AB521 (Casdatifan) Potently and Selectively Inhibits Hypoxia-Inducible Factor 2 Alpha (HIF-2α) Dependent Pro-Tumorigenic Activity

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Abstract #91

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Abstract

Background: Hypoxia-Inducible Factor 2α (HIF-2α) is a transcription factor that regulates genes critical for cell adaptation and survival in low oxygen conditions, such as those present in the solid tumor microenvironment. HIF-2α protein is stabilized and transcriptionally active in hypoxia but is targeted for proteasomal degradation in normoxic conditions by the von Hippel-Lindau (VHL) subunit of an E3 ubiquitin ligase complex. In clear cell renal cell carcinoma (ccRCC), *VHL* inactivating mutations are prevalent and cause a 'pseudohypoxic' state, wherein HIF-2α protein is stabilized, even when oxygen levels are high. Notably, HIF-2α is an established tumorigenic driver in ccRCC, and inhibition of HIF-2α has been shown to be an effective strategy to improve clinical outcomes.

Materials and Methods: Applying a pharmacophore mapping and structure-based design approach, we identified a novel and potent small molecule HIF-2α inhibitor, AB521 (casdatifan), that avidly binds to a hydrophobic pocket in the HIF-2α PAS-B domain. The potency and pharmacology of AB521 were evaluated using *in vitro* and *in vivo* experimental systems.

Results: The *in vitro* impact of AB521 on ccRCC cells was assessed using the *VHL* and *HIF1A* mutant 786-O cell line. AB521 potently inhibited HIF-2α-dependent reporter gene transcription in both low and high serum conditions with no off-target cytotoxicity. Additionally, VEGF secretion and soft agar colony formation were also inhibited by AB521. In hypoxic Hep3B hepatocellular carcinoma (HCC) cells, AB521 selectively inhibited HIF-2α, but not HIF-1α, target gene expression. AB521 also inhibited transcriptional activity of HIF-2α in relevant human primary cells, including endothelial cells and pro-tumorigenic M2-polarized macrophages. When delivered orally to mice, AB521 caused significant regression of established ccRCC xenograft tumors and dose-dependent decreases in peripheral and tumor-specific HIF-2α-associated pharmacodynamic markers. AB521 combined favorably with a clinical standard of care, the VEGF tyrosine kinase inhibitor cabozantinib, in a ccRCC xenograft model, as well as the clinical anti-PD-1 antibody, zimberelimab, in a humanized version of the ccRCC model with stably engrafted peripheral blood mononuclear cells. Outside of ccRCC, HIF-2α inhibition also provided a survival benefit in a murine model of HCC.

Mechanism of Action

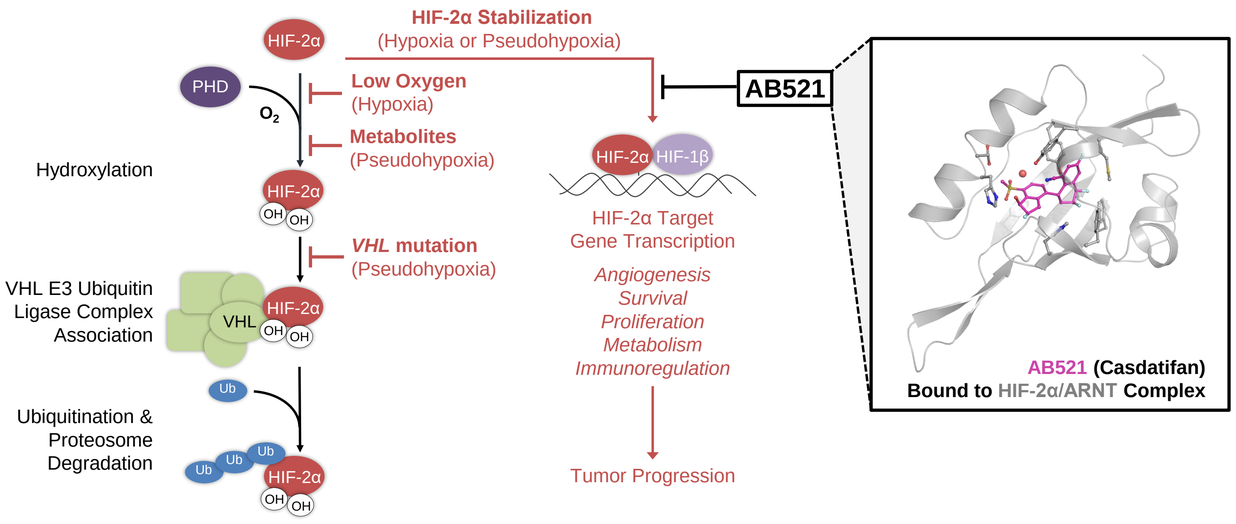

Figure 1. HIF-2α drives transcriptional adaptations to low oxygen that can be hijacked to promote tumor progression. HIF-2 is a transcription factor comprised of two proteins: a stably expressed β subunit (HIF-1β/ARNT) and an oxygen-sensitive α subunit (HIF-2α). In hypoxic or pseudohypoxic conditions, 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.

