HMPL-500, a potent and selective EZH1/EZH2 dual inhibitor, demonstrates superior anti-tumor activity in preclinical models of hematological malignancies and solid tumors

Liang Ge, Zhihu Gao, Huijing Yu, Weifang Xue, An Jiang, Shishuang Chen, Yu Zhang, Qi Ding, Tingwen Li, Jian Wang, Yang Sai, Na Yang, Weiguo Qing, Yongxin Ren, Weiguo Su

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

Abstract #254

HUTCHMED

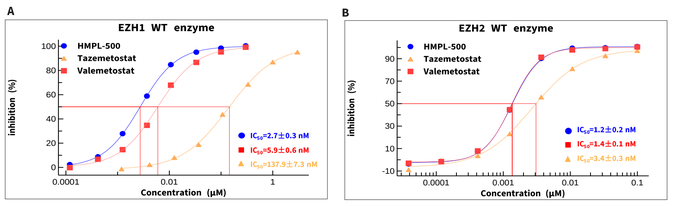
INTRODUCTION

- **Enhancer of zeste homolog 2 (EZH2) and its homolog EZH1 are indispensable for chromatin condensation and gene silencing by catalyzing mono-, di-, and tri-methylation of H3K27 (H3K27Me1/ H3K27Me2/H3K27Me3). Gain of function (GOF) mutations and overexpression of EZH2 result in H3K27Me3 accumulation, which induces tumor initiation and progression[1]. Meanwhile, loss-of-function (LOF) mutations of SWItch/Sucrose Non-Fermentable (SWI/SNF) in tumor cells contribute to the dependency on EZH2[2]. Hence, targeting EZH2 is a promising strategy for cancer treatment. However, a compensatory role of EZH1 for EZH2 has been well validated. Simultaneous depletion of EZH1 and EZH2 genes leads to a complete absence of H3K27Me3 compared to EZH2 depletion alone[3]. Thereby, targeting both EZH1 and EZH2 would be more effective in improving anti-tumor efficacy.**
- **Herein, we present preclinical characterization of HMPL-500, a highly potent and selective EZH1/EZH2 dual inhibitor, discovered and is being developed by HUTCHMED.**

RESULTS

• Biochemical activity and selectivity

➢ **HMPL-500 inhibited EZH1 and EZH2 enzyme catalytic activity with high potency and selectivity**



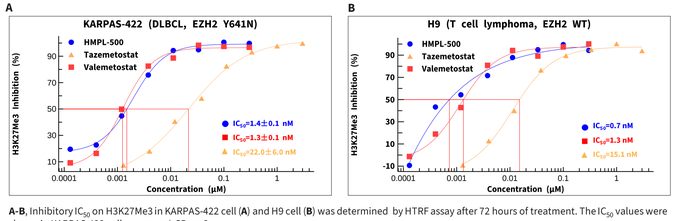
D

Selectivity	HMPL-500
31 methyltransferases other than EZH1/ EZH2	<50% inhibition at 50 μM
24 kinases	<50% inhibition at 3 μM
87 safety targets	>1000x over EZH2 WT

A-C, IC_{50} values on EZH1 (**A**), EZH2 (**B**) and EZH2 mutants (**C**) were determined by LANCE Ultra assay. The IC_{50} values were shown as mean±SD, n=3. **D**, The selectivity of HMPL-500 was evaluated with radiolabeled assays on 31 DNA and histone methyltransferases by REACTION BIO, Z'-LYTE assays on 24 kinases, and SafetyScreen 87 panel from CEREP.

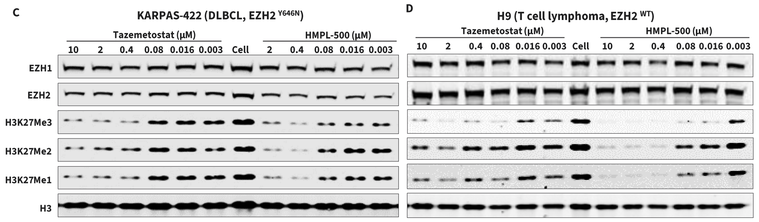
• Cellular inhibition of EZH1 and EZH2 activity

➢ **HMPL-500 potently suppressed cellular H3K27M3 with nanomolar IC_{50}**

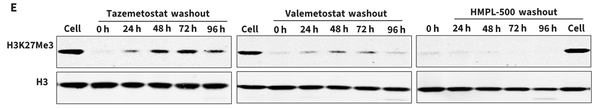


A-B, Inhibitory IC_{50} on H3K27Me3 in KARPAS-422 cell (**A**) and H9 cell (**B**) was determined by HTRF assay after 72 hours of treatment. The IC_{50} values were shown in KARPAS-422 cells as mean±SD, n=3.

