Discovery and Characterization of AB521, a Clinical-Stage, Potent, and Selective Hypoxia-Inducible Factor (HIF)-2α Inhibitor

Ken Lawson, Artur Mailyan, Guillaume Mata, Joel W Beatty, Samuel L Drew, Jeremy T A Fournier, Jaroslaw Kalisiak, Anh T Tran, Kai Yu, Brandon R Rosen, Clayton Hardman, Matthew Epplin, Kelsey E Sivick, Dana Piovesan, Suan Liu, Lixia Jin, Elaine Ginn, Cesar A Meleza, Lisa Seitz, Tzuling Cheng, Amber Pham, Mohammad Ghasemi, Elaine Paterson, Paul G Foster, Matthew J Walters, Manmohan Leleti, Jay P Powers

ACS National Meeting, Denver

August 21st, 2024

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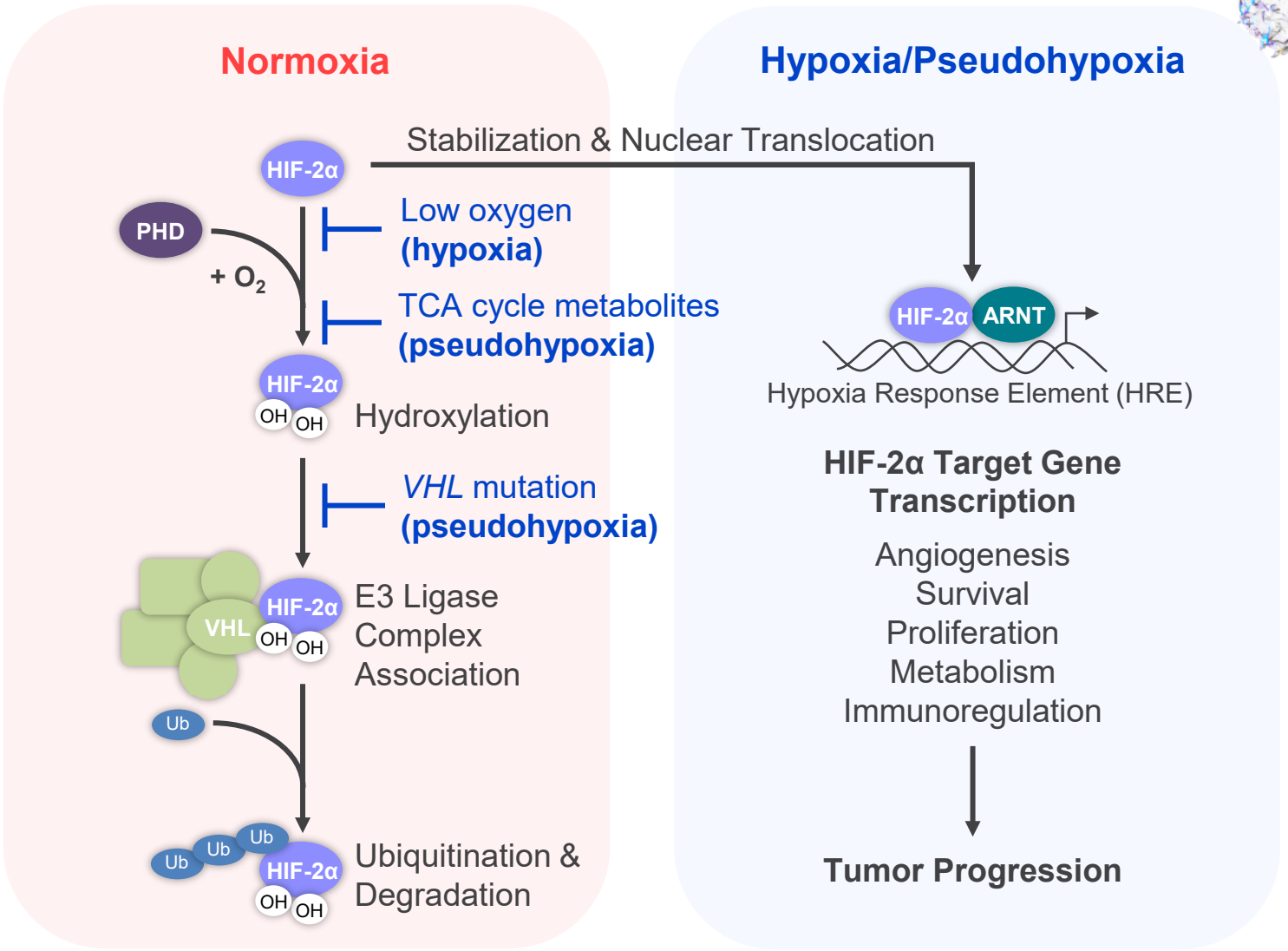
HIF-2α Drives Physiological Changes to Adapt to Low Oxygen that are Hijacked by the Tumor

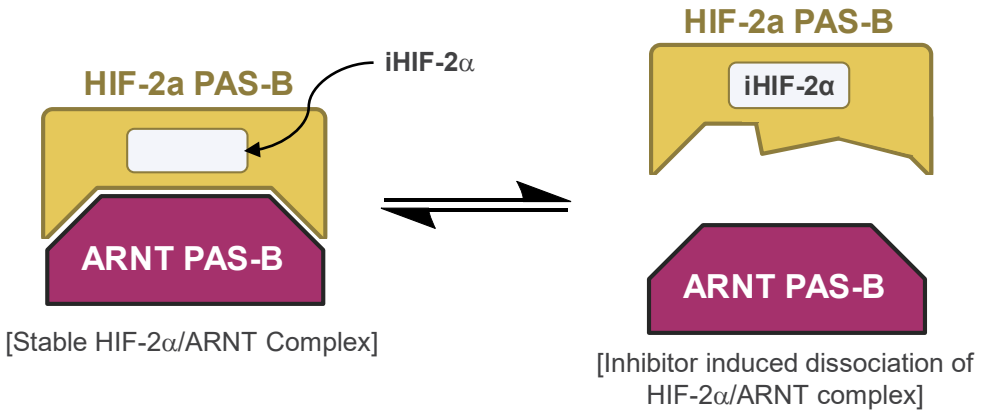
- **HIF-2α is a heterodimeric transcription factor**
  - **α subunit (HIF-2α)**: intricate post-translational regulation of protein levels
  - **β subunit (ARNT/HIF-1β)**: stable expression

- **HIF-2α mediates transcription of genes that promote tumor progression**



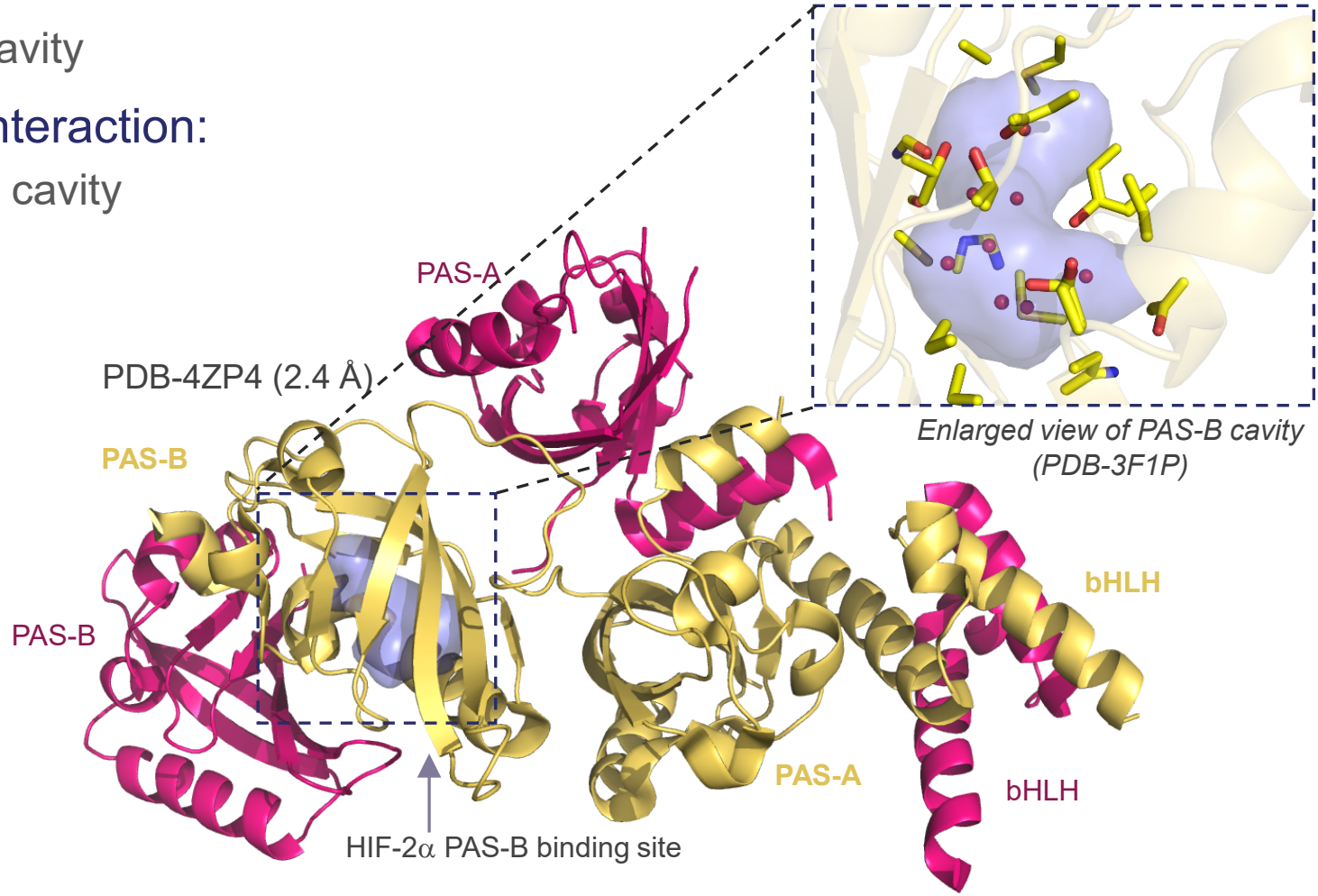
Fundamentals of Targeting the HIF-2α/ARNT Complex