AB521 Selectively Inhibits HIF-2α-Mediated Transcription and Colony Formation of Cancer Cells

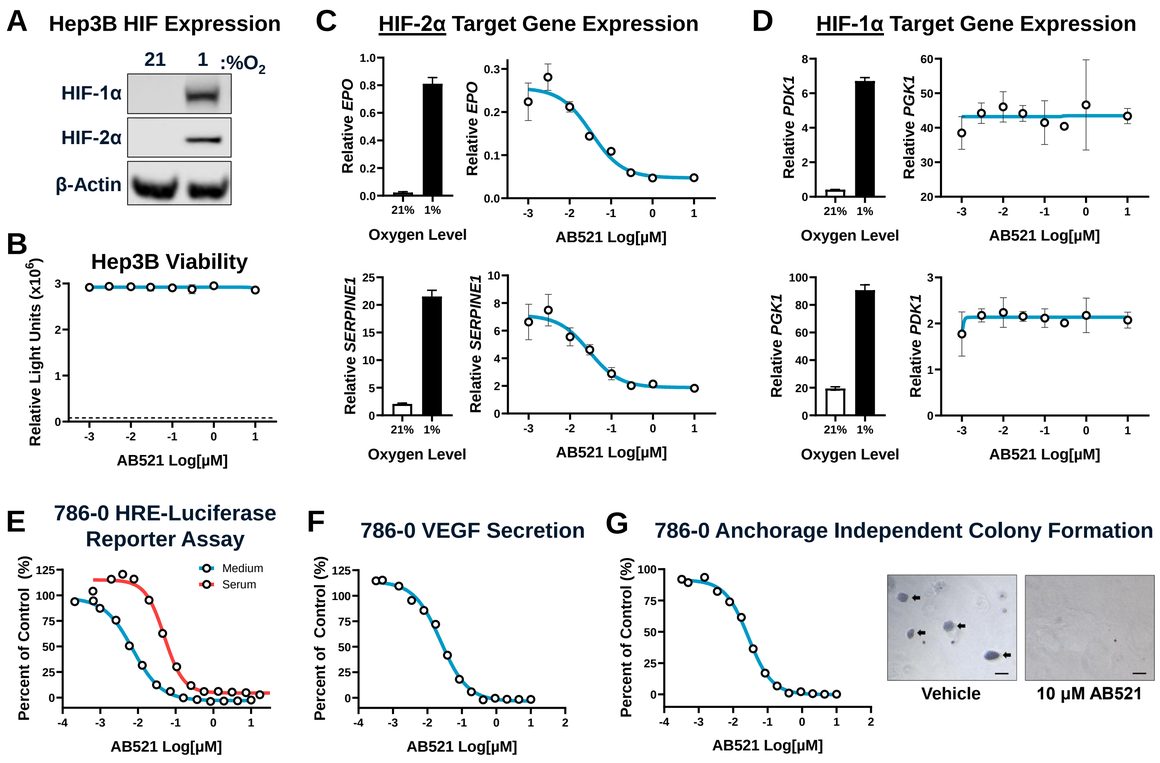

Figure 2. *In vitro* impacts of AB521 on cancer cell lines. A) HIF-1α and HIF-2α protein was stabilized under hypoxia (1% O_2) in Hep3B hepatocellular carcinoma cells. **B)** Viability of Hep3B cells was unaffected by AB521. **C)** AB521 selectively inhibited expression of HIF-2α-regulated genes. **D)** HIF-1α-regulated gene expression was unaltered by AB521 treatment. **E)** AB521 inhibited HIF-2α-dependent hypoxia-response element (HRE) reporter expression in 786-O ccRCC cells in both medium and 100% human serum conditions. **F)** HIF-2α-regulated endogenous VEGF secretion was inhibited by AB521. **G)** AB521 treatment inhibited 3D anchorage independent colony formation of 786-O cells.

