PERK INHIBITOR HC-5404 DEMONSTRATES IMMUNE-ACTIVATION AND ANTI-TUMOR EFFICACY IN COMBINATION WITH ANTI-PD1 IMMUNE CHECKPOINT INHIBITOR ANTIBODY

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ABSTRACT

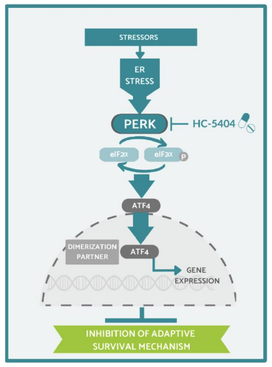
Protein kinase R-like endoplasmic reticulum kinase (PERK) is part of the unfolded protein response that facilitates cellular adaptation to ER stress. PERK is activated in cancer cells by accumulation of misfolded proteins in the ER and enables their adaptation and survival. PERK signaling has also recently been implicated in maintaining immunosuppressive functions of myeloid-derived suppressor cells (MDSCs) through inhibition of a type 1 interferon response [1] and M2-polarized macrophages through metabolic and epigenetic modification [2]. We are developing HC-5404, a highly selective and potent first-in-class, first-in-human PERK inhibitor that is currently in a phase 1 trial for solid tumors (NCT04834778). HC-5404 has demonstrated single agent and combinatorial efficacy in multiple solid tumor xenograft models. In this study, we sought to investigate the immunomodulatory effects of HC-5404. Utilizing the syngeneic bladder cancer model MB49, we evaluated efficacy and correlative immune effects of HC-5404 combined with an anti-murine-PD-1 immune checkpoint inhibitor (ICI) antibody (RMP1-14).

C57BL/6 mice were subcutaneously inoculated with MB49 cells, and treatment started on day 8 post cell inoculation. A group of animals (n=10/group) received either vehicle, HC-5404 (PO, BID), anti-PD-1 antibody (IP, every 3 days), or the combination of both. At various timepoints, animals were sacrificed, and flow cytometry was performed on blood or single cell suspensions from tumors or lymph nodes (n=6). While HC-5404 alone showed only a modest anti-tumor effect (32% TGI), the addition of HC-5404 to anti-PD-1 provided combination antitumor benefits (75% TGI) and significantly improved the effects of anti-PD-1 alone (53% TGI).

HC-5404 + anti-PD-1 treatment efficacy was correlated with increased expression of type 1 interferon receptor (IFNAR1) and increased surface calreticulin on tumor cells. Additionally, IFNAR1 expression was also significantly increased on PMN-MDSCs and tumor-associated macrophages (TAM). TAMs also showed increased expression of PD-L1 with combination treatment. Concomitant to the activation of myeloid cells, combination treatment increased the frequency of CD8 T-cells in the tumor along with increased expression of activation marker CD69 on T-cells in the tumor draining lymph node. Notably, the effect of HC-5404 on IFNAR1 was also detected on monocytes in peripheral blood, demonstrating surface expression of IFNAR1 as a potential biomarker for HC-5404 activity. In vitro evaluation of human cord blood-derived and mouse bone marrow-derived MDSCs showed a reduced inhibition of T-cells in the presence of HC-5404. Collectively, these data demonstrate the efficacious and immuno-stimulatory effects of HC-5404 co-administered with anti-PD1 mAb and outline its potential application in ICI-treated cancers.

BACKGROUND & RATIONALE

ROLE OF PERK IN THE UNFOLDED PROTEIN RESPONSE (UPR)



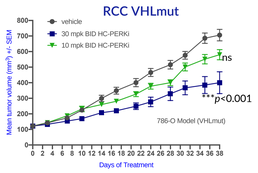
- The **Unfolded Protein Response (UPR)** is an adaptive cellular program used by cancer cells to survive by facilitating the adaptation to harsh TME as characterized by hypoxia, nutrient deprivation, oxidative stress
- Hypoxia drives accumulation of misfolded proteins in the endoplasmic reticulum (ER), leading to ER stress and activation of **PERK (protein kinase R-like endoplasmic reticulum kinase)** in the UPR pathway