- X-ray crystal structure shows internal hydrophobic cavity (~290 Å^3) with 8 water molecules[1]
  - HIF-1α lacks analogous hydrophobic cavity
- Basis for regulation of protein-protein interaction:
  - Small molecule binds to HIF-2α PAS-B cavity
    - → conformational change
  - HIF dimerization disrupted
    - → gene transcription inactive



[Stable HIF-2α/ARNT Complex]

[Inhibitor induced dissociation of HIF-2α/ARNT complex]



[1]*Proc. Natl. Acad. Sci. USA*, **2009**, 106: 450-455

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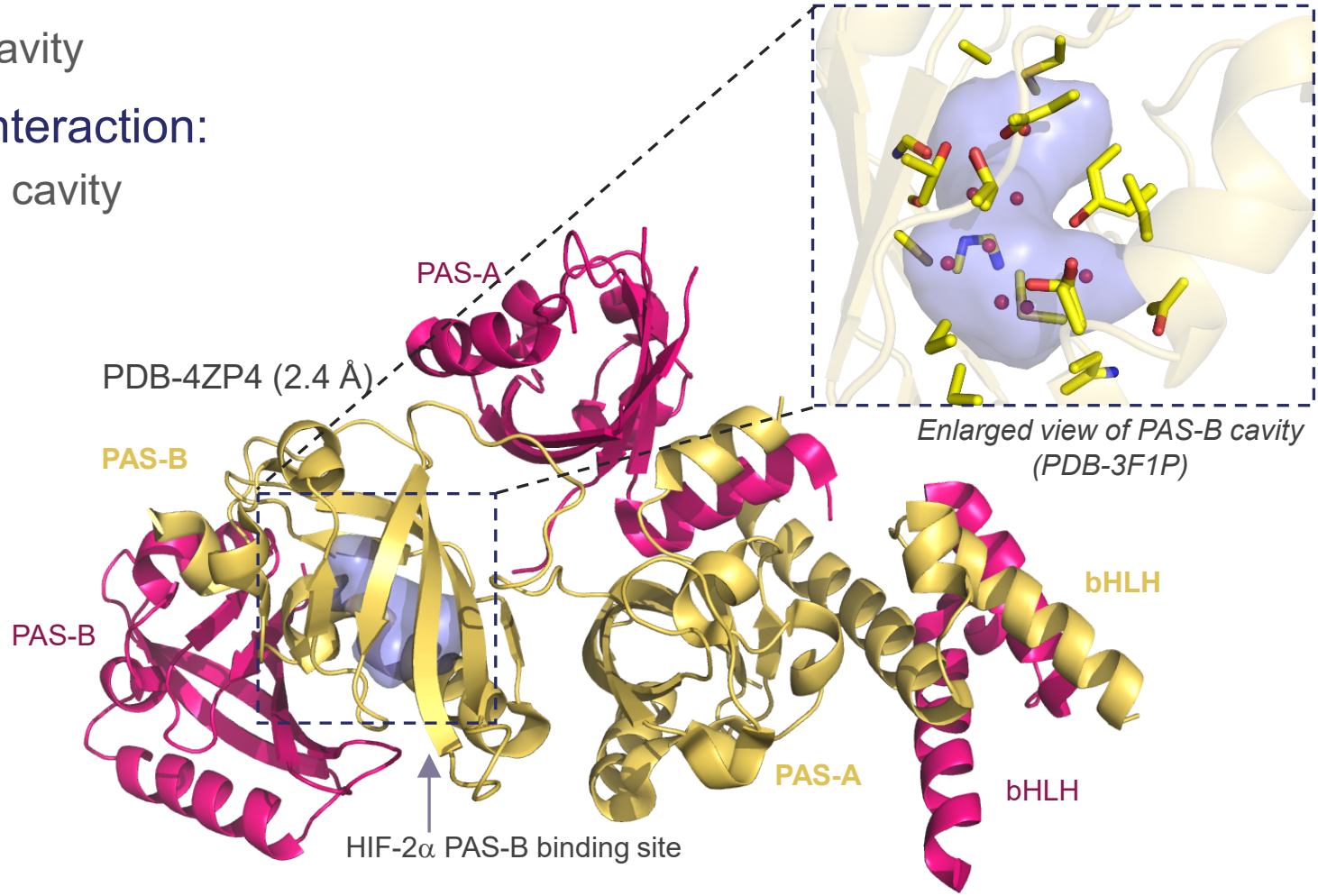
- Basis for regulation of protein-protein interaction:
  - Small molecule binds to HIF-2α PAS-B cavity
    → conformational change
  - HIF dimerization disrupted
    → gene transcription inactive
- Design challenges:
  - Small internal pocket limits ligand size
  - Binding affinity may not correlate with functional activity
  - High affinity ligands often possess undesirable physicochemical properties (high lipophilicity)



[1]*Proc. Natl. Acad. Sci. USA*, **2009**, 106: 450-455

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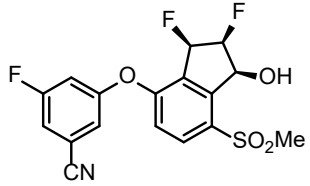
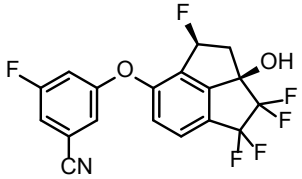
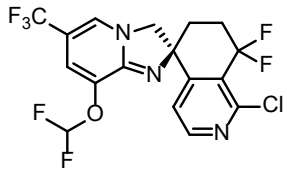
Status and Characterization of Clinical HIF-2α Inhibitors

- HIF-2α inhibition (Belzutifan) has demonstrated significant clinical activity in patients with advanced ccRCC[1]

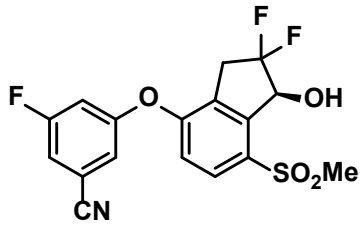
Assay	belzutifan (Peloton/Merck) 	NKT2152[a] (NiKang) 	compound A[b] (Novartis) 
ccRCC Clinical Trial Status	**Phase 3[c]**	**Phase 1/2**	**Discontinued** *(Phase 1/1b)*
HIF-2α Reporter Gene Assay[d] IC_{50} (nM)	17 ± 10 (n = 8)	5.3 ± 1.4 (n = 3)	19 ± 8.7 (n = 9)
Reporter Control[d] IC_{50} (nM)	> 10,000	> 10,000	> 10,000
HIF-2α 786-O Luc. 100% Serum IC_{50} (nM)	62 ± 6.6 (n = 4)	120 ± 13 (n = 2)	270 ± 73 (n = 9)

[a]Prepared according to WO2022086822. [b]Prepared according to WO2021220170, compound A. [c]Belzutifan is approved for treatment of VHL disease and advanced metastatic ccRCC. [d]786-O renal adenocarcinoma cells (mutant for VHL and HIF-1α) stably expressing HIF or control CMV luciferase (Luc) reporter constructs

[1]L. Albiges, et al (2023) ESMO, abstract #LBA88 (*Annals of Oncology*, **2024**, 34, S1329)

Design and Discovery of Arcus HIF-2α Inhibitors

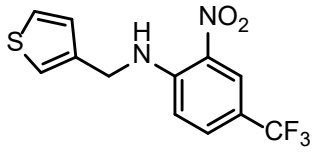
- Pharmacophore mapping and structure-aided design approach toward novel starting points



PT2385[1]

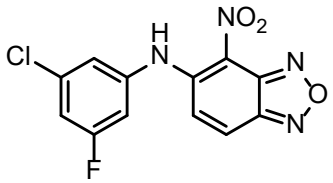
HIF-2α Cell-Based IC_{50} = 27.4 nM

[Peloton's 1st-generation HIF-2a inhibitor]



THS-017[2]

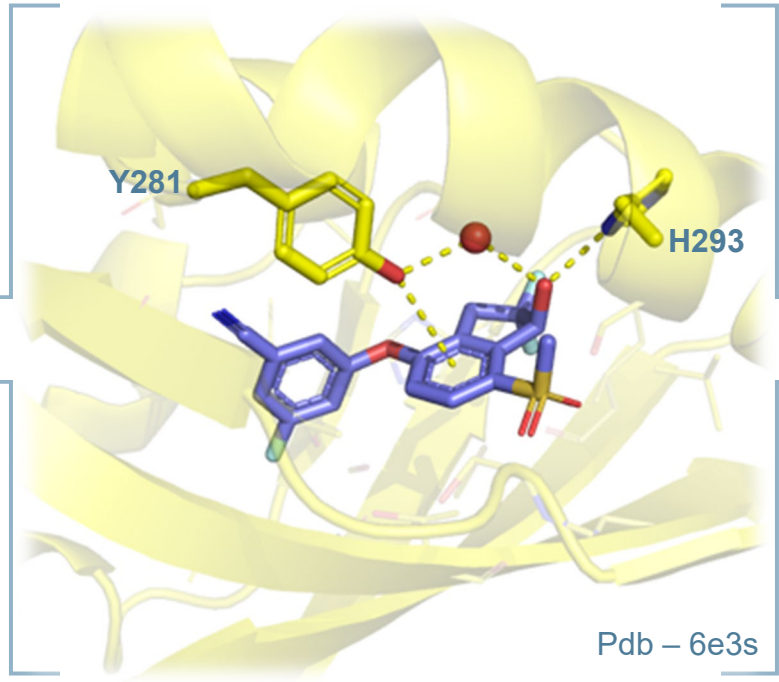
HIF-2α IC_{50} = 10,500 nM



'Compound 32'[3]

HIF-2α IC_{50} = 340 nM

PT2385 bound to HIF-2α PAS-B domain[1]

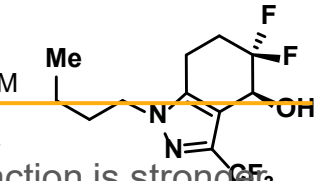


Pdb – 6e3s

Precedented HIF-2α PAS-B/inhibitor binding interactions

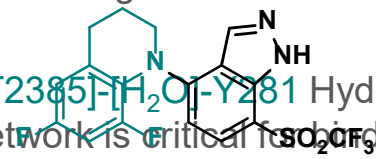
Tetrahydro-indazole Series

HIF-2α Cell-Based IC_{50} = 3,850 nM



[ACS Spring 2024 Natl. Meeting]

