BMB Reports - Manuscript Submission

Manuscript Draft

**Manuscript Number**: BMB-22-037

**Title**: Synergistic antitumor activity of sorafenib and MG149 in hepatocellular carcinoma cells

**Article Type**: Article

**Keywords**: sorafenib; MG149; ER stress; cell death; hepatocellular carcinoma

**Corresponding Author**: Jung-Ae Kim

**Authors**: Byul Moon[1,2], Mijin Park[1,2], Seung-Hyun Cho[1,3,4], Kang Mo Kim[5], Haeng Ran Seo[6], Jeong-Hoon Kim[1,3], Jung-Ae Kim[1,2,*]

**Institution**: [1]Department of functional genomics, KRIBB school of bioscience, University of science and technology,
[2]Personalized genomic medicine research center and [3]Disease target structure research center, Korea research institute of bioscience and biotechnology,
[4]R&D Center, Uppthera,
[5]Department of Gastroenterology, Asan Liver Center, Asan Medical Center, University of Ulsan College of Medicine,
[6]Advanced Biomedical Research Laboratory, Institut Pasteur Korea,

**Manuscript Type:** Article

**Title:** Synergistic antitumor activity of sorafenib and MG149 in hepatocellular carcinoma cells

**Author's name:** Byul Moon[1,2], Mijin Park[1,2], Seung-Hyun Cho[1,3,4], Kang Mo Kim[5], Haeng Ran Seo[6], Jeong-Hoon Kim[1,3*], Jung-Ae Kim[1,2*]

**Affiliation:** [1]Department of Functional Genomics, KRIBB School of Bioscience, University of Science and Technology (UST), Daejeon 34113, South Korea

[2]Personalized Genomic Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, 34141, South Korea

[3]Disease Target Structure Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, South Korea

[4]Current address: Uppthera R&D center, Incheon 21988, Republic of Korea

[5]Department of Gastroenterology, Asan Liver Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, Korea

[6]Advanced Biomedical Research Laboratory, Institut Pasteur Korea, Seongnam-si 13488, Korea

**Running Title:** Synergistic effect of MG149 and sorafenib on HCC

**Keywords:** Sorafenib, MG149, ER stress, cell death, hepatocellular carcinoma

**Corresponding Author's Information:**

J.-H. Kim (Tel: +82-42-860-4264; E-mail: jhoonkim@kribb.re.kr) and J.-A. Kim (Tel: +82-42-

879-8129; E-mail: jungaekim@kribb.re.kr)

**ABSTRACT**

Advanced hepatocellular carcinoma (HCC) is among the most challenging cancers to overcome, and there is a need for better therapeutic strategies. Among the different cancer drugs that have been used in clinics, sorafenib is considered the standard first-line drug for advanced HCC. Here, to identify a chemical compound displaying a synergistic effect with sorafenib in HCC, we screened a focused chemical library and found that MG149, a histone acetyltransferase inhibitor targeting the MYST family, exhibited the most synergistic anticancer effect with sorafenib on HCC cells. The combination of sorafenib and MG149 exerted a synergistic anti-proliferation effect on HCC cells by inducing apoptotic cell death. We revealed that cotreatment with sorafenib and MG149 aggravated endoplasmic reticulum (ER) stress to promote the death of HCC cells rather than adaptive cell survival. In addition, combined treatment with sorafenib and MG149 significantly increased the intracellular levels of unfolded proteins and reactive oxygen species, which upregulated ER stress. Collectively, these results suggest that MG149 has the potential to improve the efficacy of sorafenib in advanced HCC via the upregulation of cytotoxic ER stress.

## INTRODUCTION

Sorafenib is a clinically approved anticancer drug that has been considered the gold standard therapy in advanced hepatocellular carcinoma (HCC) (1). However, the efficacy of sorafenib in HCC is moderate, increasing the overall survival of sorafenib-treated patients by only ~3 months compared with that of the placebo groups (1, 2). For decades, extensive efforts have been made to treat advanced HCC more efficiently by generating novel anti-HCC drugs However, the efficacies of new drugs barely reach those of sorafenib in advanced HCC (3). Thus, sorafenib remains a major therapeutic option as the first-line therapy for many advanced HCC patients and is even used as a subsequent treatment for patients pretreated with new drugs such as lenvatinib (3). These findings highlight that the value in exploring a therapeutic approach to improve the efficacy of sorafenib in HCC.

Extensive studies have shown that multiple mechanisms, such as the phosphatidylinositol 3-kinase (PI3K)/AKT, Janus kinase/signal transducers and activators of transcription (JAK/STAT), transforming growth factor beta (TGF-β), and Wnt/Notch signaling pathways, are involved in sorafenib resistance in HCC cells (4). These studies showed that upregulation of these pathways is intimately linked to compensating for the anticancer effect of sorafenib. However, simultaneous targeting of these signaling pathways along with sorafenib treatment has not improved the prognosis of HCC patients in phase III clinical trials (5).

The sorafenib-induced unfolded protein response (UPR) coupled with ER stress (6) is another mechanistic axis involved in sorafenib resistance in HCC cells (7-9). ER stress accompanied by autophagy protects cells from stress-induced death, while prolonged ER stress induces cell death (10). In line with this, inhibition of autophagy was shown to increase the sensitivity of HCC cells to sorafenib (11). In addition, suppression of UPR activation via stearoyl-CoA desasturase-1 (SCD1) reduced sorafenib resistance in HCC cells (12). These studies suggest that targeting ER stress coupled with UPR is another promising approach to increase sorafenib

efficacy in HCC.