- **PERK** is an ER-resident transmembrane kinase that signals through eIF2a to block ER-dependent protein synthesis and maintain homeostasis. Various SOC cancer therapies further amplify "stress" in tumors and activate PERK as a survival mechanism
- **HC-5404** a first-in-human PERK inhibitor, currently in a Phase I clinical trial focused on solid tumors
- Inhibition of the adaptive UPR via PERK inhibition results in monotherapy and combinatorial anti-tumor activity in multiple tumor types such as renal cell carcinoma and gastric cancer

PERK-DEPENDENT STRESS ADAPTATION IN VHL$_{mut}$ RENAL CELL CARCINOMA & GASTRIC CANCER- HC-5404 AS A SINGLE AGENT

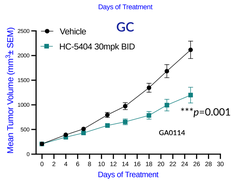
RENAL CELL CARCINOMA (RCC)

- VHL mutation (~90% of ccRCC)
- VHL$_{mut}$ drives HIF2α overexpression→increased **ER stress**
- SOC anti-angiogenic agents further increase **ER stress**
- Provides rationale for PERKi, either alone or in combination with SOC



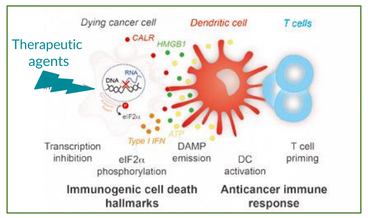
GASTRIC CANCER (GC)

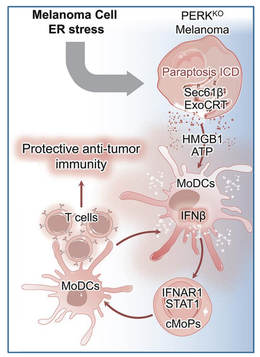
- Activation of **ER stress** observed in ~50% GC associated with metastasis and poor prognosis
- H. pylori, one of GC main risks factors, is an **ER stress** activator
- Resistance to chemotherapies
- Approved antiangiogenic therapy induces hypoxia and activates **ER stress**



BACKGROUND & RATIONALE

INTERPLAY BETWEEN ER STRESS AND IMMUNOGENIC CELL DEATH (ICD) IN IMMUNOTHERAPY





Adapted from Rodriguez et al. Cancer Cell 2022

- **Immunogenic cell death (ICD)** is a class of regulated cell death that can elicit adaptive immune response via the release of endogenous signaling molecules known as damage-associated molecular pattern (DAMP) from the dying cells
- ICD-associated DAMPs include calreticulin (CALR), ATP, type I interferon and high-mobility group box 1 (HMGB1)
- Intrinsic ER stress in tumor cells and anti-cancer therapeutic agent-induced ER stress are known inducers of ICD
- In a recent study, it has been shown that the elimination of PERK activity produces a protective anti-tumor immunity in a melanoma tumor model [3]. The authors further demonstrate that the efficacious anti-tumor T cell response are consequent to the ER-stress initiated, paraptosis-mediated ICD and its downstream type 1 interferon receptor (IFNAR1)-dependent activation and recruitment of MoDC (monocyte-lineage inflammatory DC) to the tumor

RESULTS

HC-5404 UPREGULATES SIGNALING PROTEINS ASSOCIATED WITH ICD

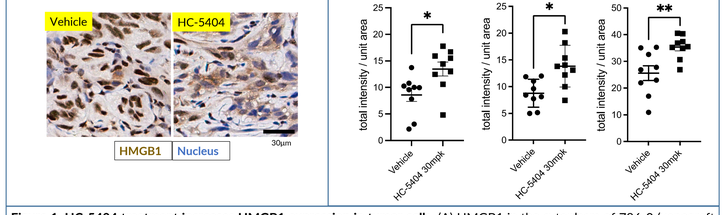


Figure 1. HC-5404 treatment increases HMGB1 expression in tumor cells. (A) HMGB1 in the cytoplasm of 786-0 (xenograft model, day 15) was detected by IHC. (B) Graphical result of IHC analysis of HMGB1 cytoplasmic expression induced by HC-5404 treatment in three different xenografts models *in vivo*. The model and date of samples collected post treatment for analysis are indicated. Unpaired student-t test was used. *P < 0.05; **P < 0.01.