- *Y281 n → π** - Interaction is stronger when aromatic ring is electron deficient[4]

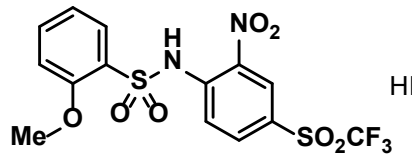


- H293-[PT2385]-[H_2O]-Y281 Hydrogen bonding network is critical for binding

Tetrahydroquinoline Series

HIF-2α Cell-Based IC_{50} = 3,850 nM

- Western arene – Hydrophobic/non-specific interactions



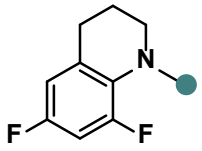
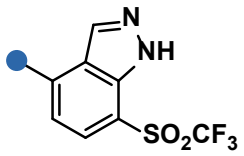
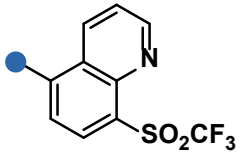
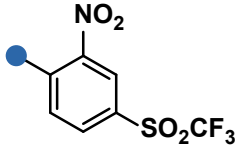
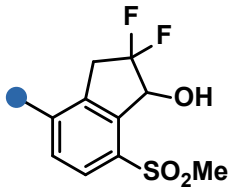
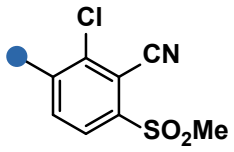
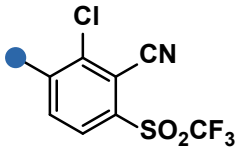
Sulfonamide Series

HIF-2α Cell-Based IC_{50} = 1,040 nM

[1]*J. Med. Chem.* **2018**, 61, 21, 9691–9721; [2]J. Am. Chem. Soc. **2009**, 131, 17649; [3]J. Am. Chem. Soc. **2013**, 56, 1739; [4]*Phys. Chem. Chem. Phys.*, **2015**, 9596

Early Tetrahydroquinoline Series SAR Demonstrates Reproducible HIF-2α Inhibition

- Initial SAR demonstrates dependence on electrostatic n → π^*_{Ar} interaction facilitated by electron deficient eastern ring
  - Incorporation of indanol motif does not improve potency

						
Compound ID	**1**	**2**	**3**	**4**	**5**	**6**
HIF-2α Biochemical IC_{50} (nM)	12,700	2,130	1,280	5,540	2,370	340
HIF-2α Cell-Based (nM)	9,350	5,820	3,850	2,890	1,630	1,560

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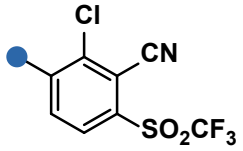
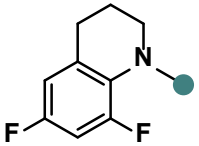
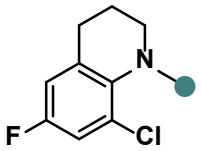
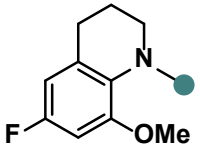
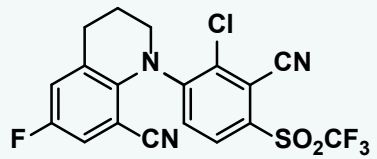
Optimization of Tetrahydroquinoline Substitution Reveals Path to Potent HIF-2α Inhibitors

- SAR of tetrahydroquinoline was found to be very sharp, with 6,8-substitution strongly preferred

				
Compound ID	**6**	**7**	**8**
HIF-2α Biochemical IC_{50} (nM)	340	470	5,010
HIF-2α Cell-Based (nM)	1,560	1,570	>10,000

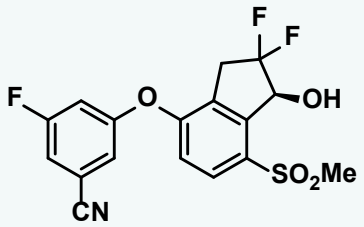
Comparison of Compound 9 and PT2385 Highlight Divergent Binding Interactions

- Superposition of PT2385 with Compound 9 show distinct binding interaction, exemplified by binding pose of western aromatic motifs



Compound 9

HIF-2α Cell-Based IC_{50} = 61 nM



PT2385[1]

HIF-2α Cell-Based IC_{50} = 27.4 nM

[Peloton's 1st-generation HIF-2α inhibitor]

[1] *J. Med. Chem.* **2018**, 61, 21, 9691–9721

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X-Ray Co-crystal Structure of Compound 9 Bound to HIF-2α/ARNT Complex

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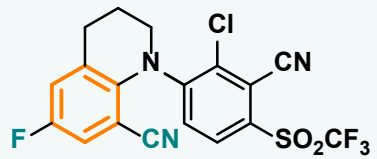

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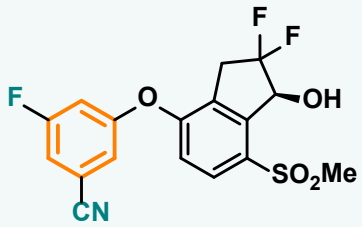

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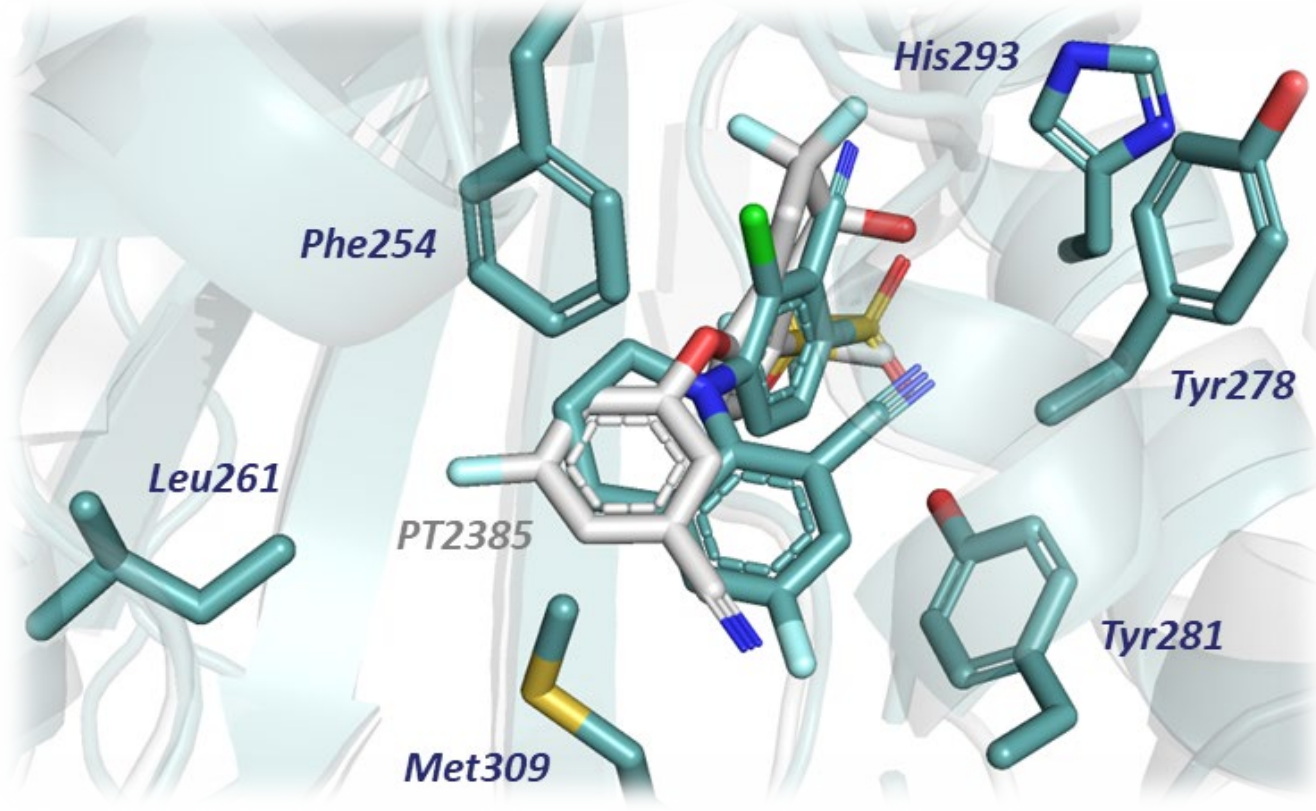

PT2385 from PDB ID: 5TBM

[1] *J. Med. Chem.* **2018**, 61, 21, 9691–9721

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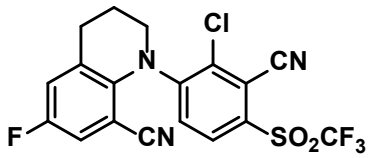
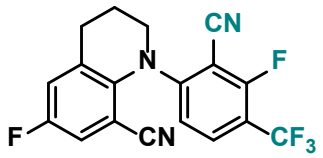
Continued Optimization of THQ Scaffold is Guided by Poor Potency in Serum and Unfavorable ADME Properties

- Metabolite ID studies identified multiple sites of oxidative metabolism





Compound ID	9	13
HIF-2α Biochemical IC_{50} (nM)	64	173
HIF-2α Cell-Based (nM)	61	144
HIF-2α Cell-Based 100% Serum IC_{50} (nM)	14,600	20,200
Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat	28 / 36	76 / 240

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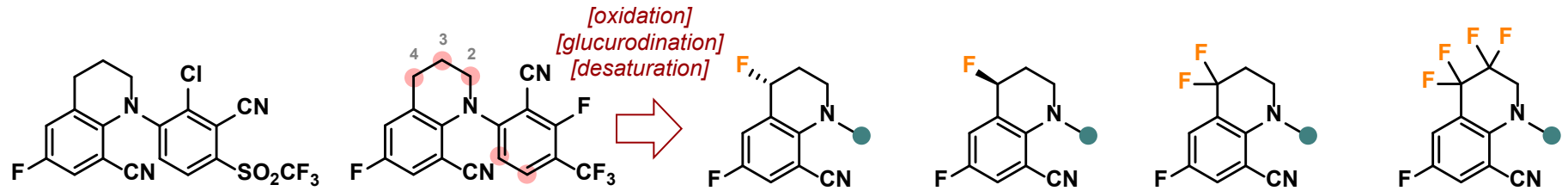
Continued Optimization of THQ Scaffold is Guided by Poor Potency in Serum and Unfavorable ADME Properties

