Tipifarnib synergizes with axitinib in clear cell renal cell carcinoma models

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BACKGROUND

- Clear cell renal cell carcinoma (ccRCC) is a highly vascularized tumor type, primarily due to loss of the Von Hippel-Lindau (VHL) gene, which is observed in ~88% of ccRCC cases[1]
- Deletion of VHL stabilizes hypoxia inducible alpha proteins, HIF1α and HIF2α, primary drivers of the angiogenic response in this cell type[1]
- Anti-angiogenic tyrosine kinase inhibitor (TKIs) such as the VEGFR1/2/3 inhibitor axitinib have demonstrated therapeutic benefit in ccRCC patients by exploiting the critical dependency of the tumors on the vasculature[2]
- Resistance to TKIs is commonly observed, necessitating development of rational combination strategies
- Tipifarnib, a farnesyltransferase inhibitor, potentially blocks hyperactivated growth factor signaling at multiple nodes[3,4]
- **Here, we utilize cell line and PDX models to establish the scope and mechanistic underpinnings of tipifarnib-axitinib effects and strengthen the scientific rationale for combining TKIs with tipifarnib in the treatment of patients with ccRCC.**

RESULTS

Tipifarnib-axitinib combination causes tumor regression or stasis in VHL-mutant and VHL-wildtype ccRCC models

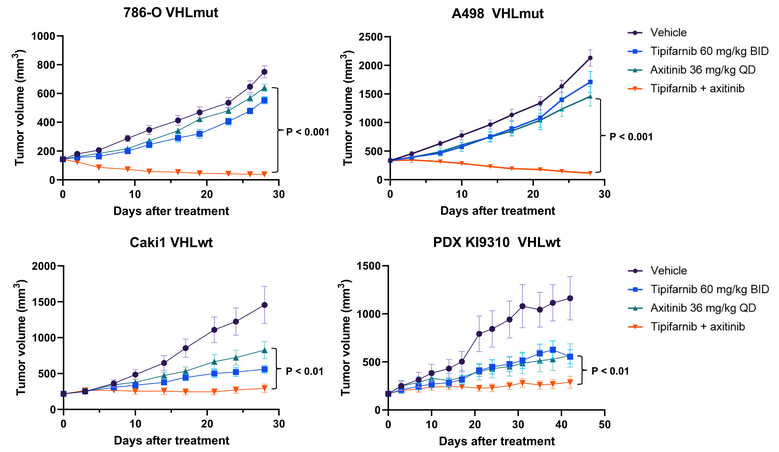

Figure 1. Continuous treatment of tipifarnib plus the anti-angiogenic TKI axitinib robustly inhibits the growth of ccRCC cell line and patient derived xenograft models. Tumor growth curves of ccRCC CDX and PDX models harboring either VHL mutant (VHLmut) or VHL wildtype (VHLwt), treated with vehicle, tipifarnib (60 mg/kg BID), axitinib (36 mg/kg QD), or the combination.

Tipifarnib enhances the anti-angiogenic activity of axitinib *in vivo*

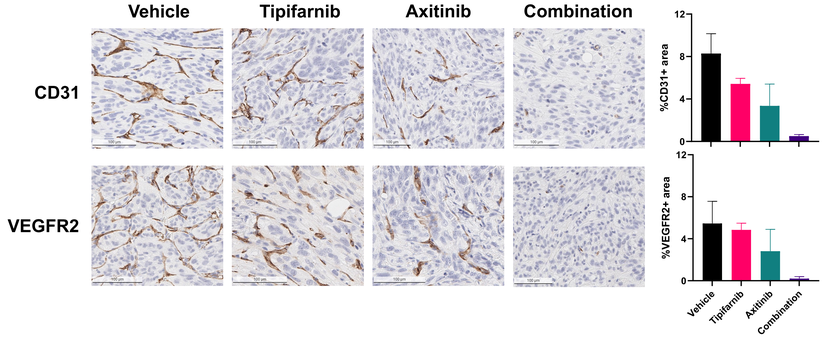

Figure 2. Tipifarnib plus axitinib combination leads to decreased expression of endothelial cell markers *in vivo*. Representative images of immunohistochemistry analysis for vascular markers CD31 and VEGFR2 in 786-O tumors collected after thirteen days of treatment with vehicle, tipifarnib (60 mg/kg BID), axitinib (36 mg/kg QD), or the combination. Right, quantitation of indicated target expression is expressed by mean percent of area with positive stain over the tumor area. Error bars represent standard deviation of the mean, n = 4.

