) suggest that AB521 may be a favorable combination partner for I-O therapeutic agents[1]
- Clinical evaluation of AB521 is expected to begin in the latter part of 2021

[1]https://arcusbio.com/publications/: Gauthier KES (2019) AACR-NCI-EORTC, Piovesan D (2020) Keystone Symposia (Hypoxia: Molecules, Mechanisms, & Disease), Piovesan D (2020) SITC