To find a new combination partner that showed a synergistic effect with sorafenib in HCC, we explored a focused chemical library and found MG149 to be a promising candidate for modulating the ER stress pathways in sorafenib-treated cells and synergistically increase the death of HCC cells.

## RESULTS

### The combination of sorafenib and MG149 mediates synergistic defects in HCC cell viability

Focused chemical libraries have been used for screening potent small molecules to target cancer cells (13, 14). To identify chemical compounds exhibiting a combinatorial effect with sorafenib on suppressing HCC, we screened a Selleckem library composed of inhibitors of different epigenetic regulators (15) for viability of Huh7 HCC cells by measuring cellular ATP with CellTiter-Glo (16). Given that the IC50 of sorafenib for Huh7 viability was ~ 3 μM (Figure 1A), we treated Huh7 cells with 10 μM of each library compound along with 3 μM of sorafenib for 72 hrs and compared the viability of the samples with those of samples treated with a single agent only. Among the 93 different small molecules in the library, anacardic acid and MG149, both of which are histone acetyltransferase (HAT) inhibitors (17, 18), were identified as those of the 4 prominent positive hits (anacardic acid, GSK126, SMI-4A, MG149), the combination of which with sorafenib increased the inhibition effect by 30% compared with treatment with a single reagent alone (Figure 1B). Anacardic acid, a natural product from cashew nut shell extract, is a non-selective inhibitor of HAT (19). Notably, GSK126, a histone methyltransferase inhibitor, was recently identified as a combination partner with sorafenib in HCC cells (20). Also, the involvement of Pim kinase, the genetic target of SMI-4A, in sorafenib resistance was previously disclosed (21). Thus, of the other hit compounds, we decided to further investigate the effect of MG149 as a potential combination drug with sorafenib.

To examine the combined effect of MG149 and sorafenib in different cellular contexts, we determined the IC50 of sorafenib on the viability of different HCC cell lines (Huh7, Hep3B, and HepG2) with or without MG149 by measuring cellular metabolic activities with EzCytox (22). In all the HCC cells tested, the combination with MG149 decreased the IC50 of sorafenib (Supplementary Figure 1). Then, to evaluate whether the combinatorial effect was synergistic,

we treated HCC cells with various concentrations of sorafenib, MG149, or combinations of both reagents in a constant ratio. As shown in Figure 1C, relative cell viability was reduced in all three HCC cell lines treated with the combination of sorafenib and MG149 in a dose-dependent manner. Given these experimental data, we calculated the combination index (CI) with CompuSyn software (23) and found that most of the CI values were less than 1 (Figure 1D, Table 1). Together, these results suggest that sorafenib in combination with MG149 had a synergistic inhibitory effect on HCC cell viability.

**Treatment with MG149 combined with sorafenib significantly induces the apoptotic death of HCC cells**

To determine whether the impaired viability of HCC cells cotreated with sorafenib and MG149 was linked to the induction of cell death, we monitored cell growth in real time for 72 hrs in the presence of propidium iodide (PI), which stained dead cells. Compared with treatment with either sorafenib or MG149 alone, the combination of sorafenib and MG149 increased PI uptake in Huh7, Hep3B, and HepG2 cells (Figure 2A). In contrast, the confluency of HCC cells cotreated with sorafenib and MG149 was significantly lower than that of cells treated with a single reagent (Figure 2B). These results indicate that the combination of sorafenib and MG149 mediated cell death rather than cell cycle arrest or quiescence.

To elucidate the cell death mechanisms induced by the combination of sorafenib and MG149, we then analyzed dead cells by staining with PI and annexin-V, which indicates early apoptotic status. Combined treatment with sorafenib and MG149 resulted in 11.03%, 21.68%, and 36.33% of Huh7, Hep3B, and HepG2 cells costained with PI and annexin-V, respectively (Figure 2C). The double-stained fractions were higher than those treated with either sorafenib or MG149 alone. The population of annexin-V-stained cells with or without PI staining was also significantly higher in cells cotreated with sorafenib and MG149 than in those treated with each

single reagent (Figure 2D).

Dead cells under sorafenib and MG149 treatment were further examined by a TUNEL assay visualizing fragmented DNA in apoptotic cells. We observed that the levels of TUNEL staining significantly increased when HCC cells were treated with a combination of sorafenib and MG149 compared with those treated with a single agent alone (Supplementary Figures 2A and 2B). Together, these results demonstrate that the combinatorial effects of sorafenib and MG149 on the apoptotic death of HCC cells were larger than those mediated by sorafenib or MG149 alone.

**The combination of sorafenib and MG149 hyperactivates endoplasmic reticulum (ER) stress signaling in HCC cells**

Sorafenib is known to induce ER stress (6), which can mitigate the efficacy of sorafenib and mediate apoptosis resistance (8, 9). Considering these previous findings, we hypothesized that MG149 might suppress sorafenib-induced ER stress involved in cell survival and thus accelerate the cytotoxic effect of sorafenib. To test this hypothesis, we determined the activity of the ER stress response in HCC cells by assessing the transcript levels of the well-known ER stress markers CHOP, IRE1a, and sXBP1 (24). Unexpectedly, while sorafenib or MG149 alone slightly increased the transcript levels, the combination of sorafenib and MG149 significantly elevated the expression of these ER stress markers (Figure 3A). Consistently, phosphorylation of IRE1a (p-IRE1a) and abundance of CHOP protein, which reflect the activity of the ER stress pathway (25), were also prominently enhanced in HCC cells cotreated with sorafenib and MG149 (Figure 3B). These results demonstrate that the combination of sorafenib and MG149 hyperactivated the ER stress response in HCC cells.

