Preclinical characterization of HMPL-295, a potent and selective ERK1/2 inhibitor

Jia Hu, Jun Ni, Yuanzhi Lv, Wenjun Li, Hui Zhang, Xin Li, Juntao Yu, Qihang Zhang, Ying Yu, Zeyu Zhong, Jian Wang, Yang Sai, Na Yang, Weiguo Qing, Yongxin Ren, Michael Shi and Weiguo Su

HUTCHMED. Building 4, 720 Cai Lun Road, Z.J. Hi-Tech Park, Shanghai, China, 201203

Abstract #1661

HUTCHMED

INTRODUCTION

- Although therapeutic agents targeting alterations of upstream kinases in the MAPK pathway have achieved great clinical success, the overall benefit is still suboptimal. Several studies have shown that reactivation of MAPK signaling is the main basis to compromise the efficacy[1, 2]. Thus, co-inhibition of ERK, the terminal master kinase of MAPK pathway, and the upstream targets may effectively shut down the MAPK signaling cascade and induce deeper and more durable anti-tumor activities.
- HMPL-295, discovered by HUTCHMED, is a potent and selective ERK1/2 inhibitor, currently being developed in phase I clinical trial (NCT04908046). Preclinical data of HMPL-295 are summarized in this poster.

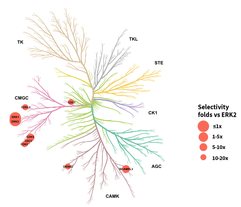
RESULTS

Figure 1. HMPL-295 is a potent and selective inhibitor of human ERK1/2

A

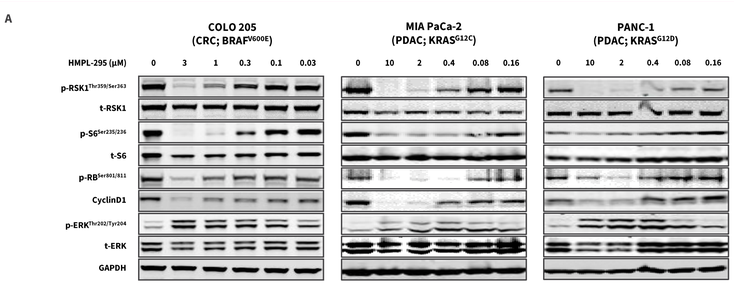
Assay		HMPL-295
Enzyme activity	ERK1	IC_{50}: 4 ± 0.04 nM
	ERK2	IC_{50}: 4 ± 1 nM
Selectivity	394 kinases	>20 folds selectivity over 384 kinases at 1 μM
	86 safety-related proteins	<35% inhibition at 1 μM
Cellular target inhibition	p-RSK in COLO 205	IC_{50}: 0.180 ± 0.005 μM

B

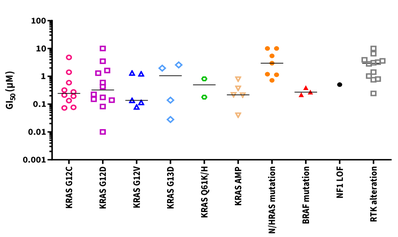


A, Inhibition of HMPL-295 on ERK 1 and ERK2 kinase was determined using Z-LYTE™ kinase assay. The IC_{50} value was shown as mean ± SD, n=3. The selectivity of HMPL-295 was evaluated against a panel of 394 kinases (KinaseProfiler™) and 86 safety-related proteins (SafetyScreen87™) at 1 μM by Eurofins. Potential off-target kinases (>90% inhibition from kinome profiling) were tested with dose-titration biochemical assays by Eurofins, and the kinases showed less than 20-fold selectivity versus ERK2 were indicated in **Figure 1B**. Cellular target inhibition on phosphorylation of RSK (p-RSK) in COLO 205 cell line was detected by ELISA. The IC_{50} value was shown as mean ± SD, n=3.