- Metabolite ID studies identified multiple sites of oxidative metabolism
  - Extensive fluorination of THQ scaffold improved *in vitro* hepatocyte stability



Compound ID	9	13	14	15	16	17
HIF-2α Biochemical IC_{50} (nM)	64	173	747	10.2	21.1	366
HIF-2α Cell-Based (nM)	61	144	1,025	34.7	14.4	490
HIF-2α Cell-Based 100% Serum IC_{50} (nM)	14,600	20,200	> 28,000	**590**	1,520	> 37,000
Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat	28 / 36	76 / 240	290 / 150	24 / 320	7.2 / 41	**< 2.7** / 23

- Fluorination of 4-position significantly improved serum potency in stereospecific manner

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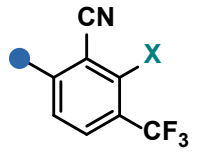
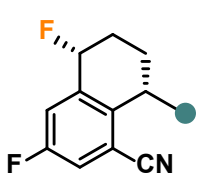
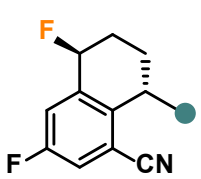
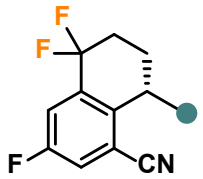
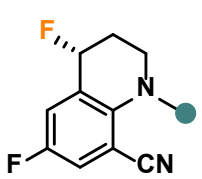
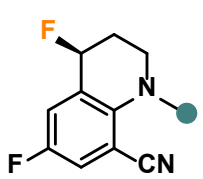
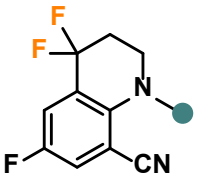
Discovery of Tetralin Series Offers Path Towards Novel HIF-2α Inhibitors

- THQ-series SAR translates well to tetralin scaffold, demonstrating continued reliance on 4-β-fluoride substitution for robust HIF-2α inhibition in physiologically relevant media



	Tetralin series X = Cl			*THQ series* X = F		
						
Compound ID	**18**	**19**	**20**	**14**	**15**	**16**
HIF-2α Biochemical IC_{50} (nM)	> 10,000	50.5	180	747	10.2	21.1
HIF-2α Cell-Based (nM)	1,410	16.3	53	1,025	34.7	14.4
HIF-2α Cell-Based 100% Serum IC_{50} (nM)	> 10,000	**638**	> 10,000	> 28,000	**590**	1,520
Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat	-	15 / 28	<2.7 / 3.5	290 / 150	24 / 320	7.2 / 41

See poster board #528 this evening (7:00-9:00PM) in general poster session for detailed THQ-series SAR

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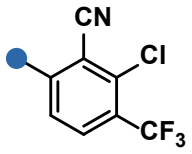
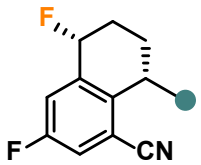
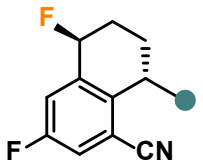
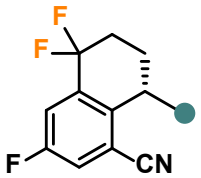
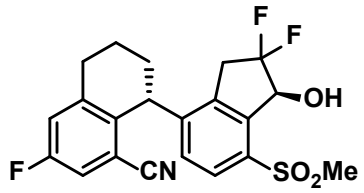
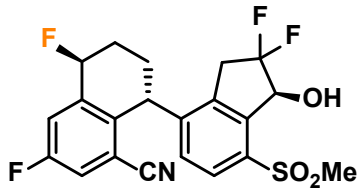
Discovery of Tetralin Series Offers Path Towards Novel HIF-2α Inhibitors

- THQ-series SAR translates well to tetralin scaffold, demonstrating continued reliance on 4-β-fluoride substitution for robust HIF-2α inhibition in physiologically relevant media

***Tetralin** series*

						
Compound ID	**18**	**19**	**20**	**21**	**22**
HIF-2α Biochemical IC_{50} (nM)	> 10,000	50.5	180	42.2	13.5
HIF-2α Cell-Based (nM)	1,410	16.3	53	56.3	4.13
HIF-2α Cell-Based 100% Serum IC_{50} (nM)	> 10,000	**638**	> 10,000	1,630	**48.1**
Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat	-	15 / 28	<2.7 / 3.5	45 / 82	2.9 / 10

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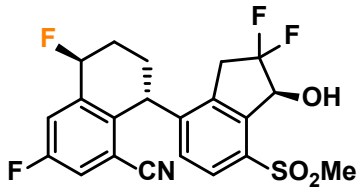
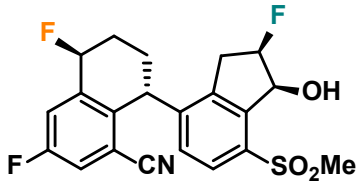
Tetralin Series Inhibitors Offer Favorable Potency and DMPK Properties

- Stereospecific fluorination of the tetralin C3-homobenzylic position further improves serum potency and metabolic stability





Compound ID	22	23
HIF-2α Cell-Based (nM)	4.13	12.1
HIF-2α Cell-Based 100% Serum IC_{50} (nM)	48.1	108
Hepatocyte CL_{int} ($\mu L/min/10^6$ cells) hu / rat	2.9 / 10	< 2.7 / 10
CYP Inh. IC_{50} (μM) 2C8 / 2C9 / 2C19 / 2D6 / 3A4	>40 / 4.5 / 12 / >40 / 35	>40 / 36 / 13 / >40 / >40
Rat PK Parameters: CL (L/h/kg) / %F	1.0 / 93%	1.9 / 80%

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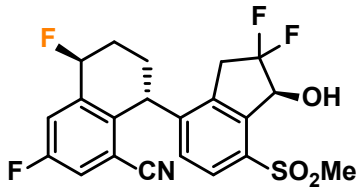
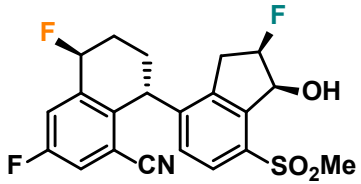
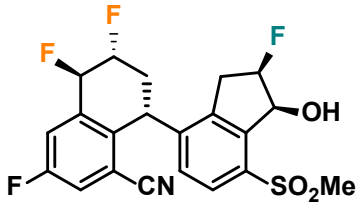
Casdatifan (AB521) Potently Inhibits HIF-2α-Mediated Gene Transcription and Exhibits a Favorable DMPK Profile

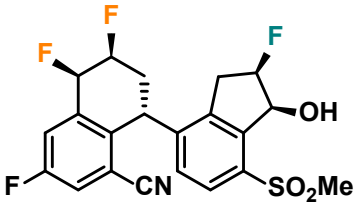
- Stereospecific fluorination of the tetralin C3-homobenzylic position further improves serum potency and metabolic stability
  - SAR efforts culminate with systematic evaluation of fluorination pattern







Casdatifan



Compound ID	22	23	24	AB521
HIF-2α Cell-Based (nM)	4.13	12.1	2,670	**8.2**
HIF-2α Cell-Based 100% Serum IC_{50} (nM)	48.1	108	> 40,000	**46.5**
Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat	2.9 / 10	< 2.7 / 10	-	**< 2.7 / < 2.7**
CYP Inh. IC_{50} (μM) 2C8 / 2C9 / 2C19 / 2D6 / 3A4	>40 / 4.5 / 12 / >40 / 35	>40 / 36 / 13 / >40 / >40	-	**>40 / 36 / 13 / >40 / >40**
Rat PK Parameters: CL (L/h/kg) / %F	1.0 / 93%	1.9 / 80%	-	**0.91 / 51%**

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Casdatifan Exhibits a Favorable DMPK Profile in Preclinical Species

- Casdatifan exhibited minimal DDI potential and was projected to be suitable for once-daily oral dosing in humans

	Hepatocytes		*In vivo*			*CYP Inhibition and Safety*	
Species	**CL_{int}** ($\mu L/min/10^6$ cells)	**$T_{1/2}$** (h)	**CL** (L/h/kg)	**Vss** (L/kg)	**$T_{1/2}$** (h)	**Assay**	**Casdatifan**
Mouse	2.7	10.8	1.22	2.2	1.4	CYP IC_{50} (μM) 2C19 / 2C8 / 2C9 / 2D6 / 3A4	>100 / >100 / 60.6 / >100 / >100
Rat	2.8	10.3	0.91	2.3	2.2	CYP TDI (% Activity loss, 30 min) 3A4 / 2C8 / 2C9 / 2D6	9.4 / 7.9 / 2.9 / 1.3
Dog	<0.7	>40	0.05	1.1	16	hERG (automatic patch clamp)	IC_{50} > 10 μM
Human	<0.7	>40	-	-	-	CEREP Safety Panel	No Findings

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).