We note that cotreatment with sorafenib and MG149 largely decreased the phosphorylation of eIF2a (p-eIF2a) in Hep3B and HepG2 cells, while treatment with either sorafenib or MG149

alone slightly induced p-eIF2a levels. Unlike the findings in Hep3B and HepG2 cells, the combination of sorafenib and MG149 did not reduce p-eIF2a in Huh7 cells. Prolonged ER stress has been known to trigger negative feedback for eIF2a activity through the expression of GADD34, a regulatory subunit of the eIF2a phosphatase PP1 (26). Given this finding, we postulated that the combination of sorafenib and MG149 might highly upregulate GADD34 expression and thus dephosphorylate eIF2a via negative feedback. Consistent with this hypothesis, we observed that cotreatment with sorafenib and MG149 highly elevated GADD34 expression in HepG2 and Hep3B cells but not in Huh7 cells (Supplementary Figure 3). These findings imply that combination treatment of sorafenib and MG149 hyperactivates ER stress signaling in HCC cells and that the magnitude of ER stress induction by the combination is dependent on cell context.

**Autophagy activity is elevated by combination treatment with sorafenib and MG149 in HCC cells**

Autophagy is an essential protective mechanism to alleviate ER stress-induced cell death (27, 28). To examine whether downregulation of autophagy might underlie the synergistic cytotoxic activity of sorafenib and MG149, we determined the effect of both drugs on the level of LC3B protein, which reflects autophagy activity (Figure 3C). In contrast to treatment with sorafenib alone, the combination of sorafenib and MG149 significantly increased the ratio of LC3B-II to LC3B-I (Figure 3D). This finding demonstrates that cotreatment with sorafenib and MG149 amplified the autophagy activity in HCC cells.

We further examined the effect of sorafenib and MG149 on autophagic flux in HepG2 cells by assessing GFP-LC3-RFP protein, a fluorescent reporter used to measure autophagic degradation (29). At 48 hrs after drug treatment, we observed that the combination of sorafenib with MG149 prominently reduced cellular GFP signals (Figure 3E). The intensity of GFP over

the internal control RFP was significantly reduced by simultaneous treatment with sorafenib and MG149 (Figure 3F). Altogether, these findings indicate that the combination of sorafenib and MG149 largely elevated rather than suppressed autophagic activity in HCC cells.

**Cotreatment with sorafenib and MG149 induces oxidative stress and misfolded protein aggregates.**

The accumulation of unfolded proteins in the ER is a major signal that activates the ER stress response pathway to cope with proteotoxic stress (28). Given that the combination of sorafenib and MG149 hyperactivated ER stress accompanied by autophagy activity, we examined whether the combination generated a greater unfolded protein burden in HCC cells. To identify whether misfolded proteins were increased by the combination treatment, we stained Huh7, HepG2, and Hep3B cells with a molecular rotor dye, ProteoStat, which becomes fluorescent when intercalated into structures of misfolded protein aggregates (30). In all three HCC cell lines, the combination of sorafenib and MG149 mediated the accumulation of misfolded protein aggresomes in the cytoplasm (Figure 3G). Aggregates detected by fluorescence imaging analysis were quantified and normalized to the number of cells measured by DAPI signals. All the cells treated with the combination displayed levels of aggresomes higher than five times that of those treated with sorafenib or MG149 alone (Supplementary Figure 4). This finding implies that the combination of sorafenib and MG149 robustly induces protein unfolding, which can activate ER stress engaged with autophagy activity.

Since protein folding is highly redox-dependent, excessive reactive oxygen species (ROS) can mediate protein misfolding to generate aggresomes (31). Thus, we measured the effect of the sorafenib/MG149 combination on the level of intracellular ROS in HCC cells. As previously reported, ROS generation was elevated in all HCC cells treated with sorafenib alone (32), but treatment with MG149 alone increased the ROS level in HepG2 cells only (Figure 3H). In

contrast, when sorafenib was coupled with MG149, the levels of ROS in HCC cells were considerably higher than the treatment of each agent alone. Collectively, these findings demonstrate that cotreatment with sorafenib and MG149 exerted an impact on the accumulation of misfolded proteins and ROS production in HCC cells.

**Sorafenib and MG149 display synergistic cytotoxic effects in patient-derived HCC cells**

We then examined the combinatorial effect of sorafenib and MG149 on patient-derived HCC cells, AMC-H1 and AMC-H2, which displayed distinctive morphology (Figure 4A) (33). Consistent with the findings of the conventional HCC cell lines described above, combined sorafenib and MG149 treatments significantly reduced the viability of AMC-H1 and AMC-H2 cells compared with sorafenib treatment alone (Figure 4B). In addition, CI calculated based on these data showed synergistic interactions between sorafenib and MG149 in patient-derived liver cancer cells (Figure 4C and Table 2). Real-time cell growth monitoring in the presence of PI showed that cotreatment with sorafenib and MG149 impaired cellular proliferation (Figure 4D) and increased the population of PI-stained dead cells compared with treatment with either agent alone (Figure 4E). These findings suggest that the combinatorial cytotoxic effect of sorafenib and MG149 would be efficacious in diverse HCC cells with different phenotypic features.