Figure 2. HMPL-295 effectively blocked ERK signaling and attenuated the growth of MAPK pathway dysregulated cancer cell lines



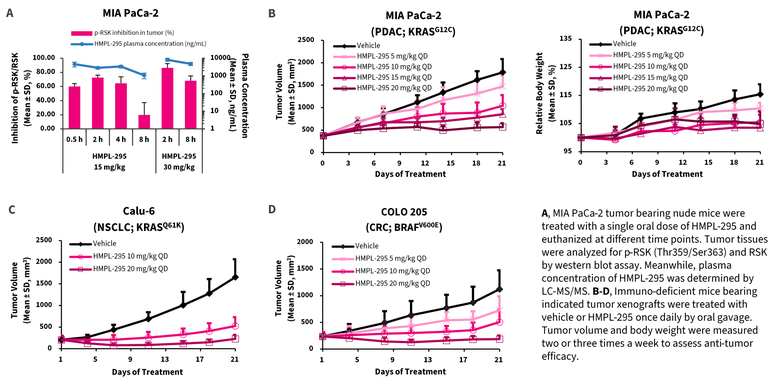
B



A, Cell lines were treated with HMPL-295 at indicated concentrations for 24 hours and lysed for western blot assay to assess the modulation on MAPK cascades. **B**, Sixty-one tumor cell lines (including colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, etc.) with MAPK pathway activation were treated with HMPL-295, and anti-proliferation activity was determined by CellTiter-Glo luminescent assay. Cell lines with RTK alterations, NRAS, HRAS or BRAF V600E mutation were cultured in standard 2-dimensional growth condition and treated with HMPL-295 for 72 hours. Cell lines with KRAS alterations, Class III BRAF or NFI loss of function (LOF) mutations were cultured in 3D spheroid growth condition and treated with HMPL-295 for 120 hours. CRC: colorectal cancer; PDAC: pancreatic ductal adenocarcinoma; NSCLC: non-small cell lung cancer.

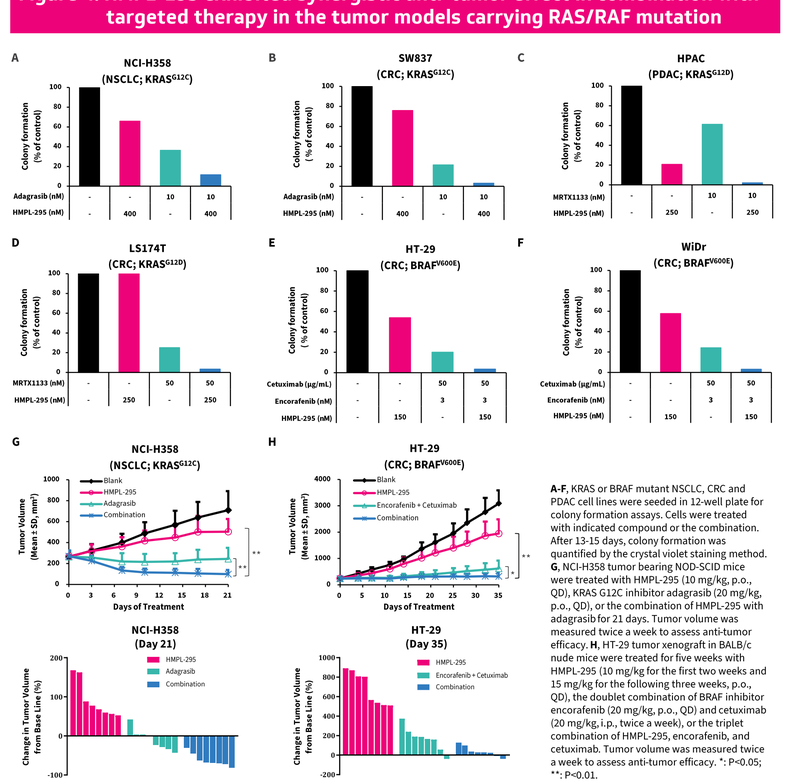
RESULTS

Figure 3. HMPL-295 dose-dependently inhibited ERK signaling and suppressed the growth of RAS/RAF mutant tumor xenografts



