968 Activation of GCN2 by HC-7366 results in significant antitumor efficacy as monotherapy and in combination with multiple standard of care agents in various solid cancer models

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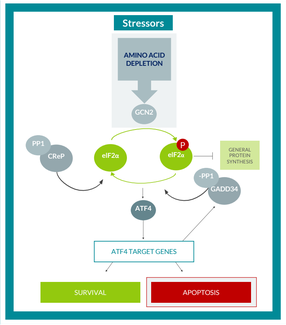
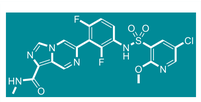
ABSTRACT

The integrated stress response (ISR) is an adaptive signaling pathway that cells utilize to respond to a wide range of extrinsic and intrinsic stresses, which are important for tumorigenesis. Activation of ISR is suggested to play a dual role in cell fate decisions. While the ISR promotes survival, prolonged activation of ISR induces apoptosis. We are developing HC-7366, a first-in-class, first-in-human GCN2 activator, and are currently evaluating it in a phase 1 clinical trial in solid tumors (NCT05121948). In this study, we present the characterization of the antitumor effects of HC-7366 in solid tumors.

In vivo efficacy studies using HC-7366 showed significant tumor growth inhibition (TGI%) in preclinical cancer models of colorectal (78-95%), head and neck (33% regression), sarcoma (80%) and prostate (65%). HC-7366 activated the ISR in tumors from treated mice as evidenced by induction of the ATF4 target genes ASNS and PSAT1. Additionally, HC-7366 induced the proapoptotic protein PUMA and reduced HIF1α and HIF2α levels. Furthermore, HC-7366 showed significant combination benefit in colorectal models when coadministered with DC101 (anti-VEGFR2 antibody), 5-fluorouracil (chemotherapy), alpelisib (PI3Ka inhibitor), or trametinib (MEK1/2 inhibitor). Using GCN2 CRISPR-knockout cells, we confirmed that the HC-7366 mediated reduction of cell growth and induction of ISR markers was dependent on GCN2. We performed multi-omics analyses to further understand the mechanism of action. Metabolomics analysis of tumors treated with HC-7366 revealed that HC-7366 altered several metabolites involved in amino acid metabolism, oxidative stress, the urea cycle, and pyrimidine biosynthesis. Additionally, proteomics analysis showed that HC-7366 significantly reduced proteins involved in oxidative phosphorylation. Analysis of the transcriptome in tumors from treated mice demonstrated that HC-7366 reduced the activity of HIF and E2F1-driven transcription, including expression of metaphase-anaphase transition genes, consistent with decreased Ki67 staining in tumors. ATF4 and JUN transcriptional activity was enhanced with HC-7366 treatment consistent with activation of ISR. Collectively, our *in vitro* and *in vivo* results demonstrate that HC-7366 is a potent GCN2 activator with strong antitumor activity across multiple solid tumor models as a monotherapy or in combination with standard of care agents.

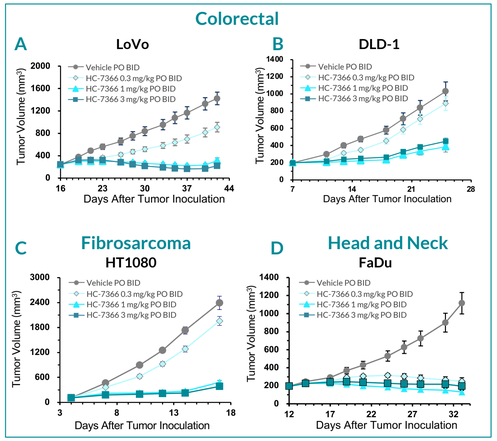
BACKGROUND





GCN2 in the Integrated Stress Response (ISR). GCN2 responds to amino acid deprivation. Activated GCN2 phosphorylates eIF2α, attenuating global translation but enhancing the translation of the transcription factor ATF4, resulting in survival or apoptosis depending on the context.