AB521 Inhibits Transcription of HIF-2α Regulated Genes in Macrophages and Endothelial Cells

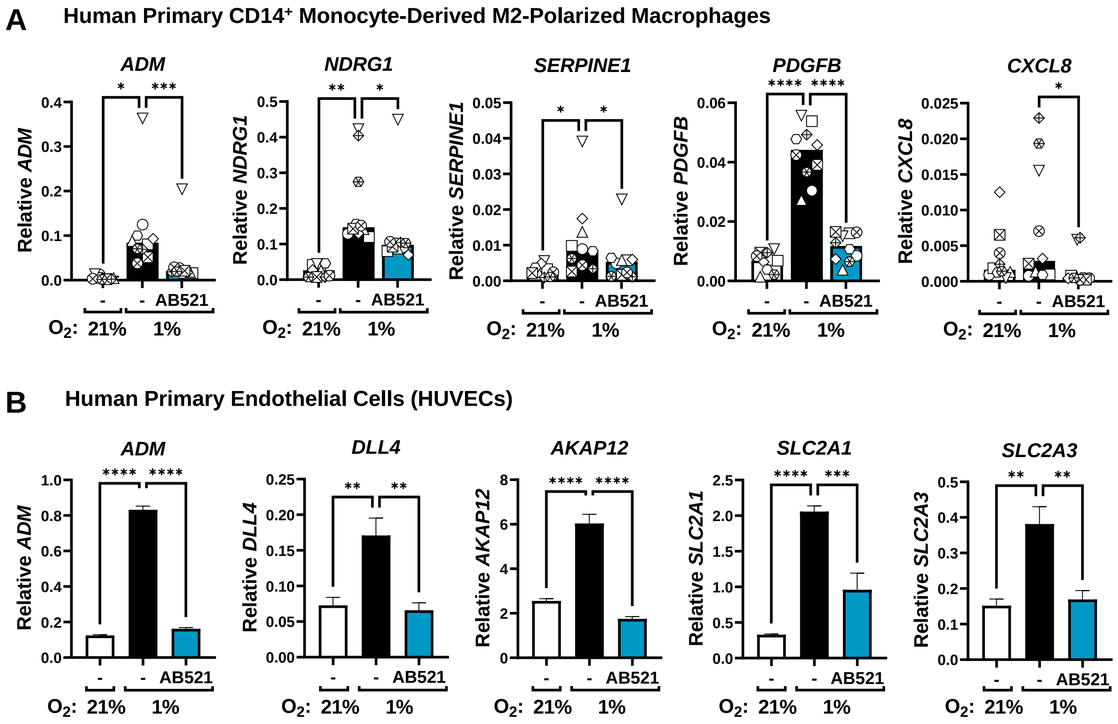

Figure 3. AB521 inhibits pro-tumorigenic gene expression in cell types relevant to the tumor microenvironment. **A)** Hypoxia-dependent upregulation of genes involved in angiogenesis (*ADM, NDRG1*), extracellular matrix remodeling (*SERPINE1, PDGFB*), and myeloid cell recruitment (*CXCL8*) was blocked with AB521 treatment in primary human M2-polarized macrophages. **B)** Hypoxia-dependent upregulation of genes involved in angiogenesis (*ADM, DLL4, AKAPL2*), and glucose metabolism (*SLC2A1, SLC2A3*) was blocked by AB521 treatment in umbilical vein endothelial cells (HUVEC). AB521 used at 1 μM, ANOVA with Dunnett's multiple comparisons * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

AB521 Shows Anti-Tumor Activity as a Single Agent and in Combination with TKI and I-O Therapies in Mice