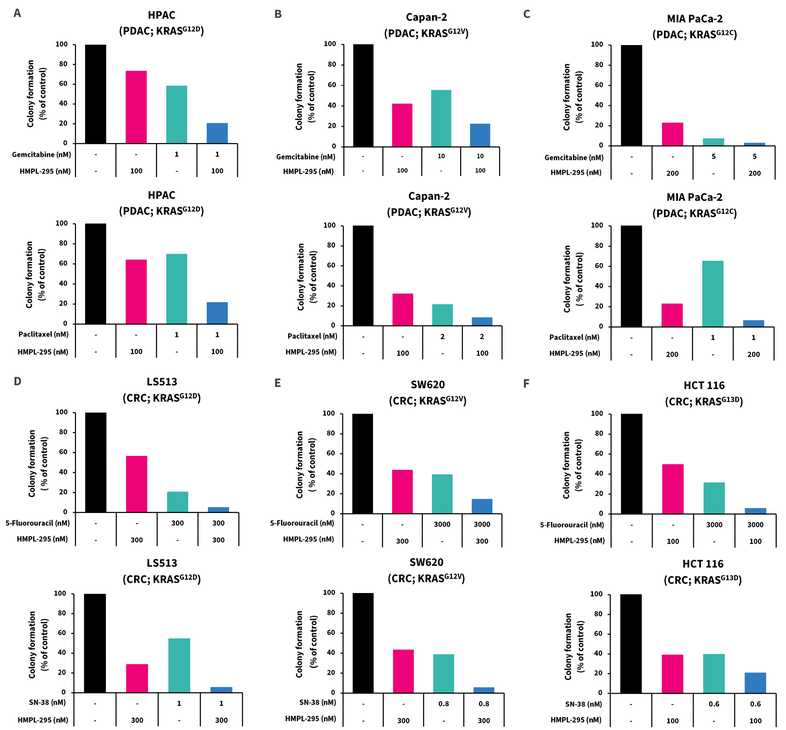
A, MIA PaCa-2 tumor bearing nude mice were treated with a single oral dose of HMPL-295 and euthanized at different time points. Tumor tissues were analyzed for p-RSK (Thr359/Ser363) and RSK by western blot assay. Meanwhile, plasma concentration of HMPL-295 was determined by LC-MS/MS. **B-D**, Immuno-deficient mice bearing indicated tumor xenografts were treated with vehicle or HMPL-295 once daily by oral gavage. Tumor volume and body weight were measured two or three times a week to assess anti-tumor efficacy.

Figure 4. HMPL-295 exhibited synergistic anti-tumor effect in combination with targeted therapy in the tumor models carrying RAS/RAF mutation



A-F, KRAS or BRAF mutant NSCLC, CRC and PDAC cell lines were seeded in 12-well plate for colony formation assays. Cells were treated with indicated compound or the combination. After 13-15 days, colony formation was quantified by the crystal violet staining method. **G**, NCI-H358 tumor bearing NOD-SCID mice were treated with HMPL-295 (10 mg/kg, p.o., QD), KRAS G12C inhibitor adagrasib (20 mg/kg, p.o., QD), or the combination of HMPL-295 with adagrasib for 21 days. Tumor volume was measured twice a week to assess anti-tumor efficacy. **H**, HT-29 tumor xenograft in BALB/c nude mice were treated for five weeks with HMPL-295 (10 mg/kg for the first two weeks and 15 mg/kg for the following three weeks, p.o., QD), the doubblet combination of BRAF inhibitor encorafenib (20 mg/kg, p.o., QD) and cetuximab (20 mg/kg, i.p., twice a week), or the triplet combination of HMPL-295, encorafenib, and cetuximab. Tumor volume was measured twice a week to assess anti-tumor efficacy. *: P<0.05; **: P<0.01.

Figure 5. HMPL-295 enhanced anti-tumor activity of standard-of-care chemotherapy in KRAS mutant tumor models



A-F, KRAS mutant PDAC and CRC cell lines were seeded in 12-well plate for colony formation assays. Cells were treated with indicated compound or the combination. After 10-18 days, colony formation was quantified by the crystal violet staining method. **G-H**, BALB/c nude mice bearing indicated tumor xenografts were treated with HMPL-295 (10 mg/kg, p.o., QD), chemotherapy (gemcitabine: 50 mg/kg, i.p., QW; nab-paclitaxel: 10 mg/kg, i.v., QW; fluorouracil: 25 mg/kg, i.v., QW; irinotecan: 5 mg/kg, i.p., twice a week), or the combination of HMPL-295 with chemotherapy. Tumor volume was measured twice a week to assess anti-tumor efficacy. **: p<0.01.

SUMMARY

- **HMPL-295 is a potent and selective ERK1/2 inhibitor with strong activity against multiple MAPK pathway activated tumor models.**
- **Combination with HMPL-295 significantly improved anti-tumor activity of targeted agents as well as standard-of-care chemotherapy in multiple tumor models with KRAS or BRAF mutation.**

References

1. Zhao Y et al. Nature. 2021;599(7886):679-683.
2. Rizos H et al. Clin Cancer Res. 2014;20(7):1965-1977.

Presented at the AACR Annual Meeting, April 5-10, 2024, San Diego, California, USA