Colorectal



RESULTS

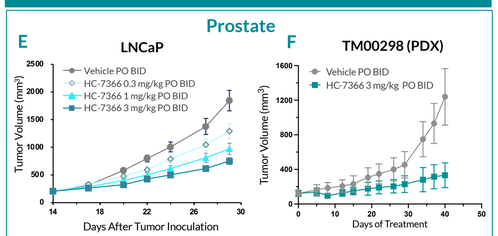


Figure 1: HC-7366 shows potent single agent activity in solid tumors including regressions. A. TGI of 94% in LoVo CRC model with single agent at 1 and 3 mg/kg, respectively. **B.** HC-7366 exhibits ~78% TGI in DLD-1 CRC model at 1 and 3 mg/kg. **C.** HC-7366 shows single agent tumor growth inhibition (TGI) of up to 80% in HT1080 fibrosarcoma model at 1 and 3 mg/kg. **D.** HC-7366 shows regression of ~33% in FaDu head and neck squamous cell model with single agent treatment at 1 mg/kg. **E.** HC-7366 showed TGI of ~61-65% at doses ≤ 3 mg/kg in LNCaP AR positive prostate xenograft model. **F.** HC-7366 achieved TGI of ~70% at 3 mg/kg in TM00298 AR positive prostate patient derived xenograft model. All treatments were administered orally and twice daily until end of study.

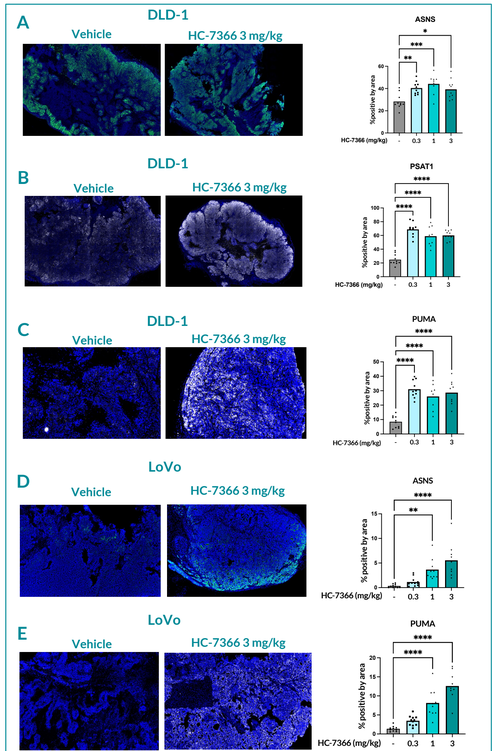


Figure 2: HC-7366 potently activates GCN2/ISR in tumors. HC-7366 treated DLD-1 tumors (Fig 1C) were evaluated for ISR markers by IHC at the end of study (day 20). HC-7366 potently induced ASNS **(A)**, PSAT1 **(B)** and PUMA **(C)**. **D.** HC-7366 significantly induced ASNS and PUMA **(E)** in end of study LoVo CRC tumors. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, one-way ANOVA.

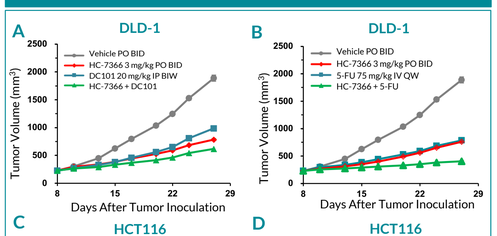


Figure 3: HC-7366 shows potent combination benefit with several standard of care agents. A. HC-7366 at 3 mg/kg inhibited tumor growth with TGI% value of ~65%, while DC101 (15 mg/kg, IP, QIW) resulted in a TGI% value of ~55% and **B.** 5-FU treatment ~68%. There was a significant combination benefit observed when 3 mg/kg HC-7366 was combined with **(A)** DC101 (~76%) or **(B)** 5-FU (~88%; tumor stasis) **C.** HC-7366 showed a trend (~38%TGI) for efficacy at 3 mg/kg dose in HCT116 CRC model. Significant combination benefit was observed when Alpelisib was co-administered with HC-7366. **D.** HC-7366 did not show efficacy at 3 mg/kg but had significant combination benefit when combined with Trametinib in HCT116 CRC xenografts. DC101 equivalent in humans is Cyramza.