## DISCUSSION

ER stress and autophagy are intimately connected cellular processes that can result in both survival and death induction (34). Mild ER stress promotes cell survival by facilitating self-protection processes such as refolding and/or autophagic removal of misfolded proteins upon UPR and ER stress activation. Unresolvable ER stress leads to the induction of proapoptotic pathways. In contrast to previous studies showing that sorafenib induces ER stress to promote the adaptation and survival of HCC cells (7, 8), we showed that sorafenib combined with MG149 hyperactivated ER stress accompanied by autophagy and led to the death of HCC cells. In addition, simultaneous treatment with MG149 and sorafenib elevated protein aggregate levels, implying that the combination of these compounds failed to refold and/or remove misfolded proteins. These results suggest that MG149 and sorafenib cooperate to upregulate the unresolvable UPR-ER stress axis and thereby result in synergistic apoptosis. Accordingly, MG149 appears to confer sorafenib-induced ER stress in HCC cells being off balance to death rather than survival. These findings suggest that enhancing ER stress can surpass sorafenib resistance in HCC cells.

MG149 is reported to be a potent inhibitor against MYST family histone acetyltransferases (HATs), Tip60 and MOF (18). Thus, our initial postulation was that inactivation of Tip60 or MOF might phenocopy the effect of MG149 on the sensitivity of HCC cells to sorafenib. However, knockdown of neither Tip60 nor MOF affected sorafenib sensitivity in HCC cells (data not shown). Simultaneous loss of Tip60 and MOF also failed to elevate sorafenib sensitivity in HCC cells (data not shown). These observations implied that the combinatorial effect of MG149 and sorafenib is unlikely to be dependent on the activity of Tip60 and MOF. Many cancer drugs have been shown to induce cytotoxic effects independent of their reported targets (35). We speculate that MG149 may interact with unidentified molecular targets and thus be involved in enhancing sorafenib sensitivity in HCC cells.

Together, we demonstrated that MG149 is a potent small molecule that combines with sorafenib to induce the synergistic death of HCC cells via the upregulation of unresolvable ER stress. Future studies to define the precise molecular targets of MG149 involved in the combinatorial effect with sorafenib on HCC cells will help develop improved therapeutic strategies to treat advanced HCC.

## MATERIALS AND METHODS

Details on the used methods are provided in the expanded Materials and Methods section in the online data supplement.

## ACKNOWLEDGMENTS

This work was supported by a grant (NRF-2019R1A2C1086151 to J.-A.K.) from the National Research Foundation, Ministry of Science and ICT, and by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program (171134054 to J.-A.K. and 1711170633 to J.-H.K and J.-A.K.).

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## FIGURE LEGENDS

**Figure 1. Synergistic effect of sorafenib and MG149 on liver cancer cell proliferation**

(A) Huh7 cells were treated with various concentrations of sorafenib for 72 hrs to determine the IC50 value for sorafenib. (B) A total of 93 small molecules in the focused chemical library were ordered by 'Combination score'. (C) Cotreatment of sorafenib and MG149 in HCC cells decreased cell viability in a dose-dependent manner. (D) Synergism between sorafenib and MG149 was confirmed in all HCC cell lines. The CI value was calculated using CompuSyn software (28) (n=3). *, $p<0.05$, **, $p<0.01$, ****, $p<0.0001$, ns, not statistically significant

**Figure 2. Combination of sorafenib with MG149 induces synergistic liver cancer cell death**

(A) Cotreatment with sorafenib and MG149 facilitates cell death in all HCC cell lines. Representative images were acquired after 72 hrs of incubation with DMSO (0.1%) or drugs. Scale bars denote 300 μm. (B) Cell confluence under the indicated treatment for 72 hrs was determined by live-cell imaging analysis (n=3). (C) Apoptotic cell death was analyzed by flow cytometry. Representative data are displayed. (D) The percentage of cells positively labeled with annexin-V was quantified from three independent FACS analyses (n=3). ****, $p<0.0001$

**Figure 3. Combination of sorafenib and MG149 enhances ER stress response.**

(A) The relative mRNA levels of CHOP, IRE1, and sXBP1 (splicing form of XBP1) were determined by qPCR analysis (n=3). (B) The protein levels of IRE1α, phospho-eIF2α, and CHOP in cells were determined by western blots. (C) The expression level of LC3B was examined using western blot analysis. (D) The ratio of LC3B-II and LC3B-I was calculated from the densitometric analysis of Figure 3C. (n=3). (E) Representative fluorescent images revealed increased autophagic flux in the combination treatment (reduced intensity of GFP).

(F) The relative GFP-LC3/RFP ratio was quantified by analysis of the intensity of GFP and RFP signals from treated cells in Figure 3E (n=10). (G) In all HCC cells, augmentation of aggresomes in combination with sorafenib and MG149 was detected by a PROTEOSTAT® aggresome detection kit. (H) Increased intracellular ROS levels were found after combination treatment with sorafenib and MG149 compared with single drug treatment. **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$, ns, not statistically significant

**Figure 4. The combination of sorafenib with MG149 synergistically suppresses the viability of patient-derived liver cancer cells**

(A) Cell morphology of patient-derived liver cancer cell lines (AMC-H1 and AMC-H2). (B) MG149 enhanced the sensitivity to sorafenib at various concentrations in two patient-derived liver cancer cell lines. (n=3). (C) At various concentrations, the synergistic effect between sorafenib and MG149 was corroborated by combination index (CI) values. (D) Relative cell confluency was considerably decreased in sorafenib+MG149 in AMC-H1 and AMC-H2 cells (n=3). (E) PI images were acquired from IncuCyte Zoom software at the end of drug treatment. **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$, ns, not statistically significant