HC-5404 INDUCES IFNAR1 EXPRESSION ON PERIPHERAL BLOOD MONOCYTE

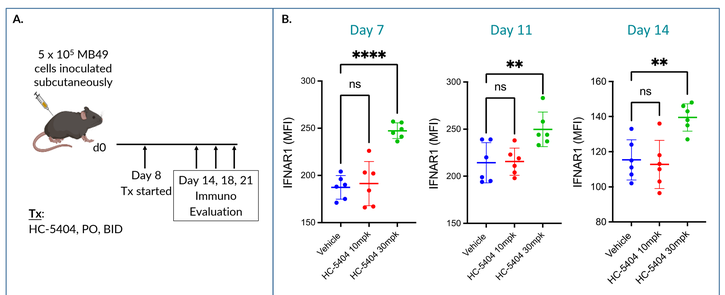


Figure 2. HC-5404 induces dose-dependent of increase in IFNAR1 expression on peripheral blood monocytes. (A) Study design. (B) Whole blood was collected on the indicated date post treatment (day 7, 11 and 14 are equivalent to day 14, 18 and 21 post inoculation) in MB49 bladder cancer model. IFNAR1 expression was determined by flow cytometry and gated on CD14+ monocytes. One-way ANOVA with Dunnett for multiple comparison was used. **P < 0.01; ****p<0.001

EFFECT OF HC-5404 ON GENE EXPRESSION IN MB49 TUMOR

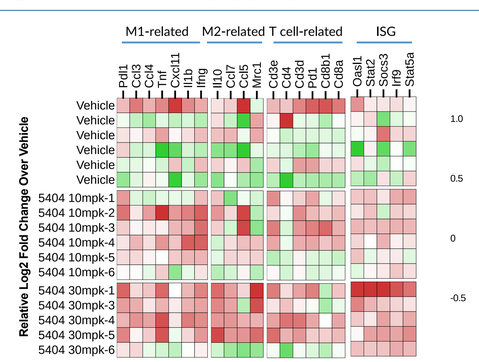
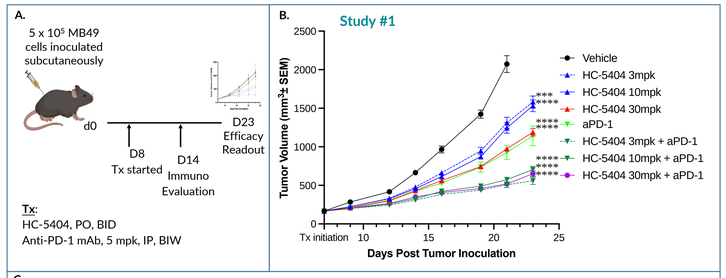
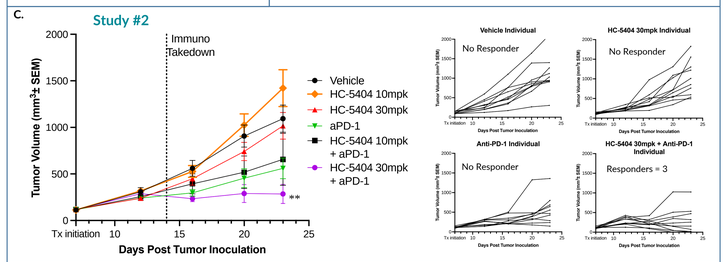


Figure 3. HC5404 treatment induces expression of myeloid (M1- and M2- related), lymphoid (T cell-related), and interferon stimulated genes (ISG). RNA samples from day 7 (post treatment) MB49 tumors were analyzed for the transcriptional changes of indicated genes by QuantiGene Plex assay on a Luminex platform. Results are presented in a heatmap showing the Log2 fold changes over vehicle.