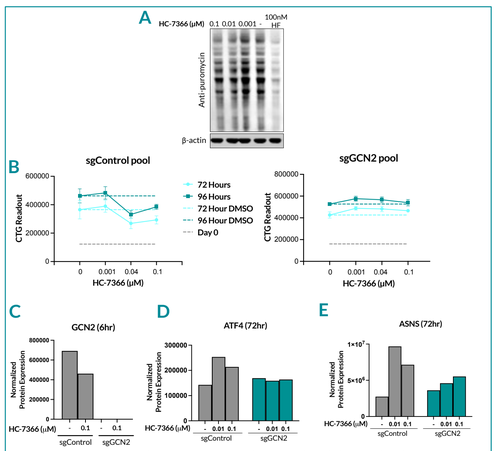


Figure 4: HC-7366 reduces protein synthesis and viability and induces ISR in a GCN2-dependent manner. A. HC-7366 potently reduced puromycin labeling of newly synthesized proteins at 100 nM in FaDu cells after 6hrs. 100nM Halofuginone (HF) treatment for 6hrs was used as a control. **B.** FaDu cells were treated with different concentrations of HC-7366 and viability was assessed by Cell Titer Glo (CTG) assay after 72 and 96 hour of treatment. HC-7366 potently reduced viability in CRISPR control cells. This response was reversed in GCN2 CRISPR knockout cells. **C.** Protein expression of GCN2 in WT and CRISPR GCN2 knockout cells as measured by JESS confirms knockout status of GCN2 CRISPR cells. **D.** HC-7366 mediated activation of ISR was assessed by looking at ATF4 and its downstream target ASNS (**E**) protein expression which showed a GCN2-dependent increase in both ATF4 and ASNS with HC-7366 at 0.01 and 0.1 µM concentration.

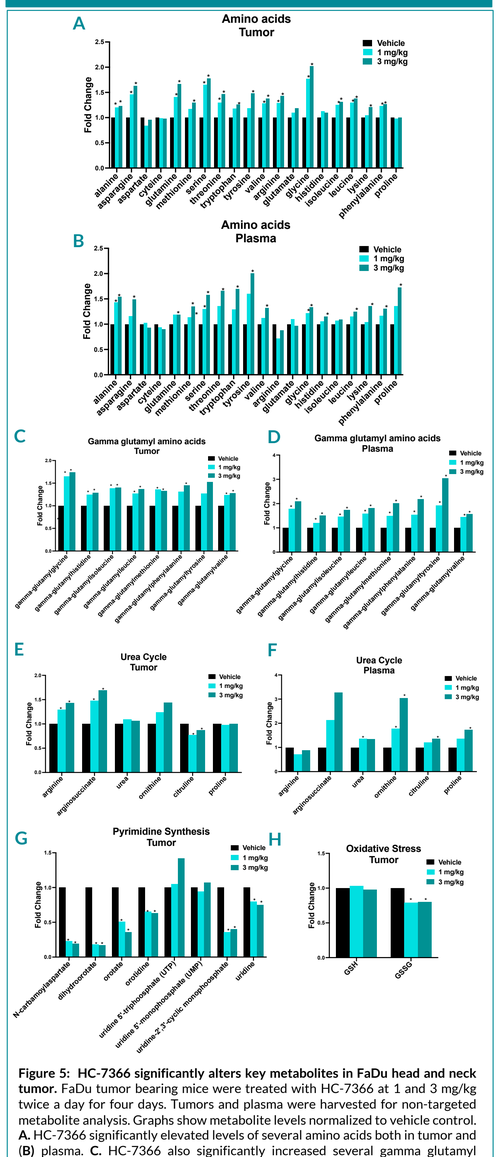


Figure 5: HC-7366 significantly alters key metabolites in FaDu head and neck tumor. FaDu tumor bearing mice were treated with HC-7366 at 1 and 3 mg/kg twice a day for four days. Tumors and plasma were harvested for non-targeted metabolite analysis. Graphs show metabolite levels normalized to vehicle control. **A.** HC-7366 significantly elevated levels of several amino acids both in tumor and **(B)** plasma. **C.** HC-7366 also significantly increased several gamma glutamyl amino acid levels in both tumor and **(D)** plasma. **E.** HC-7366 significantly increased levels of arginine, arginosuccinate and citruline in tumors and significantly increased urea levels in the plasma **(F). G.** HC-7366 significantly reduced pyrimidine synthesis metabolites in tumors. **H.** HC-7366 significantly reduced oxidized glutathione levels but did not alter reduced glutathione levels in tumor. Statistically significant changes have an asterisk * (p ≤ 0.05)