**REFERENCES**


1. Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., de Oliveira, A. C., Santoro, A., Raoul, J. L., Forner, A., Schwartz, M., Porta, C., Zeuzem, S., Bolondi, L., Greten, T. F., Galle, P. R., Seitz, J. F., Borbath, I., Häussinger, D., Giannaris, T., Shan, M., Moscovici, M., Voliotis, D. and Bruix, J. (2008) Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* **359,** 378-390.
2. Cheng, A. L., Kang, Y. K., Chen, Z., Tsao, C. J., Qin, S., Kim, J. S., Luo, R., Feng, J., Ye, S., Yang, T. S., Xu, J., Sun, Y., Liang, H., Liu, J., Wang, J., Tak, W. Y., Pan, H., Burock, K., Zou, J., Voliotis, D. and Guan, Z. (2009) Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* **10,** 25-34.
3. Rimassa, L. and Wörns, M.-A. (2020) Navigating the new landscape of second-line treatment in advanced hepatocellular carcinoma. *Liver International* **40,** 1800-1811.
4. Xia, S., Pan, Y., Liang, Y., Xu, J. and Cai, X. (2020) The microenvironmental and metabolic aspects of sorafenib resistance in hepatocellular carcinoma. *EBioMedicine* **51,** 102610.
5. Huang, A., Yang, X. R., Chung, W. Y., Dennison, A. R. and Zhou, J. (2020) Targeted therapy for hepatocellular carcinoma. *Signal Transduct Target Ther* **5,** 146.
6. Rahmani, M., Davis, E. M., Crabtree, T. R., Habibi, J. R., Nguyen, T. K., Dent, P. and Grant, S. (2007) The kinase inhibitor sorafenib induces cell death through a process involving induction of endoplasmic reticulum stress. *Mol Cell Biol* **27,** 5499-5513.
7. Zhou, T., Lv, X., Guo, X., Ruan, B., Liu, D., Ding, R., Gao, Y., Ding, J., Dou, K. and Chen, Y. (2015) RACK1 modulates apoptosis induced by sorafenib in HCC cells by interfering with the IRE1/XBP1 axis. *Oncol Rep* **33,** 3006-3014.
8. Won, J. K., Yu, S. J., Hwang, C. Y., Cho, S. H., Park, S. M., Kim, K., Choi, W. M., Cho,

H., Cho, E. J., Lee, J. H., Lee, K. B., Kim, Y. J., Suh, K. S., Jang, J. J., Kim, C. Y., Yoon, J. H. and Cho, K. H. (2017) Protein disulfide isomerase inhibition synergistically enhances the efficacy of sorafenib for hepatocellular carcinoma. *Hepatology* **66,** 855-868.

9. Zhou, B., Lu, Q., Liu, J., Fan, L., Wang, Y., Wei, W., Wang, H. and Sun, G. (2019) Melatonin Increases the Sensitivity of Hepatocellular Carcinoma to Sorafenib through the PERK-ATF4-Beclin1 Pathway. *Int J Biol Sci* **15,** 1905-1920.

10. Hetz, C. and Papa, F. R. (2018) The Unfolded Protein Response and Cell Fate Control. *Mol Cell* **69,** 169-181.

11. Shimizu, S., Takehara, T., Hikita, H., Kodama, T., Tsunematsu, H., Miyagi, T., Hosui, A., Ishida, H., Tatsumi, T., Kanto, T., Hiramatsu, N., Fujita, N., Yoshimori, T. and Hayashi, N. (2012) Inhibition of autophagy potentiates the antitumor effect of the multikinase inhibitor sorafenib in hepatocellular carcinoma. *Int J Cancer* **131,** 548-557.

12. Ma, M. K. F., Lau, E. Y. T., Leung, D. H. W., Lo, J., Ho, N. P. Y., Cheng, L. K. W., Ma, S., Lin, C. H., Copland, J. A., Ding, J., Lo, R. C. L., Ng, I. O. L. and Lee, T. K. W. (2017) Stearoyl-CoA desaturase regulates sorafenib resistance via modulation of ER stress-induced differentiation. *J Hepatol* **67,** 979-990.

13. Tanaka, K., Yu, H. A., Yang, S., Han, S., Selcuklu, S. D., Kim, K., Ramani, S., Ganesan, Y. T., Moyer, A., Sinha, S., Xie, Y., Ishizawa, K., Osmanbeyoglu, H. U., Lyu, Y., Roper, N., Guha, U., Rudin, C. M., Kris, M. G., Hsieh, J. J. and Cheng, E. H. (2021) Targeting Aurora B kinase prevents and overcomes resistance to EGFR inhibitors in lung cancer by enhancing BIM- and PUMA-mediated apoptosis. *Cancer Cell* **39,** 1245-1261.e1246.

14. Harris, I. S., Endress, J. E., Coloff, J. L., Selfors, L. M., McBrayer, S. K., Rosenbluth, J. M., Takahashi, N., Dhakal, S., Koduri, V., Oser, M. G., Schauer, N. J., Doherty, L. M., Hong, A. L., Kang, Y. P., Younger, S. T., Doench, J. G., Hahn, W. C., Buhrlage, S.

J., DeNicola, G. M., Kaelin, W. G., Jr. and Brugge, J. S. (2019) Deubiquitinases Maintain Protein Homeostasis and Survival of Cancer Cells upon Glutathione Depletion. *Cell Metab* **29,** 1166-1181.e1166.