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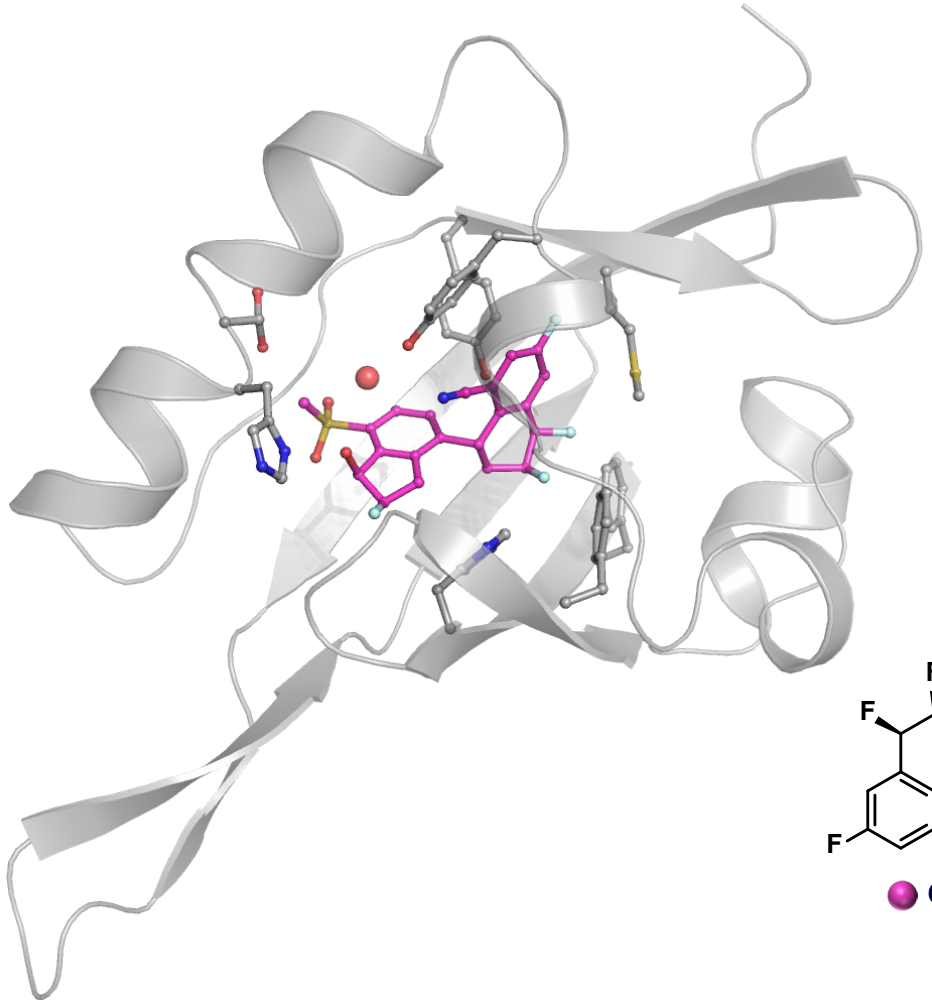
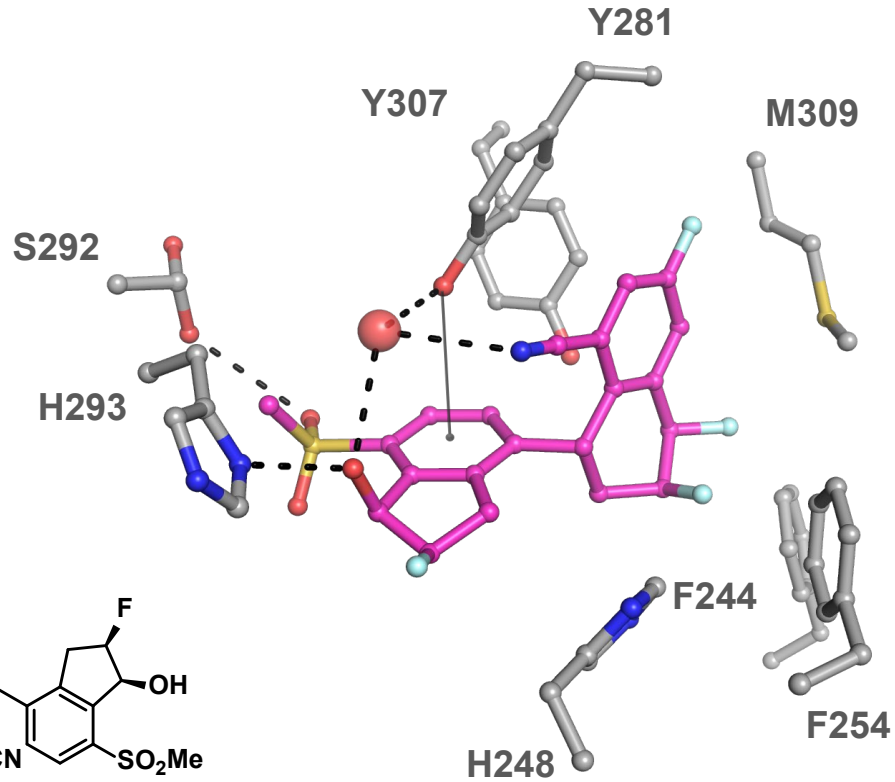
Casdatifan Avidly Binds the HIF-2α/ARNT Complex

- Casdatifan bound to HIF-α/ARNT complex (1.9 Å resolution)





Casdatifan
[AB521]

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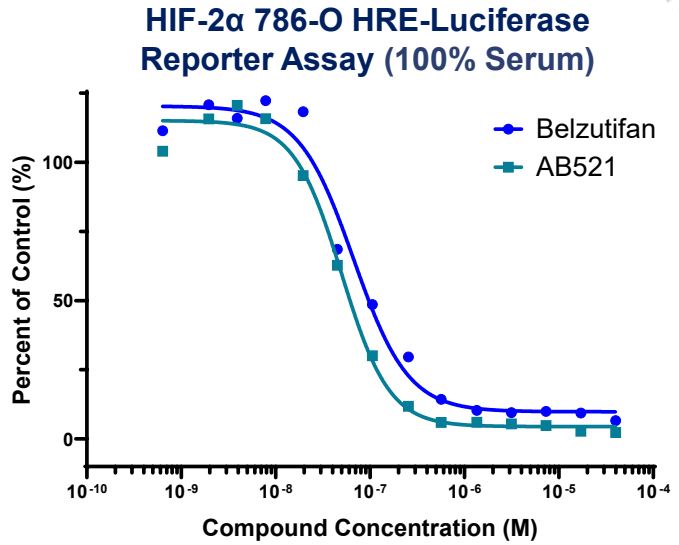
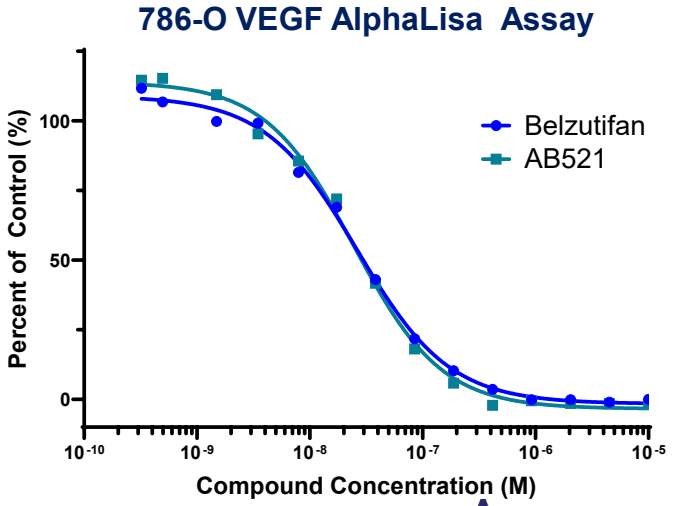
Casdatifan is a Potent and Selective HIF-2α Inhibitor *in vitro*

- Cas strongly binds the PAS-B domain and inhibits HIF-2α function *in vitro*
  - Cas inhibits HIF-2α, but not HIF-1α, mediated transcription

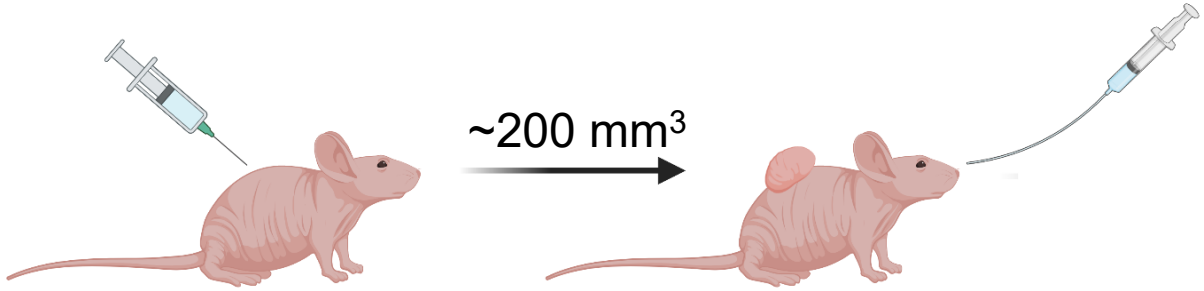
	SAR Assay	**casdatifan** (mean ± SD)	**belzutifan** (mean ± SD)
Cell-based assays	786-O HRE Luc Reporter IC_{50} (nM)	8.2 ± 2.5	16.9 ± 10.1
	786-O HRE Luc Reporter IC_{50} [100% Serum] (nM)	46.5 ± 14.2	61.8 ± 6.6
	786-O Control Luc Reporter IC_{50} (nM)	>10,000	>10,000
	786-O VEGF-A Secretion IC_{50} (nM)	28.9 ± 3.6	47.7 ± 30.8
	Hep3B *EPO* (HIF-2α-specific) Transcript IC_{50} (nM)	35.9 ± 5.0	39.0 ± 9.7
	Hep3B *PDK1* (HIF-1α-specific) Transcript IC_{50} (nM)	>10,000	>10,000
Binding assays	Thermal Shift Assay ΔT_M (°C)	14.7 ± 0.6	12.1 ± 0.3
	MicroScale Thermophoresis K_D (nM)	2.4 ± 0.8	15.4 ± 2.7
	Isothermal Titration K_D (nM)	53.6 ± 17.9	58.3 ± 19.3
	Scintillation Proximity Assay IC_{50} (nM)	16.6 ± 5.0	22.3 ± 5.6





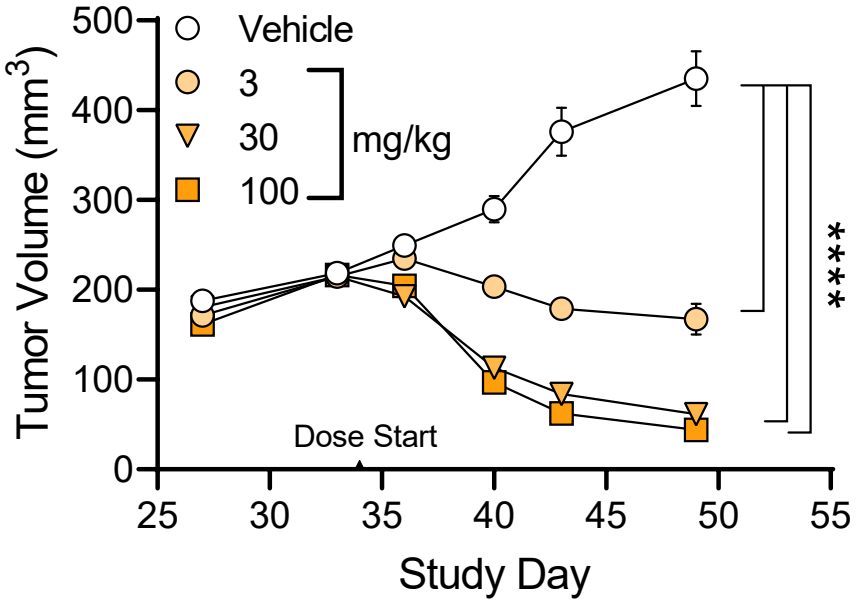
Dose-dependent Tumor Control is Exacted by AB521 in *VHL*-mutated ccRCC Xenograft Models