RESULTS

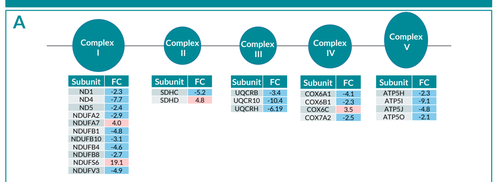
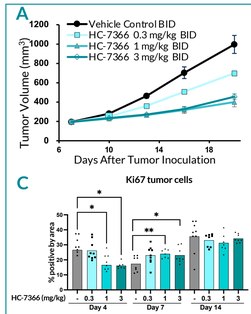
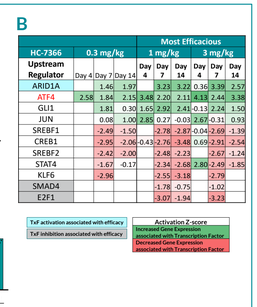


Figure 6: HC-7366 activating dose reduces proteins involved in oxidative phosphorylation. FaDu tumor bearing mice were treated with HC-7366 at 3 mg/kg twice a day for four days. Tumors were harvested for non-targeted proteomic analysis. **A.** Several protein subunits of the electron transport chain are decreased in the 3 mg/kg tumor (relative to vehicle). Data suggest that the GCN2 activating dose of 3 mg/kg HC-7366 inhibits mitochondrial respiration in tumors, representing a possible MOA that is unique to this dose. FC= fold change, blue color filling indicates downregulated proteins, red color filling indicates upregulated proteins.





HC-7366	0.3 mg/kg			Most Efficacious 1 mg/kg			Most Efficacious 3 mg/kg		
Upstream Regulator	Day 4	Day 7	Day 14	Day 4	Day 7	Day 14	Day 4	Day 7	Day 14
ARID1A		1.46	1.97		3.23	3.22	0.36	3.39	2.57
ATF4	2.58	1.84	2.15	3.48	2.20	2.11	4.13	2.44	3.38
GLI1		1.81	0.30	1.65	2.92	2.41	0.13	2.24	1.50
JUN		0.08	1.00	2.85	0.27	-0.03	2.67	-0.31	0.93
SREBF1		-2.49	-1.50		-2.78	-2.87	-0.04	-2.69	-1.39
CREB1		-2.95	-2.06	-0.43	-2.76	-3.48	0.69	-2.91	-2.54
SREBF2		-2.42	-2.00		-2.48	-2.23		-2.67	-1.24
STAT4		-1.67	-0.17		-2.34	-2.68	2.80	-2.49	-1.85
KLF6		-2.96			-2.55	-3.18		-2.79	
SMAD4					-1.78	-0.75		-1.02	
E2F1					-3.07	-1.94		-3.23	

TxF activation associated with efficacy
TxF inhibition associated with efficacy

Activation Z-score
Increased Gene Expression associated with Transcription Factor
Decreased Gene Expression associated with Transcription Factor

Figure 7: RNAseq analysis of HC-7366 treated tumor shows reduction of SMAD4 and E2F1 signature while inducing ATF4 and ARID1A signature in DLD-1 CRC tumor model. A. DLD-1 tumor bearing mice were treated with HC-7366 at 0.3, 1 and 3 mg/kg twice a day for 14 days. Efficacy graph is shown. Tumors were harvested for RNAseq analysis at Day 4, Day 7 and Day 14. **B.** RNAseq analysis of upstream regulators of differentially expressed genes showed that ATF4 was significantly induced at all doses of HC-7366. Certain transcription factors associated with efficacy. These included ARID1A, SMAD4 and E2F1. **C.** IHC staining of Ki67 positive cells in DLD-1 tumor sections shows that HC-7366 significantly reduces Ki67 positive cancer cells on Day 4 at the most efficacious doses of 1 and 3 mg/kg but not at the less efficacious dose of 0.3 mg/kg.

SUMMARY/CONCLUSIONS

- The GCN2 modulator HC-7366 has potent monotherapy efficacy in multiple solid cancer models
- HC-7366 treatment leads to ISR activation as evidenced by multiple, clinically translatable PD markers (ASNS and PSAT1) and upregulates apoptosis markers such as PUMA
- HC-7366 shows significant combinatorial benefit with DC101, 5FU, Trametinib and Alpelisb in CRC models
- HC-7366 significantly altered several metabolites involved in amino acid metabolism, oxidative stress, pyrimidine synthesis and urea cycle
- Transcriptomic analysis in HC-7366 treated tumors demonstrated that HC-7366 reduced the activity of SMAD4 and E2F1-driven transcription, including expression of metaphase-anaphase transition genes, consistent with decreased Ki67 staining in tumors
- HC-7366 is a first in human GCN2 modulator that is currently in a phase1 clinical trial focused on solid tumors (NCT05121948)

ACKNOWLEDGMENT

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