15. Broux, M., Prieto, C., Demeyer, S., Vanden Bempt, M., Alberti-Servera, L., Lodewijckx, I., Vandepoel, R., Mentens, N., Gielen, O., Jacobs, K., Geerdens, E., Vicente, C., de Bock, C. E. and Cools, J. (2019) Suz12 inactivation cooperates with JAK3 mutant signaling in the development of T-cell acute lymphoblastic leukemia. *Blood* **134,** 1323-1336.

16. Niles, A. L., Moravec, R. A. and Riss, T. L. (2009) In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high throughput screening. *Curr Chem Genomics* **3,** 33-41.

17. Sun, Y., Jiang, X., Chen, S. and Price, B. D. (2006) Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation. *FEBS Letters* **580,** 4353-4356.

18. Ghizzoni, M., Wu, J., Gao, T., Haisma, H. J., Dekker, F. J. and George Zheng, Y. (2012) 6-alkylsalicylates are selective Tip60 inhibitors and target the acetyl-CoA binding site. *European Journal of Medicinal Chemistry* **47,** 337-344.

19. Balasubramanyam, K., Swaminathan, V., Ranganathan, A. and Kundu, T. K. (2003) Small Molecule Modulators of Histone Acetyltransferase p300 *. *Journal of Biological Chemistry* **278,** 19134-19140.

20. Kusakabe, Y., Chiba, T., Oshima, M., Koide, S., Rizq, O., Aoyama, K., Ao, J., Kaneko, T., Kanzaki, H., Kanayama, K., Maeda, T., Saito, T., Nakagawa, R., Kobayashi, K., Kiyono, S., Nakamura, M., Ogasawara, S., Suzuki, E., Nakamoto, S., Yasui, S., Mikata, R., Muroyama, R., Kanda, T., Maruyama, H., Kato, J., Mimura, N., Ma, A., Jin, J., Zen, Y., Otsuka, M., Kaneda, A., Iwama, A. and Kato, N. (2021) EZH1/2 inhibition augments

the anti-tumor effects of sorafenib in hepatocellular carcinoma. *Scientific Reports* **11,** 21396.

21. Green, A. S., Maciel, T. T., Hospital, M. A., Yin, C., Mazed, F., Townsend, E. C., Pilorge, S., Lambert, M., Paubelle, E., Jacquel, A., Zylbersztejn, F., Decroocq, J., Poulain, L., Sujobert, P., Jacque, N., Adam, K., So, J. C., Kosmider, O., Auberger, P., Hermine, O., Weinstock, D. M., Lacombe, C., Mayeux, P., Vanasse, G. J., Leung, A. Y., Moura, I. C., Bouscary, D. and Tamburini, J. (2015) Pim kinases modulate resistance to FLT3 tyrosine kinase inhibitors in FLT3-ITD acute myeloid leukemia. *Sci Adv* **1,** e1500221.
22. Yu, H., Kim, D. J., Choi, H. Y., Kim, S. M., Rahaman, M. I., Kim, Y. H. and Kim, S. W. (2021) Prospective pharmacological methodology for establishing and evaluating anti-cancer drug resistant cell lines. *BMC Cancer* **21,** 1049.
23. Chou, T.-C. (2010) Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. *Cancer Research* **70,** 440-446.
24. Oslowski, C. M. and Urano, F. (2011) Measuring ER stress and the unfolded protein response using mammalian tissue culture system. *Methods Enzymol* **490,** 71-92.
25. Oyadomari, S. and Mori, M. (2004) Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death & Differentiation* **11,** 381-389.
26. Novoa, I., Zeng, H., Harding, H. P. and Ron, D. (2001) Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J Cell Biol* **153,** 1011-1022.
27. Ogata, M., Hino, S., Saito, A., Morikawa, K., Kondo, S., Kanemoto, S., Murakami, T., Taniguchi, M., Tanii, I., Yoshinaga, K., Shiosaka, S., Hammarback, J. A., Urano, F. and Imaizumi, K. (2006) Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* **26,** 9220-9231.
28. Hetz, C., Chevet, E. and Oakes, S. A. (2015) Proteostasis control by the unfolded

protein response. *Nat Cell Biol* **17,** 829-838.

29. Kaizuka, T., Morishita, H., Hama, Y., Tsukamoto, S., Matsui, T., Toyota, Y., Kodama, A., Ishihara, T., Mizushima, T. and Mizushima, N. (2016) An Autophagic Flux Probe that Releases an Internal Control. *Mol Cell* **64,** 835-849.

30. Shen, D., Coleman, J., Chan, E., Nicholson, T. P., Dai, L., Sheppard, P. W. and Patton, W. F. (2011) Novel Cell- and Tissue-Based Assays for Detecting Misfolded and Aggregated Protein Accumulation Within Aggresomes and Inclusion Bodies. *Cell Biochemistry and Biophysics* **60,** 173-185.

31. Wang, C., Yu, J., Huo, L., Wang, L., Feng, W. and Wang, C.-c. (2012) Human Protein-disulfide Isomerase Is a Redox-regulated Chaperone Activated by Oxidation of Domain a'. *Journal of Biological Chemistry* **287,** 1139-1149.

32. Coriat, R., Nicco, C., Chéreau, C., Mir, O., Alexandre, J., Ropert, S., Weill, B., Chaussade, S., Goldwasser, F. and Batteux, F. (2012) Sorafenib-Induced Hepatocellular Carcinoma Cell Death Depends on Reactive Oxygen Species Production *In Vitro* and *In Vivo*. *Molecular Cancer Therapeutics* **11,** 2284-2293.

