#7

THE PERK INHIBITOR HC-5404 SENSITIZES CLEAR CELL RENAL CELL CARCINOMA TUMOR MODELS TO ANTIANGIOGENIC TYROSINE KINASE INHIBITORS

Michael E. Stokes[1], Veronica Calvo[1], Crissy Dudgeon[1], Sho Fujisawa[1], Sharon Huang[1], Jose Malagon-Lopez[1], Leyi Shen[1], Kirk A. Staschke[2,3], Mark J. Mulvihill[1], Alan C. Rigby[1], Nandita Bose[1] , Eric Lightcap[1], David Surguladze[1]

[1]HiberCell, Inc., New York City, NY, 10019. [2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, [3]Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN 46202, USA.

ABSTRACT

Antiangiogenic agents form the backbone of standard of care for advanced clear cell renal cell carcinoma (ccRCC), but their clinical impact is limited by primary and secondary resistance mechanisms that remain a critical problem. Furthermore, the approvals for VEGFR-targeting receptor tyrosine kinase inhibitors (VEGFR-TKIs), cabozantinib in second-line, and tivozanib in third-line RCC patients were based on modest objective response rates and median progression-free survival. There is an urgent need for novel mechanisms that target adaptive tumor responses that drive resistance to these agents, as well as combination drug partners that improve outcomes for patients.

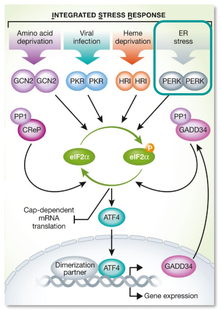
As part of their mechanism, VEGFR-TKIs induce hypoxia and nutrient-deprivation that drives endoplasmic reticulum (ER) stress. Tumors can evade deleterious ER stress by activating PERK branch of the integrated stress response, which arrests global translation and restores homeostasis. We hypothesized that inhibiting PERK would enhance the antitumor activity of VEGFR-TKIs *in vivo* and tested this using HC-5404, a potent and selective PERK inhibitor currently in Ph1 clinical testing (NCT04834778). Here, we present preclinical evidence that supports combining HC-5404 with VEGFR-TKIs in ccRCC. We demonstrate that axitinib, cabozantinib, lenvatinib, and sunitinib all activate PERK in 786-O ccRCC xenografts in a dose-responsive manner. The addition of HC-5404 significantly enhanced the tumor growth inhibition (TGI) of VEGFR-TKIs across multiple ccRCC tumor models, resulting in tumor stasis or regression in combination groups. Expression profiling and IHC analysis of tumor sections revealed that HC-5404 enhanced the antiangiogenic effects of axitinib and lenvatinib in 786-O tumors, highlighting the protective role of PERK in response to antiangiogenics.

To evaluate whether the combination treatments could benefit a diverse patient population, sensitivity to HC-5404 and axitinib was evaluated across a panel of patient-derived xenograft (PDX) models. This experiment confirmed widespread responsiveness to the combination treatment that in some cases achieved ≥50% tumor regression. As tumor progression on VEGFR-TKIs limits the success of these agents in the clinic, we evaluated the effect of adding HC-5404 to tumors that have previously progressed on axitinib. In this study, 786-O xenografts were treated with axitinib for 2 weeks and nonresponders were rerandomized into groups of either single agent or combination of HC-5404 and axitinib. The combination treatment significantly improved TGI relative to either monotherapy, resulting in tumor regression of ~20%. Taken together, these findings highlight that by disrupting an adaptive stress response evoked by VEGFR-TKIs, HC-5404 presents a clinical opportunity to enhance the antitumor effects of well-established standard of care therapies in ccRCC.

BACKGROUND

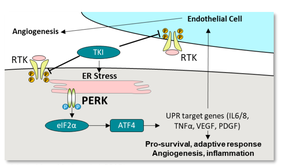
PERK IS AN ISR KINASE THAT DRIVES ER STRESS RESPONSE

- The Integrated Stress Response (ISR) consists of four closely-related kinases that mediate tumor response to cellular stress[1].
- ISR kinases (PERK, GCN2, PKR, HRI) autophosphorylate in response to stress and inhibit protein translation while activating stress mitigating pathways.
- PERK is activated by ER stress, which is caused by misfolded proteins in the ER lumen.
- ATF4 is induced by the ISR to mediate stress response and is a hallmark of tumor stress. If stress persists, cells undergo ATF4/CHOP-mediated cell death.
- HC-5404 is a potent and selective PERK inhibitor[2], formerly known as LY-4, that is currently in Ph1 trials (NCT04834778).
- Here, HC-5404 is used to demonstrate the role PERK plays in mitigating effects of antiangiogenic agents and evaluate clinical opportunity to combine with VEGFR-TKIs.