Implant nude mice with 786-O and A-498 human ccRCC tumors

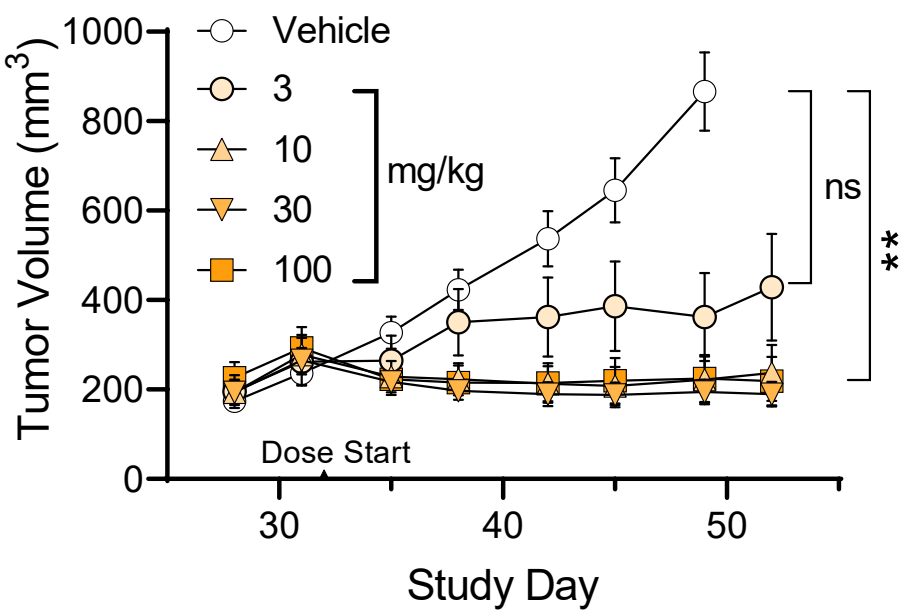


Daily oral gavage with **AB521**

786-O



A-498



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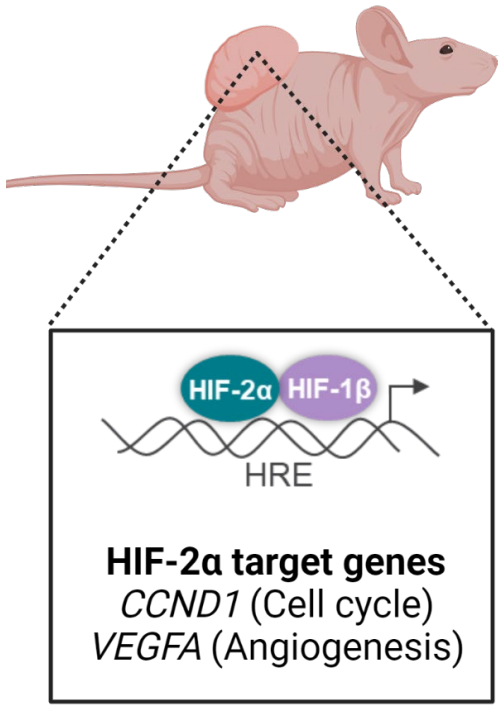
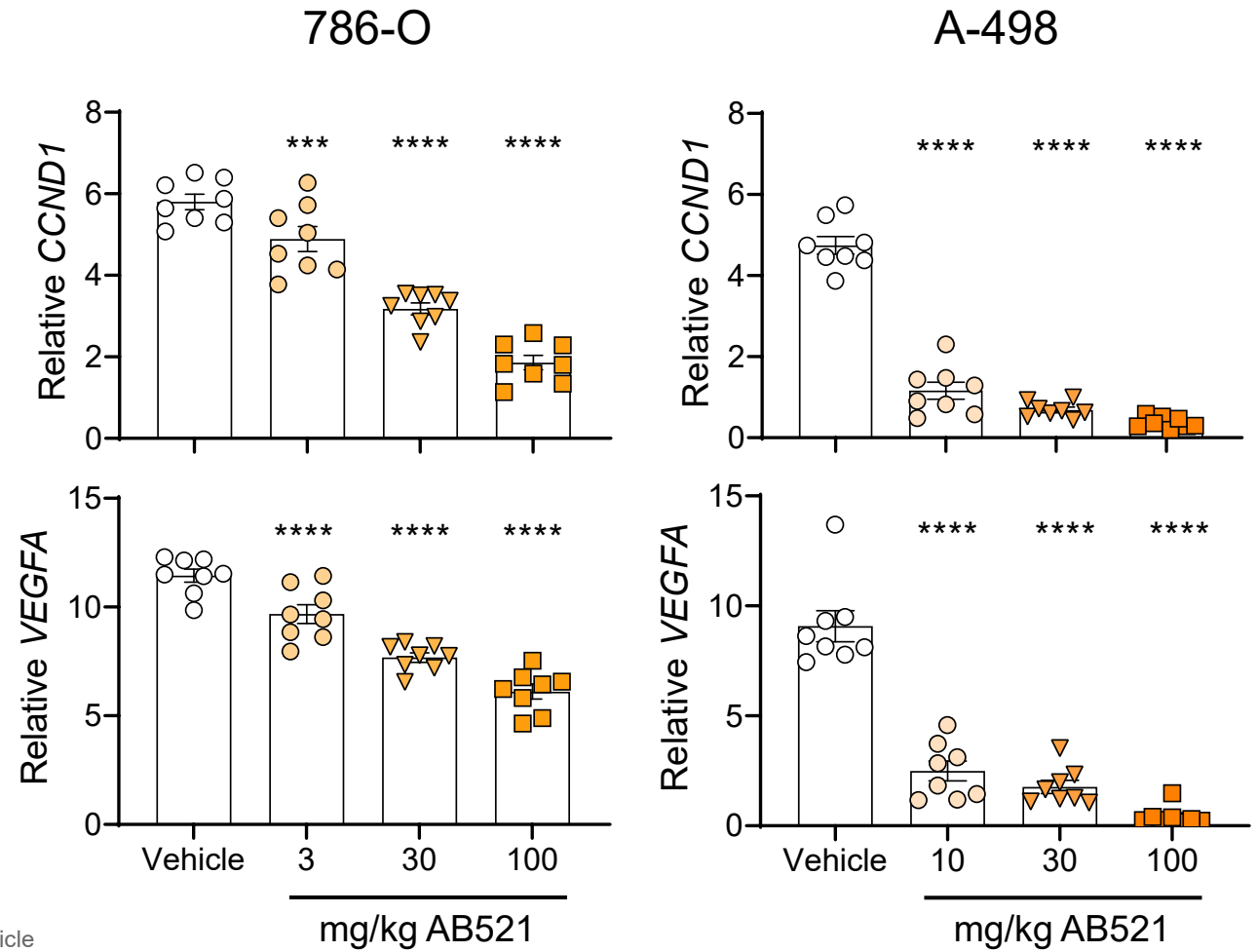
Pharmacodynamics: Dose-dependent Decrease in HIF-2α Targets by AB521 in the Tumor

Tumor Tissue
(24 hours after single dose)

