HMPL-506, a novel, highly potent and differentiated menin-MLL inhibitor for the treatment of *MLL-rearranged* and *NPM1* mutant acute leukemia in preclinical models

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Introduction

- Mixed-lineage leukemia (*MLL*), also known as lysine methyltransferase 2A, *KMT2A*) gene rearrangements (*MLL-r*) occur in 5%-10% acute leukemias, particularly common in infant leukemia (70%-80%) and are associated with poor prognosis. *Nucleophosmin 1* mutations (*NPM1m*) are the most common genetic alterations in acute myeloid leukemia (AML) with about 30% of incidence.
- There is no approved targeted therapy regimen for *MLL-r* leukemia and *NPM1m* AML, indicating an unmet medical need for both leukemia subtypes.
- *MLL-r* and *NPM1m* leukemias are characterized with over-expression of *HOX* genes and their cofactor *MEIS1*, which are downstream genes controlled by the interaction of menin-MLL to drive leukemogenesis. Menin interacts with MLL-fusion or MLL-wildtype protein to regulate *HOXA9* and *MEIS1* expression through histone H3 lysine 4 (H3K4) and H3K79 methylation. Recent reports showed positive clinical results from small molecule inhibitors revumenib (SNDX-5613) and zifitomenib (KO-539) which demonstrated that blocking menin-MLL interaction could be a novel target therapy for *MLL-r* or *NPM1m* leukemia[1,2].
- HMPL-506 is a highly potent and differentiated small molecule compound targeting menin-MLL interaction. Here we introduce its pre-clinical anti-tumor activities against *MLL-r* and *NPM1m* leukemia.

Materials and methods

- Biochemical activity was determined by Fluorescence Polarization (FP) based binding assay between menin protein and MLL peptide encompassing the entire menin binding motif.
- The menin-MLL target gene *MEIS1*, *HOXA9* and differentiation gene *CD11b* was investigated by Real-time PCR in MV-4-11 cells.
- The cell proliferation inhibition was tested by CellTiter-Glo in *MLL-r* and *NPM1* mutant cells.
- *In vivo* anti-tumor activities were evaluated in MV-4-11 or MOLM-13 cell line derived xenograft models.
- The plasma and tumor concentrations were determined by liquid chromatography–mass spectrometry (LC–MS) method.
- HMPL-506 on hERG potassium channel was assessed in CHO (Chinese hamster ovary) cells expressing hERG potassium channel by the manual patch-clamp assay in the concentration range of 3 to 100 μM.
- The selectivity profile of HMPL-506 was evaluated in a DNA and histone methyltransferase panel composed of 34 targets, a kinase panel of 24 representative kinases, and a safety panel composed of 87 proteins including GPCRs (G-protein-coupled receptors), ion channels, transporters, and enzymes.

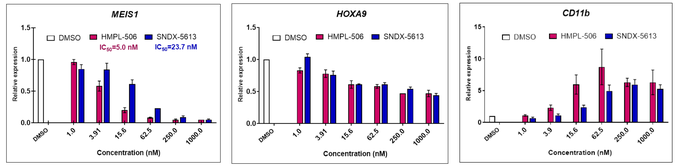
Results

A. HMPL-506 displayed strong inhibition on menin-MLL interaction and cell growth of *MLL-r* and *NPM1m* leukemia cells

Compound	Biochemical Inhibition of Menin-MLL Binding	Cellular Growth Inhibition GI_{50} (nM)					
	IC_{50} (nM, N=3)	OCI-AML-3 (*NPM1m*)	MV-4-11 (*MLL-AF4*)	MOLM-13 (*MLL-AF9*)	RS4;11 (*MLL-AF4*)	K562 (*MLL-WT*)	HL60 (*MLL-WT*)
HMPL-506	1.0±0.2	22.4	3.0	9.5	12.1	>10,000	>10,000
SNDX-5613	1.5±0.2	191.2	10.5	26.6	66.3	>10,000	>10,000
KO-539	4.0±0.8	80.5	9.5	26.5	40.1	1,407.6	1,698.7
JNJ-75276617	1.1±0.6	71.8	4.9	14.4	34.3	>10,000	>10,000
DSP-5336	2.2±0.8	83.8	5.2	17.4	35.7	>10,000	>10,000

IC_{50}: half-maximal inhibitory concentration; GI_{50}: half-maximal growth inhibitory concentration. SNDX-5613, KO-539, JNJ-75276617 and DSP-5336 compounds are other menin-MLL inhibitors.

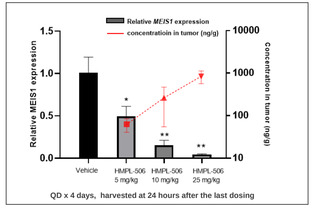
B. HMPL-506 potently down-regulated menin-MLL targeted genes and up-regulated differentiation marker in *MLL-r* leukemia cells



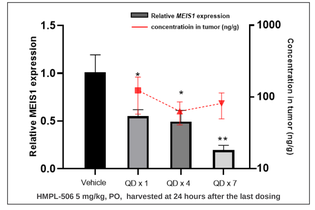
The expression levels of *MEIS1*, *HOXA9* and *CD11b* were investigated by Real-time PCR in MV-4-11 cells harboring *MLL-AF4* fusion after 48 hours treatment.