RESULTS

PERK INHIBITOR HC-5404 SHOWS COMBINATION BENEFIT WITH ANTI-PD-1 IN A SYNGENEIC BLADDER CANCER MODEL



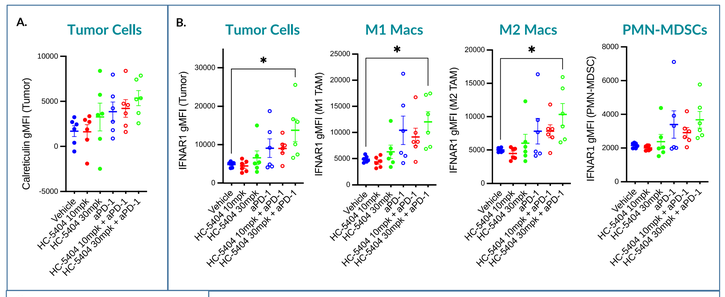


D.

TGI %	Treatment			
	Vehicle	HC-5404 30 mpk	aPD-1	HC-5404 30 mpk + aPD-1
Study #1	0.00	52.99	55.27	**74.96**
Study #2	0.00	10.64	50.75	**74.93**
Average	0.00	31.82	53.01	**74.95**

Figure 4. HC-5404 demonstrates combination effect with anti-PD-1 in the syngeneic MB49 model. (A) Study design. Anti-PD-1 mAb was from BioXell (cat # BE0146; clone # RPM1.14). (B) Dose study of HC-5404 in combination with anti-PD-1. (C) HC-5404 + anti-PD-1 efficacy study with selective individual responses. Number of responders in the treatment group are indicated. (D) Table of TGI % from two independent studies. One-way ANOVA with Dunnett for multiple comparison was used. **P < 0.01; ***P < 0.001; ****P < 0.0001.

MODULATION OF EXPESSION OF CALRETICULIN (CALR), IFNAR1 AND PD-L1 IN HC-5404 + ANTI-PD-1–TREATED MB49 TUMORS



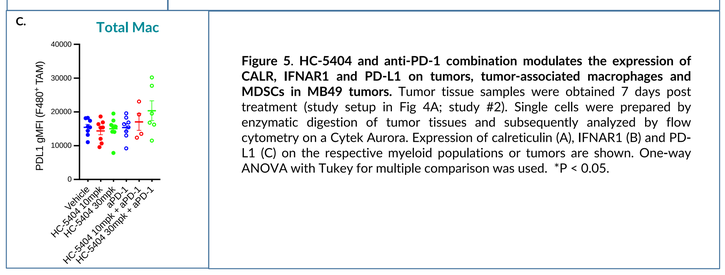


Figure 5. HC-5404 and anti-PD-1 combination modulates the expression of CALR, IFNAR1 and PD-L1 on tumors, tumor-associated macrophages and MDSCs in MB49 tumors. Tumor tissue samples were obtained 7 days post treatment (study setup in Fig 4A; study #2). Single cells were prepared by enzymatic digestion of tumor tissues and subsequently analyzed by flow cytometry on a Cytek Aurora. Expression of calreticulin (A), IFNAR1 (B) and PD-L1 (C) on the respective myeloid populations or tumors are shown. One-way ANOVA with Tukey for multiple comparison was used. *P < 0.05.

EFFECT OF HC-5404 + PD-1 COMBO TREATMENT ON LYMPHOID POPULATIONS IN MB49 TUMOR

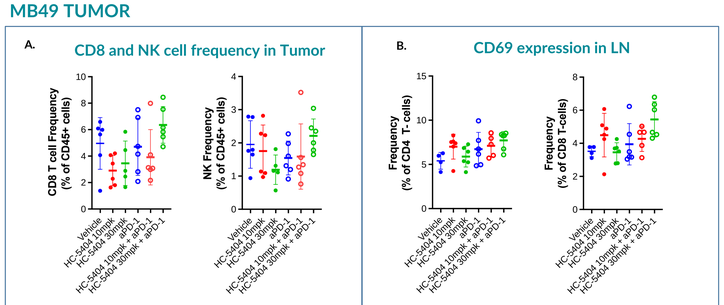


Figure 6. HC-5404 + anti-PD-1 combo treatment regulates the frequency of tumor infiltrated CD8 T and NK cells and the early T-cell activation in LN. (A) Frequency of CD8 T and NK cells on day 7 tumor samples (study #2) and (B) CD69 expression on CD4 and CD8 T cells of day 7 LN samples (study #2) were determined by flow cytometry.