Adapted from Pakos-Zebrucka et al 2016[1]

TUMORS RELY ON PERK TO MITIGATE STRESS FROM ANTIANGIOGENICS



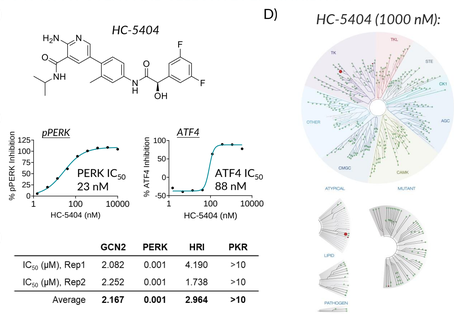
Adapted from Makhov et al. 2018[4]

- Antiangiogenic VEGFR-TKIs disrupt tumor vasculature and block the flow of oxygen and nutrients, driving an ISR response[3].
- PERK activation by VEGFR-TKIs is a mechanism to overcome inhibitory ER stress.
- **Hypothesis: PERK inhibition enhances the antitumor effects of VEGFR-TKIs**

CITATIONS
[1]Pakos-Zebrucka *et al.* (2016) The integrated stress response. *EMBO Reports*
[2]Calvo *et al.* (2021) Discovery of 2-amino-3-amido-5-aryl-pyridines as highly potent, orally bioavailable and efficacious PERK inhibitors. *BMCL*
[3]Fels *et al.* (2006) The PERK/pelF2a/ATF4 module of the UPR in hypoxia resistance and tumor growth. *Cancer Biol. Ther.*
[4]Makhov *et al.* (2018) The convergent roles of NF-kB and ER stress in sunitinib-mediated expression of pro-tumorigenic cytokines and refractory phenotype in renal cell carcinoma. *Cell Death & Disease*

RESULTS

HC-5404 IS A POTENT AND SELECTIVE PERK INHIBITOR



	GCN2	PERK	HRI	PKR
IC_{50} (μM), Rep1	2.082	0.001	4.190	>10
IC_{50} (μM), Rep2	2.252	0.001	1.738	>10
Average	**2.167**	**0.001**	**2.964**	**>10**

Figure 1: HC-5404 is a potent and selective PERK inhibitor. A) HC-5404 molecular structure. B) Western blot analysis of pPERK and ATF4 in HEK293 cells treated with HC-5404 in presence of 1 μM tunicamycin. C) HC-5404 is selective for PERK relative to four closely-related ISR kinases in FRET-based biochemical assays. D) Biochemical TreeSpot kinome panel assays demonstrate selectivity of HC-5404 against >400 kinases. NB: PERK is not included in the panel, and zero interactions were observed when HC-5404 assayed at 100 nM.

HC-5404 HAS FAVORABLE IN VIVO PROPERTIES

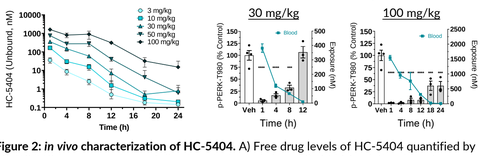


Figure 2: *in vivo* characterization of HC-5404. A) Free drug levels of HC-5404 quantified by LC-MS/MS in mouse plasma following single oral dose. B) PK/PD relationship of mouse plasma exposure and pPERK levels in mouse pancreas following single oral administration of HC-5404 at 30 and 100 mg/kg.