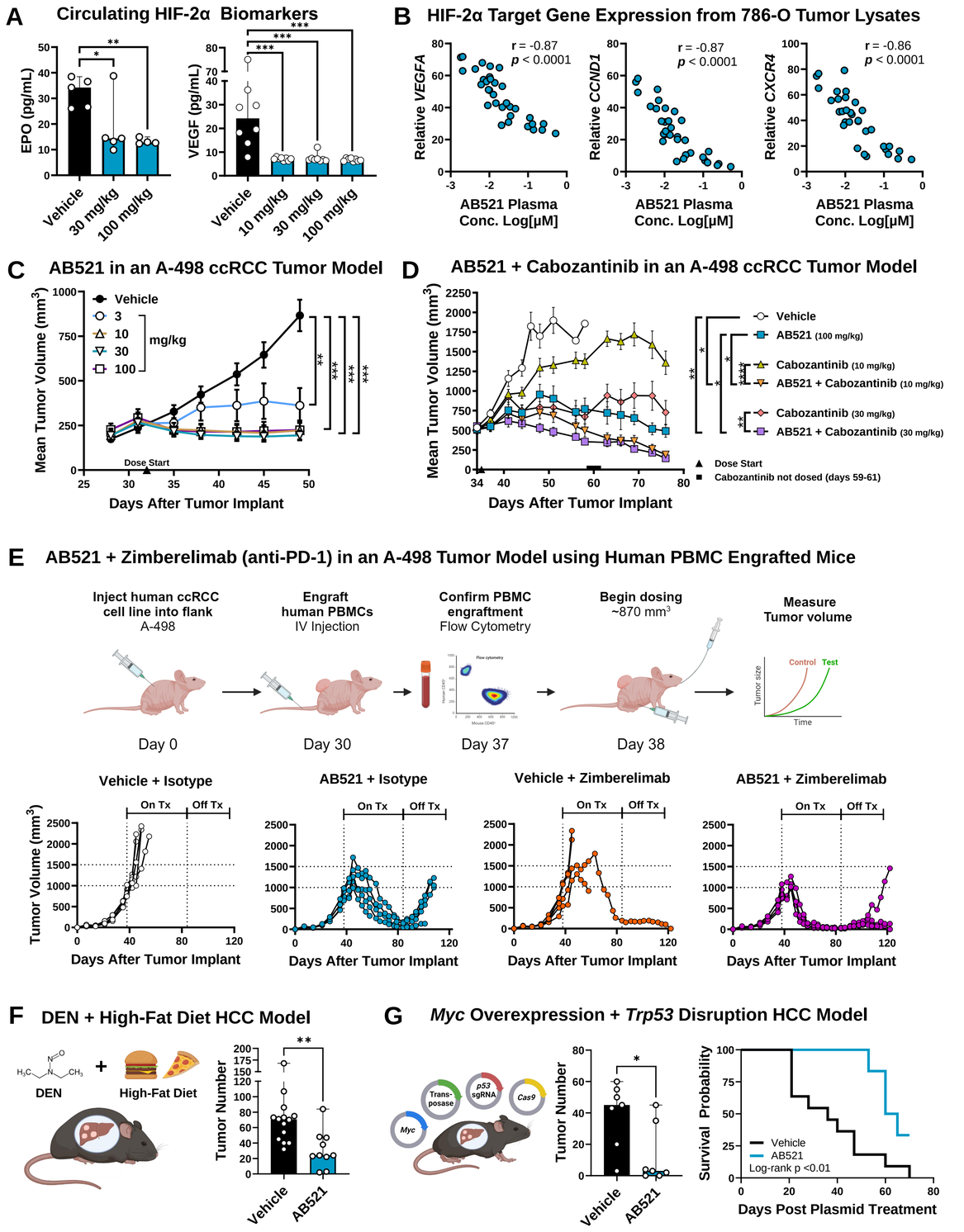

Figure 4. AB521 demonstrates anti-tumor activity in pre-clinical *in vivo* models. A) Mouse EPO and ccRCC tumor derived VEGF in plasma of mice after 8-hours (EPO) or 10-days (VEGF) of AB521 treatment. **B)** Relationship between AB521 plasma concentrations at trough and HIF-2α target gene expression in 786-O xenograft lysates. **C)** Efficacy of AB521 in an A-498 ccRCC xenograft model. **D)** Combinatorial efficacy of AB521 with the tyrosine kinase inhibitor cabozantinib in an A-498 xenograft model. **E)** Evaluation of AB521 (30 mg/kg, PO QD) in combination with zimberelimab (anti-PD-1, 10 mg/kg IP Q3D) in an A-498 xenograft model using human peripheral blood mononuclear cell (PBMC) engrafted mice. **F)** AB521 therapeutic efficacy in a diethylnitrosamine (DEN) and high-fat diet driven hepatocellular carcinoma (HCC) model. **G)** AB521 therapeutic efficacy and impact on survival in a *Myc* and *Trp53* driven HCC model.

Conclusions

- **AB521 is a novel, potent, selective, and orally bioavailable HIF-2α inhibitor**
- **Anti-tumor efficacy of AB521 as a single agent and in combination with clinically relevant therapies was observed in preclinical mouse tumor growth models**
- **AB521 (Casdatifan) is in clinical trials for ccRCC and other solid tumors**
  - **ARC-20: NCT05536141, STELLAR-009: NCT0691796**
  - **Upcoming trials in ccRCC: PEAK-1 Phase 3 in combination with cabozantinib and Phase 1 in combination with volrustomig, an investigational PD-1/CTLA-4 bispecific antibody**

Figure 1, 4E, 4F-G Made with BioRender