Tipifarnib and axitinib inhibit endothelial cell proliferation and tube formation *in vitro*

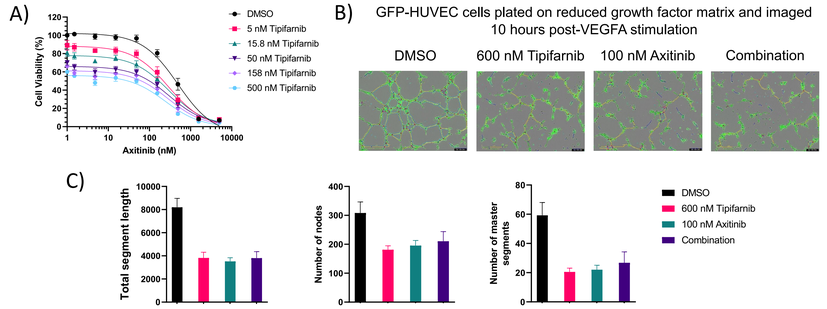

Figure 3. Tipifarnib and axitinib inhibit human umbilical vein endothelial cell (HUVEC) proliferation and tube formation *in vitro*. A) Percent cell viability of HUVEC cells after seven days of continuous treatment with varying concentrations of axitinib and tipifarnib. **B)** Representative images of tubes formed by GFP-HUVECs on matrix proteins after treatment with vehicle, 600 nM tipifarnib, 100 nM axitinib, or the combination. **C)** Quantitation of total segment length, number of nodes and number of master segments formed by GFP-HUVEC tubes in B. Analysis was performed using the ImageJ plugin Angiogenesis Analyzer. Bars represent the mean ± SEM, n = 4.

Tipifarnib blunts mTOR signaling and induces cell cycle arrest in endothelial cells

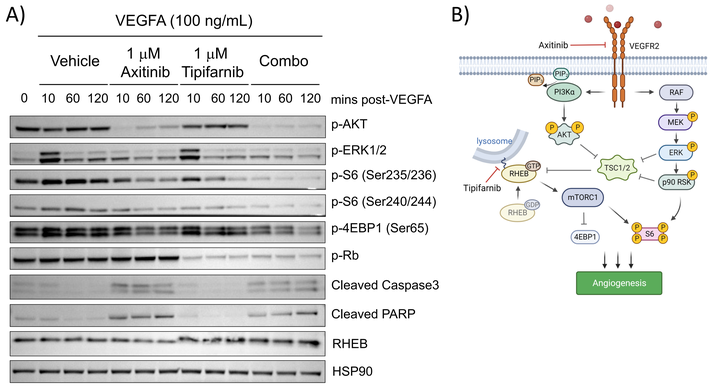

Figure 4. Combined tipifarnib and axitinib treatment inhibits mTOR signaling more potently than axitinib alone. A) Immunoblots of indicated MAPK/PI3K pathway components and apoptotic markers in serum-starved HUVEC cells treated with axitinib for one hour in the absence or presence of tipifarnib (24-hour treatment) then stimulated with VEGFA for 10, 60, or 120 minutes. Shift in RHEB mobility is indicative of defarnesylation. HSP90 serves as the loading control. **B)** Schematic of MAPK and PI3K signaling nodes that can be inhibited by tipifarnib and axitinib in endothelial cells.

CONCLUSIONS

- Tipifarnib and axitinib synergize to induce tumor regression or stasis in ccRCC CDX and PDX models.
- Tipifarnib enhances the anti-angiogenic activity of axitinib *in vivo*, as observed by decreased expression of vascular markers in 786-O tumors.
- The effect of the combination can in part be explained by the anti-angiogenic activity of tipifarnib. Mechanistically, while axitinib induces apoptosis of endothelial cells, tipifarnib inhibits mTOR signaling and increases cell cycle arrest.
- The combination of tipifarnib and axitinib holds potential for the treatment of ccRCC. Ongoing studies aim to further define the basis of the combination's synergy.

References
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4) Smith AE, et al. "Tipifarnib potentiates the antitumor effects of PI3Kα inhibition in *PIK3CA*- and *HRAS*-dysregulated HNSCC via convergent inhibition of mTOR activity." BioRxiv 2023.01.17.523964.