PERK INHIBITION DRIVES AN ATF4-MEDIATED TUMOR STRESS RESPONSE

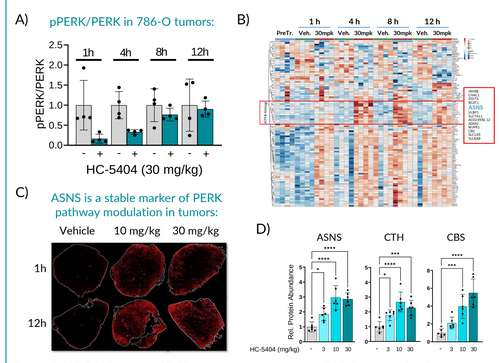


Figure 3: HC-5404 induces ATF4 tumor stress response in 786-O xenografts. A) SimpleWestern (JESS) quantification of pPERK and PERK in xenografts following treatment with HC-5404. Tumors sampled at indicated time-points following treatment at 30 mg/kg, PO, BID for 15 days. B) RNASeq analysis: Heatmap of expression response to HC-5404 across time. In red box, multiple ATF4 targets identified by IPA Analysis. C) IHC analysis of ASNS in 786-O xenografts. D) SimpleWestern (JESS) validation of ATF4 targets show dose-dependent response to HC-5404.

PERK INHIBITION ENHANCES ANTITUMOR EFFECT OF VEGFR-TKIs

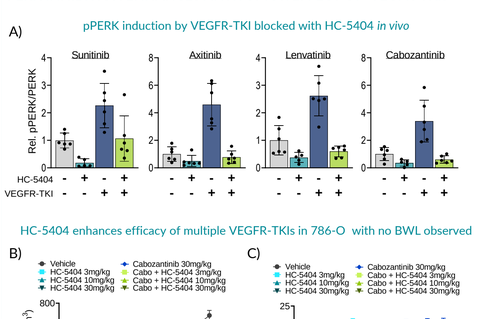


Figure 4: HC-5404 combines with VEGFR-TKIs to inhibit tumor growth in 786-O RCC xenografts. A) SimpleWestern protein analysis of pPERK and PERK in 786-O xenografts treated with VEGFR-TKIs, sampled after 7 days of treatment. B) 786-O RCC xenografts treated with HC-5404 at three dose levels in combination with cabozantinib (30 mg/kg; PO; QD) as indicated. C) Mouse body weight measurements from cabozantinib study. NB: No treatment related BWL was observed for any VEGFRi combinations tested. D) 786-O xenografts treated with HC-5404 (30 mg/kg; PO; BID) and sunitinib (40 mg/kg; PO; QD), lenvatinib (10 mg/kg; PO; BID), or axitinib (30 mg/kg; PO; BID). E) ATF4, DDIT3, and CHAC1 transcript abundance in response to HC-5404 and cabozantinib (30 mg/kg; PO; QD).

INHIBITION OF VEGFR2 SUFFICIENT FOR COMBINATION BENEFIT

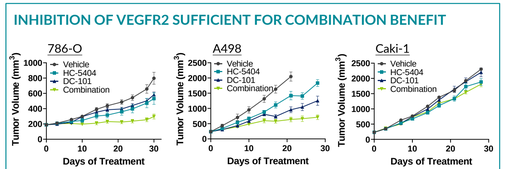


Figure 5: HC-5404 combines with antiangiogenic monoclonal antibody selectively targeting mouse VEGFR-2. RCC tumor models treated with HC-5404 (30 mg/kg; PO; BID) and DC-101 (15 mg/kg; IP; BIW) for 28 days. Shown here are mean tumor volumes ± SEM.