➢ **HMPL-500 exhibited more complete H3K27 methylation inhibition than tazemetostat**



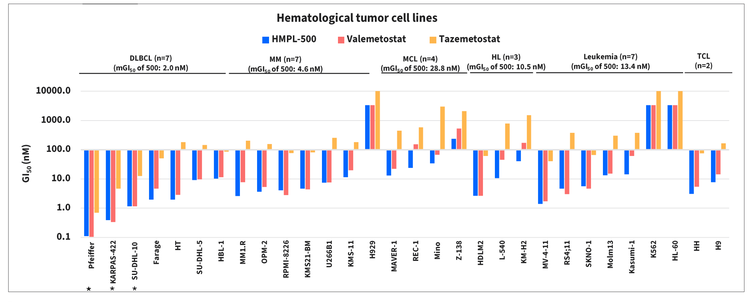
➢ **HMPL-500 displayed more sustained H3K27Me3 inhibition than tazemetostat and valemetostat**



C-D, The Inhibition on EZH1/EZH2 and H3K27Me1/2/3 was evaluated by western blot after 72 hours of treatment. **E**, Wash-out assay was performed in Hela cell expressing wild-type EZH2 to evaluate the duration of H3K27Me3 inhibition. After 96 hours of treatment at 10 uM of Tazemetostat and 1 μM of Valemetostat or HMPL-500 to achieve complete inhibition, the compounds were washed thoroughly and H3K27Me3 signal was detected at different time points (0-96 h) by Western blot experiment.

• *In vitro* tumor cell growth inhibition

➢ **HMPL-500 showed robust *in vitro* anti-proliferation activity against multiple hematological and solid tumor cell lines, and arrested G1 phase cell cycle in KARPAS-422 tumor model**



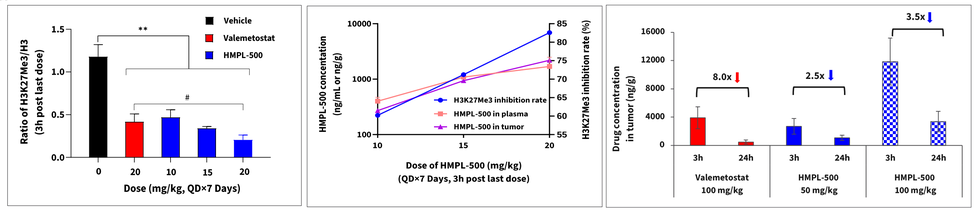
A-B, *in vitro* anti-proliferation activity against 30 hematological (**A**) and 14 solid (**B**) tumor cell lines was assessed with CellTiter-Glo assay or Celigo image cytometer after compounds treatment for 11-16 days. *, the cell lines carry EZH2 GOF mutations (**A**). #, the cell lines harbor SWI/SNF alterations (**B**). The median GI_{50} values (mGI_{50}) of HMPL-500 were shown in the A and B, for the tumor types with ≥ 3 cell line models. **C**, cell cycle analysis was performed by Celigo for quantitative analysis of cellular DNA using propidium iodide (PI) after compound treatment for 4 days on KARPAS-422 cell line. Abbreviation: DLBCL = Diffuse Large B-Cell Lymphoma, HL = Hodgkin's Lymphoma, MM = Multiple Myeloma, MCL = Mantle Cell Lymphoma, TCL = T Cell Lymphoma, SCLC=Small cell lung cancer, OC=Ovarian cancer, PC=Prostate cancer, BC=Breast cancer, MRT=malignant rhabdoid tumor, UC=urothelial cancer, CRC=Colorectal cancer, HCC=Hepatocellular carcinoma

RESULTS

• *In vivo* pharmacokinetics/pharmacodynamics (PK/PD)