33. Song, Y., Kim, J.-S., Kim, S.-H., Park, Y. K., Yu, E., Kim, K.-H., Seo, E.-J., Oh, H.-B., Lee, H. C., Kim, K. M. and Seo, H. R. (2018) Patient-derived multicellular tumor spheroids towards optimized treatment for patients with hepatocellular carcinoma. *Journal of Experimental & Clinical Cancer Research* **37,** 109.

34. Corazzari, M., Gagliardi, M., Fimia, G. M. and Piacentini, M. (2017) Endoplasmic Reticulum Stress, Unfolded Protein Response, and Cancer Cell Fate. *Frontiers in Oncology* **7**.

35. Lin, A., Giuliano, C. J., Palladino, A., John, K. M., Abramowicz, C., Yuan, M. L., Sausville, E. L., Lukow, D. A., Liu, L., Chait, A. R., Galluzzo, Z. C., Tucker, C. and Sheltzer, J. M. (2019) Off-target toxicity is a common mechanism of action of cancer

drugs undergoing clinical trials. *Science Translational Medicine* **11,** eaaw8412.

**Figure 1.**

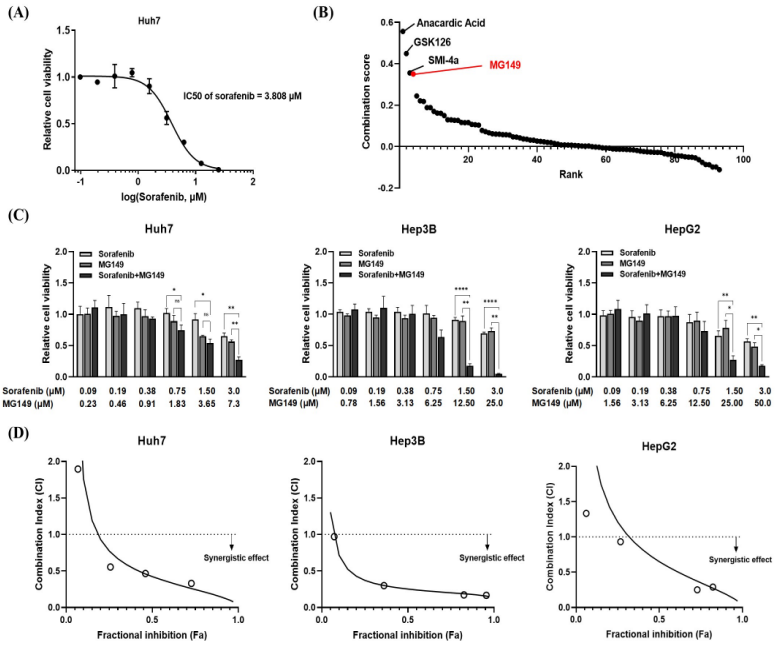



Fig. 1. Figure 1

**Figure 2.**

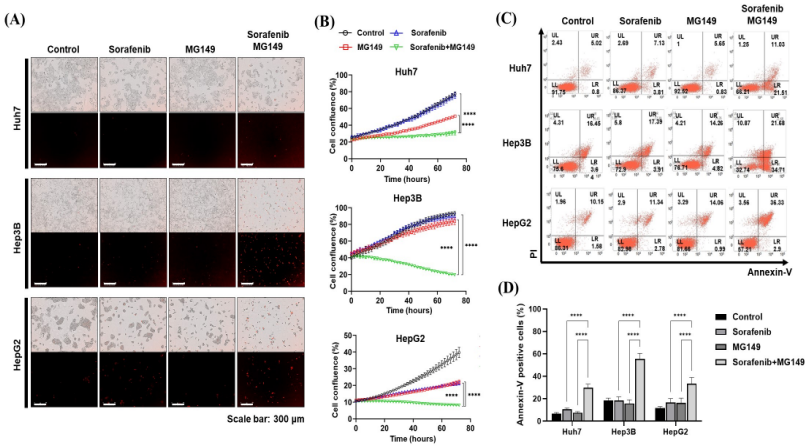



Fig. 2. Figure 2

**Figure 3.**

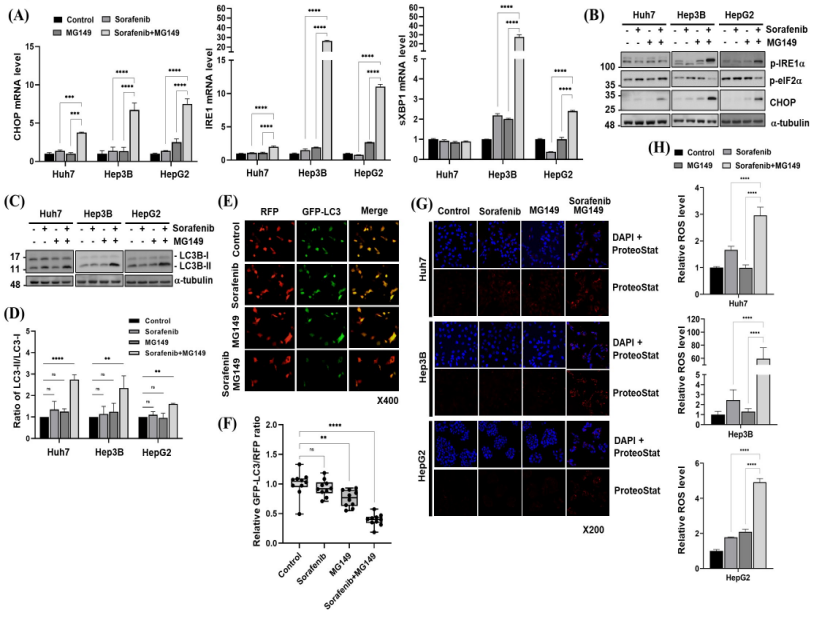



Fig. 3. Figure 3

**Figure 4.**

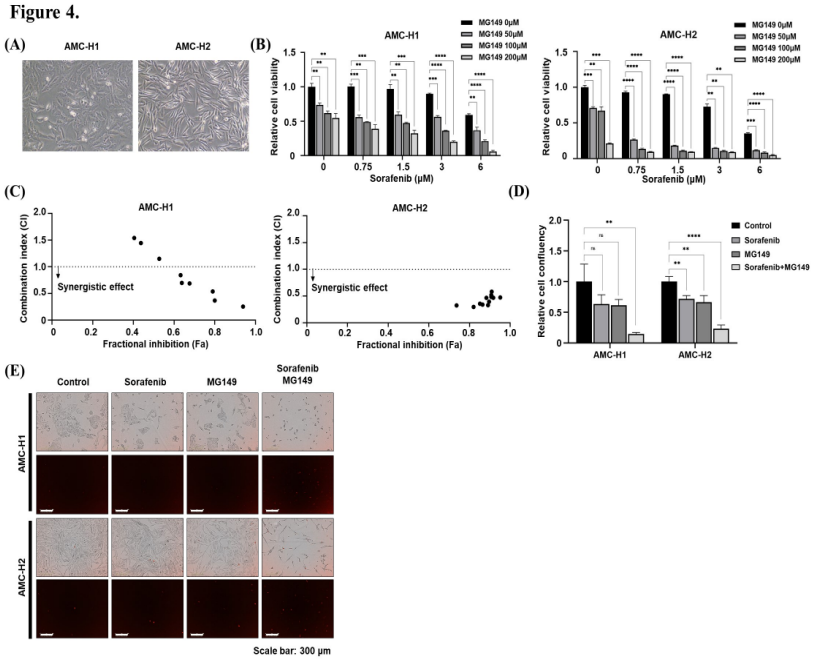
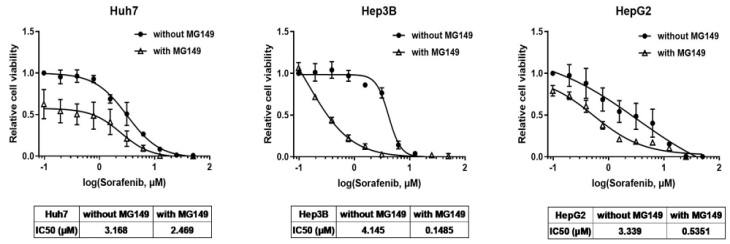



Fig. 4. Figure 4

**Supplementary Figure 1.**



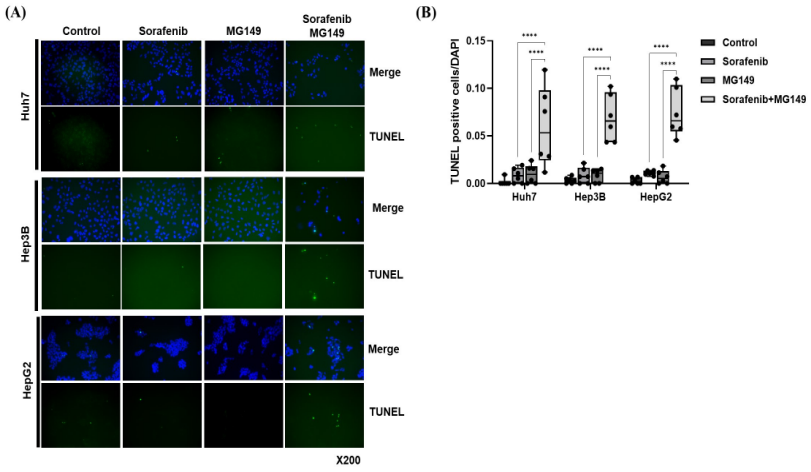

| Huh7 | without MG149 | with MG149 |
|---|---|---|
| IC50 (μM) | 3.168 | 2.469 |

| Hep3B | without MG149 | with MG149 |
|---|---|---|
| IC50 (μM) | 4.145 | 0.1485 |

| HepG2 | without MG149 | with MG149 |
|---|---|---|
| IC50 (μM) | 3.339 | 0.5351 |

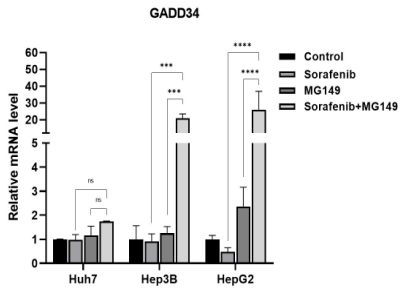
Sup. 1.

**Supplementary Figure 2.**



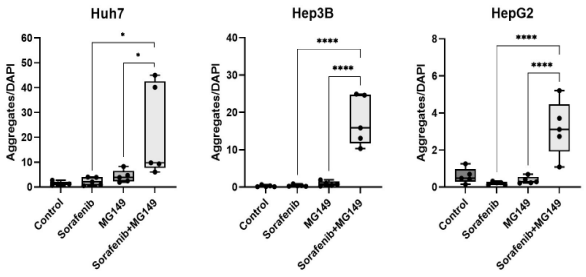

Sup. 2.

**Supplementary Figure 3.**




Sup. 3.

**Supplementary Figure 4.**




Sup. 4.