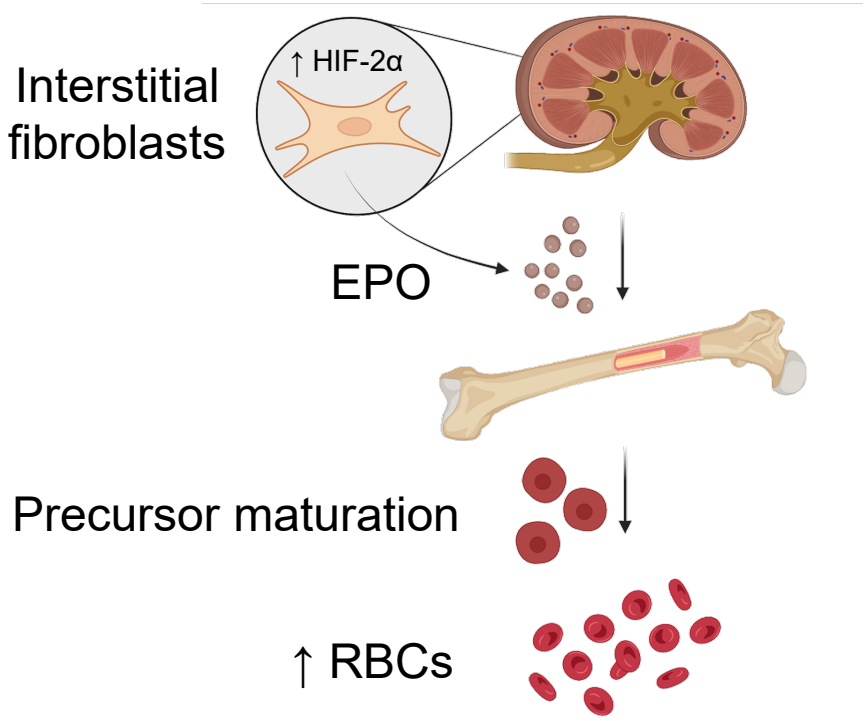


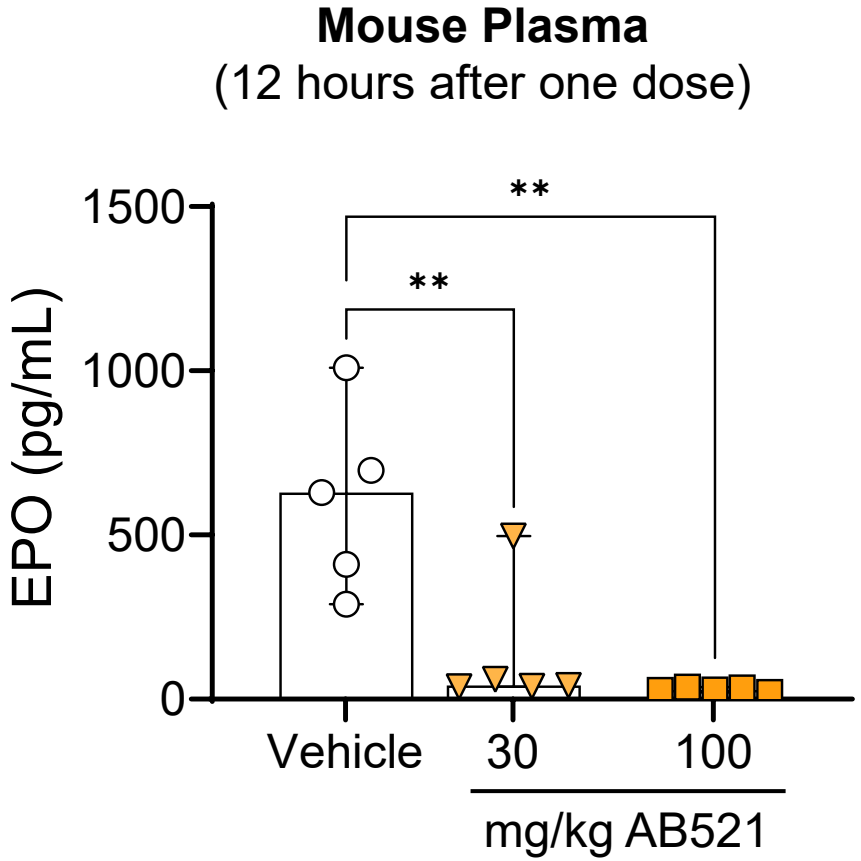


Statistics vs Vehicle

Erythropoietin (EPO) is a Useful Peripheral Biomarker to Assess HIF-2α Inhibition

- Erythropoietin (EPO) is a secreted hormone essential for red blood cell production

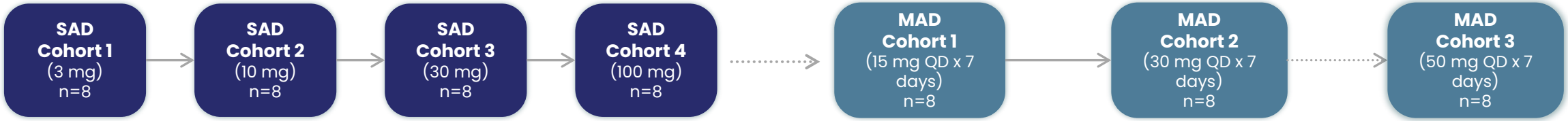




ARC-14 Phase 1 Study of AB521 in Healthy Volunteers

SAD (single ascending dose)

MAD (multiple ascending dose)



SAD and MAD

- Cohorts of 8 subjects, randomized 3:1 AB521:placebo
- Evaluate safety and PK for single/multiple ascending doses of AB521
- PK/PD modeling based on exposure and changes in erythropoietin, Hgb levels

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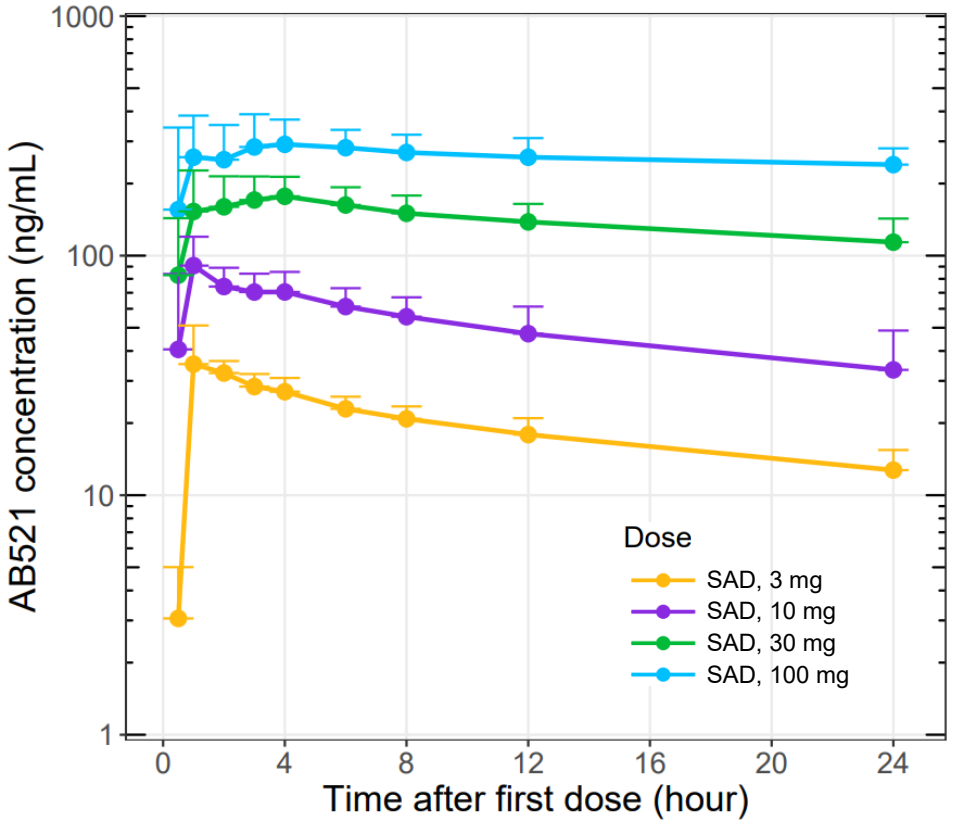
Pharmacokinetic and Pharmacodynamic Parameters Associated with Cas in Human Healthy Volunteers

- Mean apparent terminal half-life is 18 to 24 hours, supporting once-daily (QD) dosing
- The peak-to-trough ratio is low (~2) over 24 hours

Single ascending dose (SAD) PK



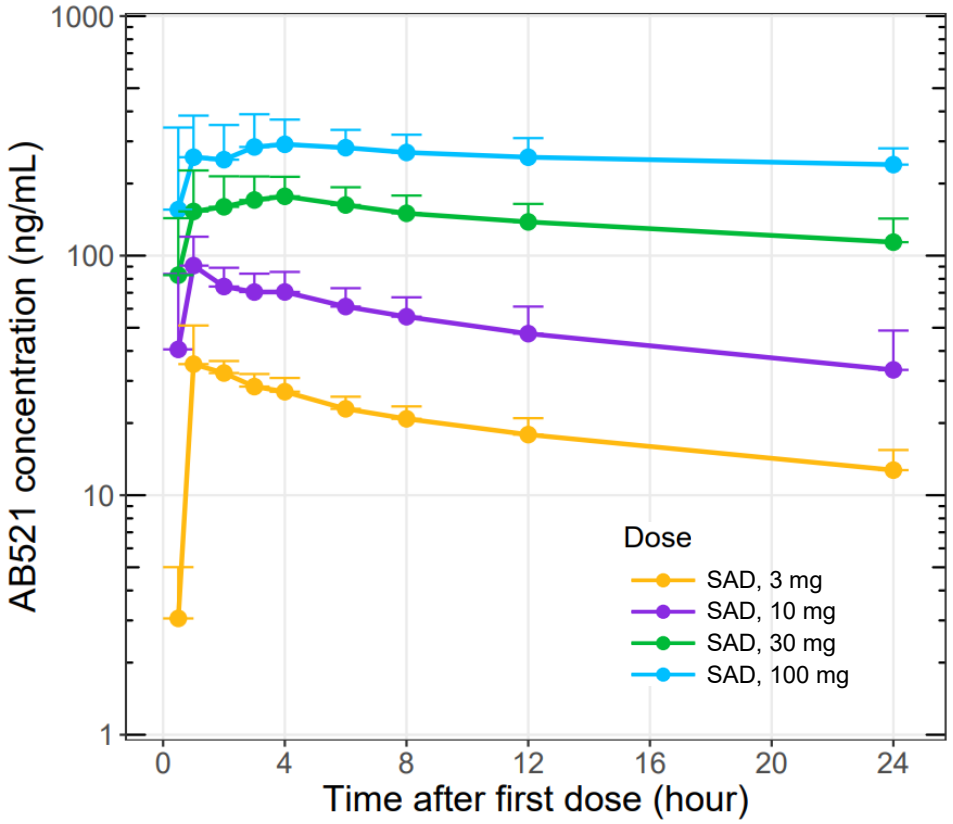
Dose (mg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{INF} (h*ng/mL)	$T_{1/2}$ (h)
3	**39.5** (24%)	**1.0** (1.0-2.0)	**822** (25%)	**20.1** (20%)
10	**95.0** (29%)	**1.0** (0.5-6.0)	**2180** (44%)	**17.9** (42%)
30	**190** (26%)	**3.0** (1.0-6.0)	**6650** (28%)	**18.2** (20%)
100	**338** (28%)	**3.5** (0.5-6.0)	**15200** (23%)	**23.8** (15%)

(NCT05117554/ARC-14)

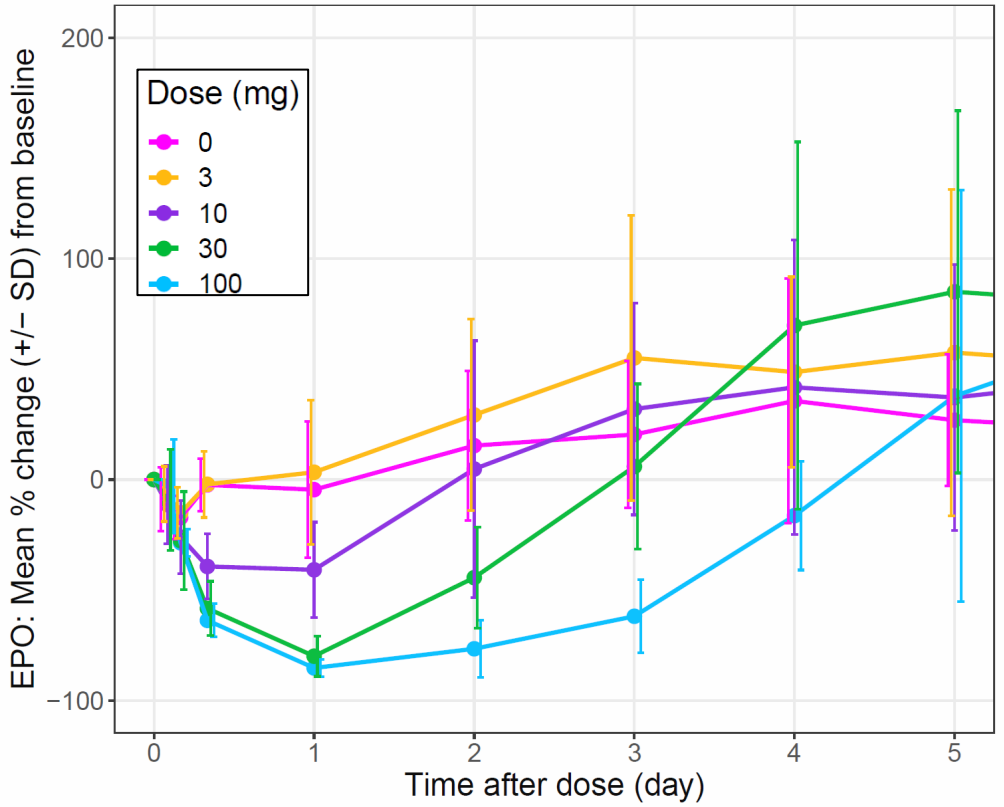
Pharmacokinetic and Pharmacodynamic Parameters Associated with Cas in Human Healthy Volunteers