➢ **HMPL-500 robustly suppressed H3K27Me3 in a dose- and concentration-dependent manner**

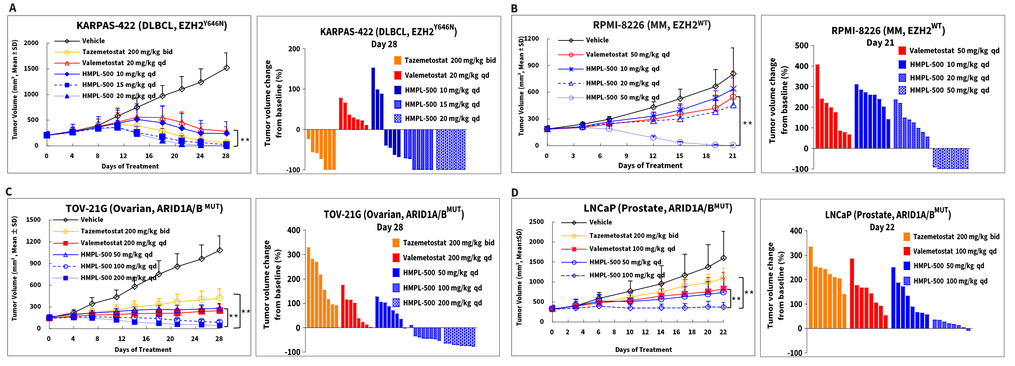
➢ **HMPL-500 showed higher and long-lasting tumor exposure compared to valemetostat**



A, KARPAS-422 tumor-bearing mice were orally dosed with 10, 15 and 20 mg/kg of HMPL-500 and 20 mg/kg of valemetostat once daily (QD) for 7 days, samples were collected 3 hours post last dose. H3K27Me3 level was determined by HTRF assay and normalized to the H3 level. Statistical analysis was performed with Student's t test, **: P<0.01, #: p<0.05. **B**, PK/PD correlation was determined by evaluating H3K27Me3 inhibition rate (relative to vehicle) and tumor and plasma concentration of HMPL-500 using LC-MS/MS. **C**, LNCaP tumor-bearing mice were orally dosed with 50 and 100 mg/kg of HMPL-500 and 100 mg/kg of valemetostat QD for 22 days, samples were collected 3 and 24 hours post last dose and tumor drug concentration were determined using LC-MS/MS. Decreased folds of tumor drug concentration (ng/g) from 3 to 24 hours were indicated above the columns.

• *In vivo* anti-tumor efficacy in multiple hematological and solid tumor xenograft models

➢ **HMPL-500 demonstrated stronger anti-tumor activity than valemetostat at the same dose level and than tazemetostat at lower dose level in multiple tumor models**



A-D, *In vivo* anti-tumor activity in various xenograft models, including lymphoma, multiple myeloma, and solid tumors. The indicated xenograft models were orally dosed with vehicle, HMPL-500 accompanied with Tazemetostat and/or Valemetostat. Tumor volume was measured twice weekly to assess anti-tumor efficacy. Tumor growth curve of KARPAS-422 (**A**), RPMI-8226 (**B**), TOV-21G (**C**) and LNCaP (**D**) xenograft models treated with indicated doses of compounds were shown. Statistical analysis was performed with Student's t test, **: P<0.01. Waterfall plots depicted the percent change in tumor size from baseline in all animals treated with compounds at the end of the study.

SUMMARY

- **HMPL-500 is a highly potent and selective EZH1/EZH2 dual inhibitor with superior *in vitro* and *in vivo* anti-tumor activity across multiple preclinical models of hematological malignancies and solid tumors.**
- **Compared to tazemetostat and valemetostat, HMPL-500 displayed more sustained target inhibition in tumor cells after compound wash-out and more potent anti-tumor efficacy and long-lasting tumor exposure in mice. The stronger *in vivo* anti-tumor activity of HMPL-500 might be attributed to the sustained target inhibition and favorable pharmacokinetics properties.**

References

1. Cold Spring Harb Perspect Med. 2016;6(9):a026575.
2. Nat Genet. 2017;49(2):213-222.
3. Mol Cell. 2008;32(4):491-502.

36th EORTC-NCI-AACR Symposium in Barcelona, Spain from Oct 23-25, 2024