RESULTS

HC-5404 + PD-1 COMBO TREATMENT ENHANCES DC & T CELL FUNCTIONS IN LN

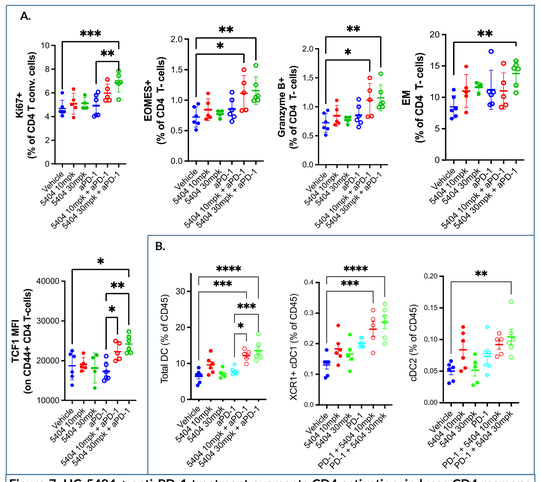


Figure 7. HC-5404 + anti-PD-1 treatment augments CD4 activation, induces CD4 memory T cells, and expands DC populations in the LN of MB49 tumor bearing mice. LN cells from day 14 post treatment samples were analyzed by flow cytometry. (A) Expression of Ki67, EOMES, and Granzyme B on CD4+ T cell, effector memory (EM) population and TCF-1 expression on activated T cells were shown. EM CD4 cells are defined as CD62L$^-$ CD44$^+$ CD4$^+$. (B) Total DC is identified as CD11c$^+$ MHCII$^+$ CD11b$^{dim/-}$, cDC1 is identified as XCR1$^+$ DC, and cDC2 is identified as XCR1$^-$ CD24$^+$ DC. One-way ANOVA with Tukey for multiple comparison was used. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

HC-5404 MODIFIES THE FUNCTION OF HUMAN MDSC *IN VITRO*

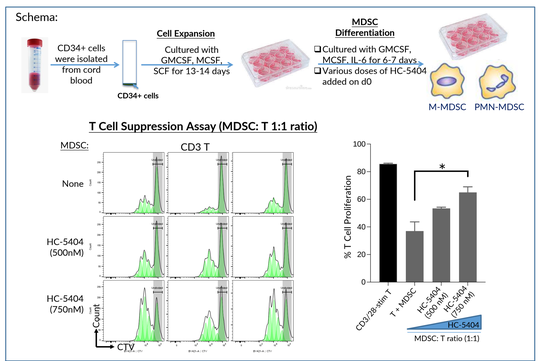


Figure 8. HC-5404 reduces the suppressive capacity of cultured human MDSC *in vitro*. Schema of the preparation of differentiated human MDSC from cord blood. MDSC suppressive activity was measured by a standard T cell suppression Assay. Briefly, cell trace violet (CTV)-labeled human CD3 T cells were activated with Dynabead Human T-activator and co-cultured with MDSC for 3-5 days. T cell proliferation was analyzed via CTV dilution by flow cytometry. Histogram plots of CTV dilution in CD3 T cells and the summary graph are shown. One-way ANOVA with Dunnett for multiple comparison was used. *P < 0.05.

CONCLUSIONS

Our results show

1. HC-5404 demonstrates combination benefits with anti-PD-1 treatment in the syngeneic MB49 mouse bladder model
2. HC-5404 + anti-PD-1 combo treatment efficacy correlates with increased expression of IFNAR1 and increased surface calreticulin on tumor cells
3. The combo treatment also induces
   - Upregulation of IFNAR1 expression on PMN-MDSCs and TAM, and PD-L1 expression on TAM
   - Regulation of CD8 T cell frequency in the tumor along with an upward trend in expression of activation marker CD69 on T-cells in LN
4. HC-5404 as single agent activates the ICD pathway, induces IFNAR1 on peripheral blood monocytes, modulates myeloid, lymphoid and ISG gene signature and diminishes human MDSC suppressiveness *in vitro*.

Taken together, the current study shows the efficacious and immuno-stimulatory effects of HC-5404 co-administered with anti-PD1 mAb and outline its potential application in ICI-treated cancers.

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