- Dose-dependent reductions in serum EPO were observed following a single dose at 10 to 100 mg, with mean maximum reduction from baseline up to 85%

Single ascending dose (SAD) PK

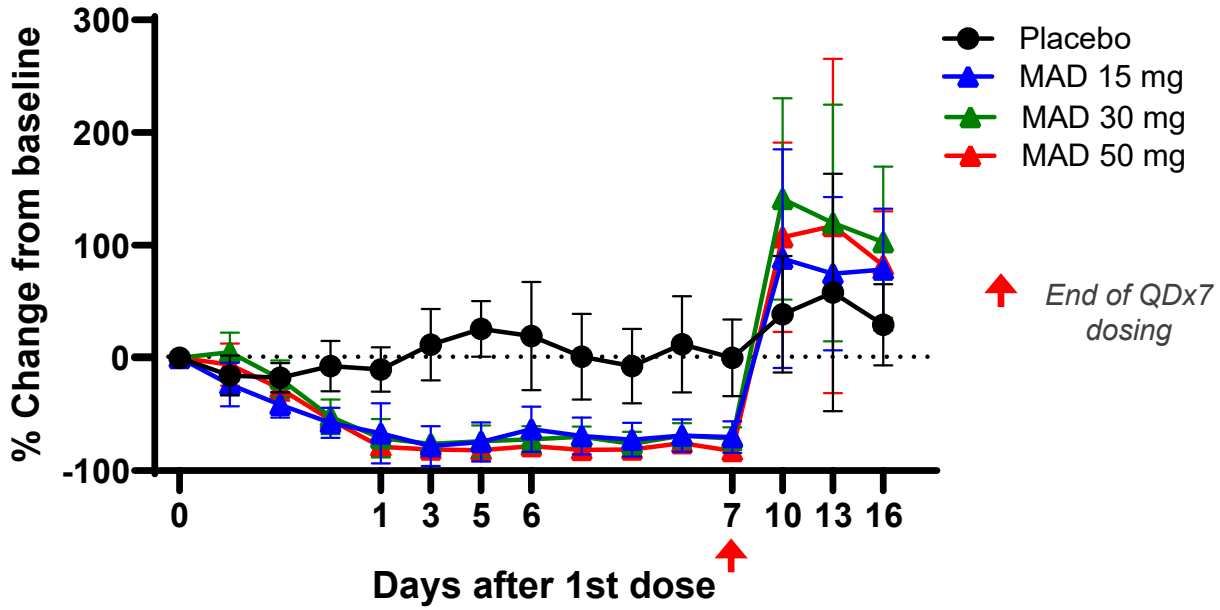


Mean serum EPO reduction-time profiles after single dose of casdatifan



Casdatifan Exhibits Potential Best-in-Class PK/PD Profile

- MAD cohort subjects were dosed 15, 30, or 50 mg cas daily for seven days
- A casdatifan dose of 100 mg, selected for further development, allows exploration of the full therapeutic potential of HIF-2α inhibition
  - A 20 mg daily dose of casdatifan is predicted to provide a similar PD effect as 120 mg belzutifan



MAD Dose (mg)	Average %EPO Reduction (Day 7, Mean ± SD)
Placebo	0.1 ± 34
15	-70 ± 14
30	-72 ± 10
50	-83 ± 5
Belz 120 mg‡ (day 15)	-64 ± 22

‡Marathe, D.D. et *al J. Clin. Pharmacol.*, **2024** (10.1002/jcph.2459)

- Cas is under evaluation in patients with clear cell renal cell carcinoma (ccRCC) and other solid tumors in an ongoing Phase 1 study, ARC-20 (NCT05536141)

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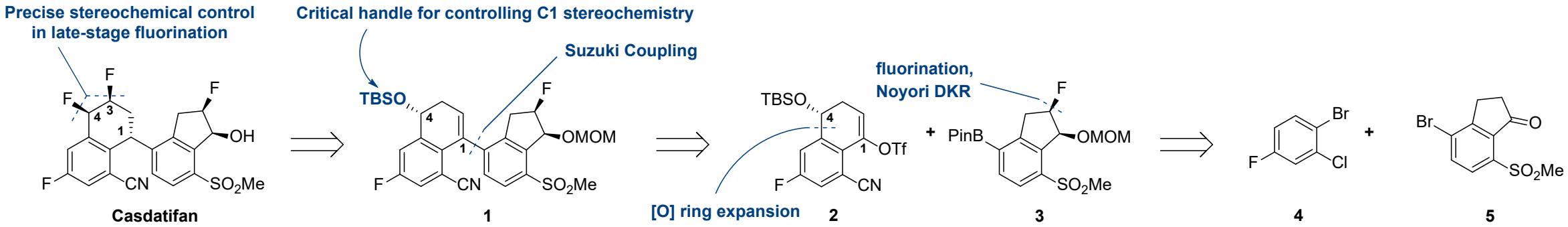
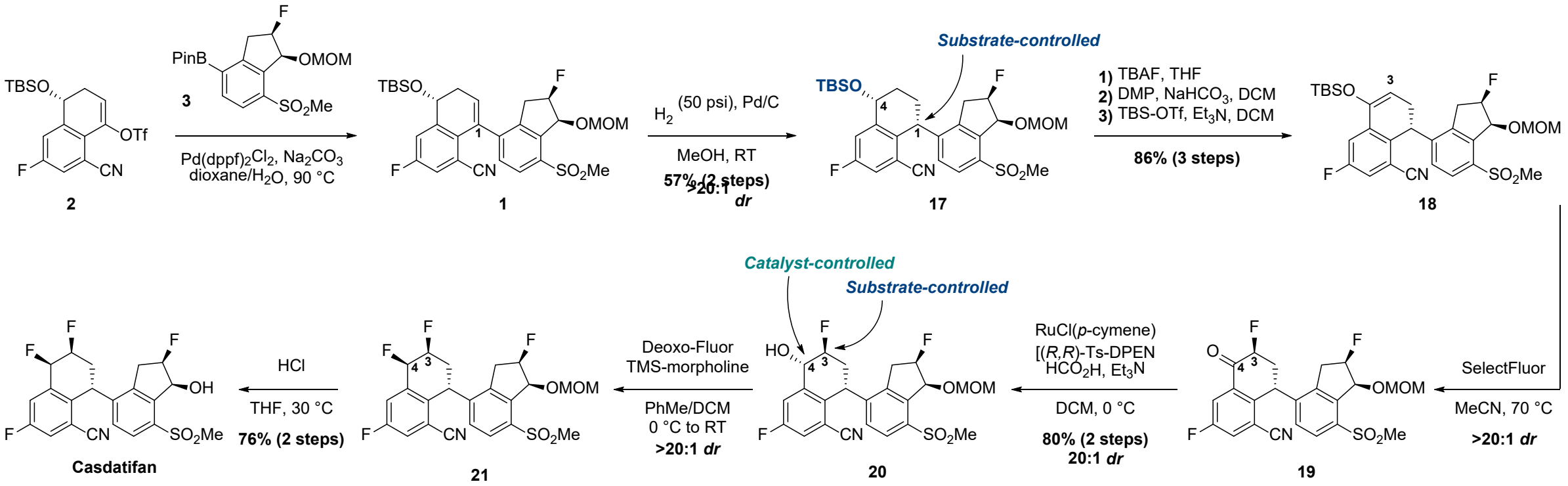
Casdatifan Presents Significant Synthetic Challenges

- 4 highly substituted ring systems bearing 5 stereocenters
- Convergent synthesis – fragments prepared in parallel
  - Strategic installation of remote stereocenter on tetralin fragment enables definition of C1 methine configuration
- Combination of substrate and catalyst control employed to control configuration of *cis*-vicinal difluoride in late-stage fluorination



Casdatifan Presents Significant Synthetic Challenges

- Synthesis proceeds in 21 total steps
- Each stereocenter is set with high stereoselectivity (99% *ee*/>20:1 *dr*) using a combination of substrate and catalyst control



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Casdatifan is a Clinical Stage, Potent, and Selective HIF-2α Inhibitor with Best-in-Class Potential

- HIF-2α is a transcription factor that is an oncogenic driver in clear cell renal cell carcinoma (ccRCC)
- A structure-based design and pharmacophore mapping strategy was employed to identify novel starting points for discovery chemistry
  - Iterative SAR optimization of tetrahydroquinoline and tetralin scaffolds led to the discovery of AB521
  - DMPK properties and HIF-2α potency was highly dependent on specific skeletal fluorination patterns
- Mean terminal half-life in human was 18 to 24 hours, supporting once-daily dosing
- Cas showed dose-proportional increases in exposure over the evaluated range
- Potent HIF-2α inhibition has been demonstrated in healthy volunteers (ARC-14) with dose-dependent reductions in serum EPO
  - A 20 mg daily dose of casdatifan is predicted to provide similar PD effect as 120 mg belzutifan

Thank You to the Arcus Drug Discovery Teams

- Medicinal Chemistry
- Discovery Pharmacology
- Biology
- Biophysics
- DMPK
- Translational Science
- Clinical Pharmacology
- Clinical Science and Development

Annual Research Retreat
Asilomar, CA
(Circa 2023)

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