SENSITIVITY TO HC-5404/VEGFR-TKI COMBINATIONS ACROSS DIVERSE RCC MODELS IS INDEPENDENT OF VHL MUTATION STATUS

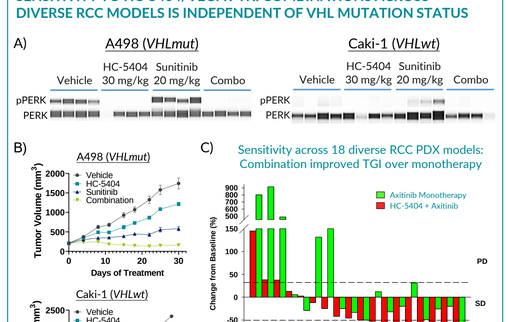


Figure 6: Sensitivity to VEGFR-TKIs and HC-5404 combination is independent of *VHL* mutation status. A) pPERK and total PERK abundance evaluated by SimpleWestern in RCC tumor xenografts treated for seven days with HC-5404, sunitinib, or combinations as indicated. B) A498 and Caki-1 RCC tumor xenografts treated with HC-5404 and sunitinib as indicated in panel A. Results suggested VHL mutation may drive sensitivity to HC-5404/VEGFR-TKI combination, so tested across panel of 18 diverse PDX models. C) Waterfall plot illustrating relative change in tumor volume of 18 RCC PDX models following 28 days of treatment with either axitinib (30 mg/kg; PO; BID), HC-5404 (30 mg/kg; PO; BID), or a combination thereof. Models ranked on % change tumor volume in combination group. Progressive Disease (PD) = ≥30% increase from baseline; Stable Disease (SD) = ≤30% increase and ≤50% regression; Partial Response (PR) = ≥50% regression. Models that are *VHL* wild-type are indicated by an arrow.

HC-5404 ENHANCES THE ANTIANGIOGENIC EFFECT OF VEGFR-TKIs

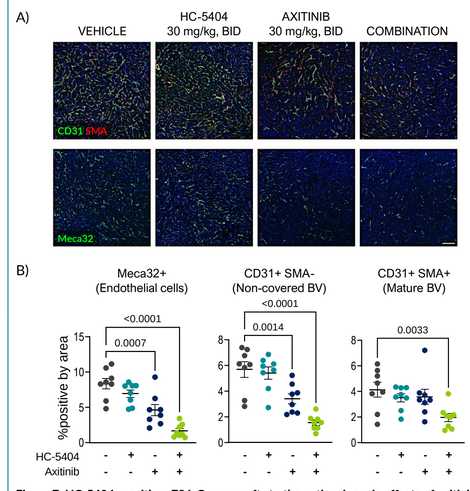


Figure 7: HC-5404 sensitizes 786-O xenografts to the antiangiogenic effects of axitinib. A) IHC images of 786-O xenograft sections stained for Meca32, CD31, and SMA. Scale bar=150 μm. B) Quantification of IHC staining. Graphs indicate proportion of cells stained positive by area.

XENOGRAFTS THAT PROGRESS ON AXITINIB REGRESS FOLLOWING ADDITION OF HC-5404

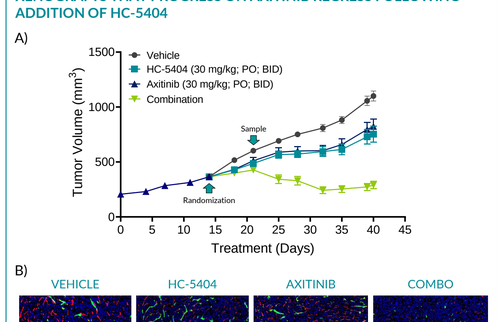


Figure 8: Addition of HC-5404 to axitinib regimen induces tumor regression. A) 786-O tumor growth across the study period. Xenografts that progressed in presence of axitinib (30 mg/kg; PO; BID) for 14 days were rerandomized and transferred to indicated treatment groups for an additional 28 days. B) IHC images of xenograft sections stained with antibodies specific for CD31 and SMA. C) Quantification of proportion of cells that stained positive for Meca32, CD31+SMA- (immature BVs), or CD31+SMA+ (mature BVs) in panel B. D) Images and quantification of IHC staining of tumor sections using antibodies specific for the pericyte markers NG2 and MCAM.

SUMMARY/CONCLUSIONS

- PERK inhibition by HC-5404 increases sensitivity of RCC tumor models to VEGFR-TKIs.
- PERK inhibition exacerbates tumor stress, resulting in ATF4-driven response and possible CHOP-mediated cell death.
- HC-5404 enhances the antiangiogenic effects of VEGFR-TKIs, particularly in mature vessels and pericyte cells which can be recalcitrant to VEGFRi.
- Tumors that advance on axitinib retain sensitivity to HC-5404, highlighting potential for later lines of therapy.
- Findings support a clinical rationale to combine HC-5404 with standard-of-care VEGFR-TKIs in RCC.

HC-5404 inhibits protective effects of PERK, exacerbating stress and driving cell death