C. HMPL-506 exhibited strong and sustained target inhibition in MV-4-11 xenograft model

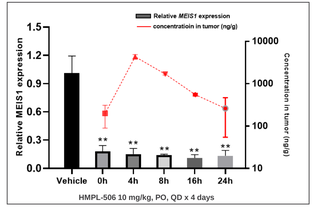
a) Dose dependent target inhibition correlating with drug concentration in tumor tissue



b) Longer dosing duration led to stronger target inhibition



c) Continuous treatment achieved durable and strong *MEIS1* inhibition within the 24 hours dosing interval

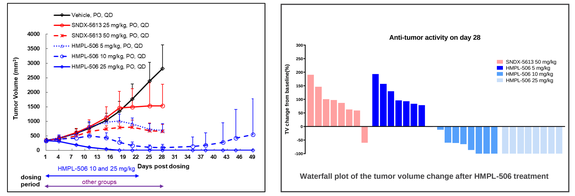


In the subcutaneous tumor model of MV-4-11, *MEIS1* expression in tumor tissues after oral administration (PO) of HMPL-506 was investigated:

a) the target inhibition at the different dose levels (5, 10 and 25 mg/kg) with the same duration of administration (QD x 4 days);
b) the target inhibition at the same dose (5 mg/kg) with the different durations of administration (QD x 1 day, QD x 4 days, QD x 7 days);
c) the target inhibition at 0, 4, 8, 16, and 24 hours after 4 consecutive days oral administration of HMPL-506 at 10 mg/kg.

MEIS1 expression was analyzed by RT-PCR assay. The concentrations of HMPL-506 in tumor were measured by LC-MS. The *MEIS1* expression level was normalized with *GAPDH* and calculated relative to vehicle. The difference in *MEIS1* expression level was compared to the vehicle, *P<0.05, **P<0.01. Error bars represent mean ± SD.

D. HMPL-506 showed robust and sustained anti-tumor efficacy in MV-4-11 subcutaneous tumor model



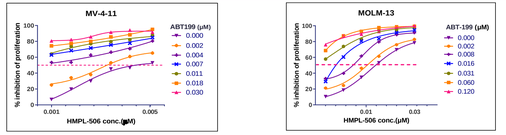
- **HMPL-506 demonstrated remarkable anti-tumor efficacy in a dose-dependent manner in MV-4-11 xenograft model.**
- **HMPL-506 at 10 and 25 mg/kg induced high tumor regression rate and maintained robust anti-tumor efficacy up to Day 49 after drug withdrawal from Day 23.**

The tumor model was established by inoculating MV-4-11 tumor cells subcutaneously in BALB/c nude mice. HMPL-506 was orally administered at the doses of 5, 10 and 25 mg/kg, QD. Reference compound SNDX-5613 was orally administered at 25 and 50 mg/kg, QD. N=8 mice/group. All animals were tolerant during the treatment. TV: tumor volume; PO: oral gavage; QD: once daily

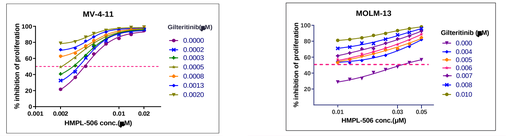
E. *In vitro* and *in vivo* combination effect of HMPL-506 with targeted agents in *MLL-r* leukemias

- **HMPL-506 induced synergistic effects on MV-4-11/MOLM-13 cell proliferation in combination with BCL-2 inhibitor, FLT3 inhibitor, chemotherapy, or CDK4/6 inhibitor**

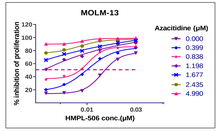
HMPL-506 + ABT199 (Venetoclax, BCL-2 inhibitor)



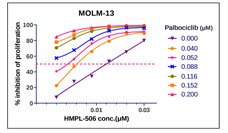
HMPL-506 + Gilteritinib (FLT3 inhibitor)



HMPL-506 + Azacitidine

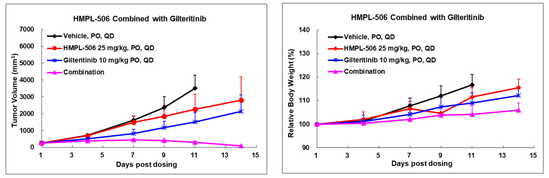


HMPL-506 + Palbociclib (CDK4/6 inhibitor)



Tumor cell proliferation inhibitions were tested by CTG cell viability assay in MV-4-11 or MOLM-13 leukemia cells treated with HMPL-506 in combination with ABT199 (BCL-2 inhibitor), Gilteritinib (FLT3 inhibitor), Azacitidine (Chemotherapy), Palbociclib (CDK4/6 inhibitor).

- **Synergistically improved anti-tumor effect by combination treatment of HMPL-506 and FLT3 inhibitor Gilteritinib in MOLM-13 subcutaneous tumor model**



The tumor model was established by inoculating MOLM-13 tumor cells subcutaneously in male BALB/c nude mice. Each group was treated with vehicle, HMPL-506 (25 mg/kg; PO; QD), Gilteritinib (10 mg/kg; PO; QD) or their combined. N=8 mice/group. All animals were tolerant during the treatment.

F. HMPL-506 demonstrated no hERG inhibition and high selectivity among methyltransferases, multiple kinases, and safety related targets

Selectivity and safety	HMPL-506
hERG patch clamp (IC_{50})	62.45 μM
34 Methyltransferases	No inhibition was found at 10 μM
24 Kinases	No inhibition was found at 10 μM
87 Safety-associated proteins	*M2 and NK1 receptor:* >300 selectivity fold over *MEIS1* inhibition *Other targets:* No significant inhibition at 10 μM

Summary

- **HMPL-506 is a highly potent and selective inhibitor blocking menin and MLL interaction.**
- **Compared to the competitors tested, HMPL-506 showed the most potent anti-tumor activities against both *MLL-r* and *NPM1m* tumor models.**
- **HMPL-506 demonstrated clear synergistic effects on MV-4-11 and/or MOLM-13 AML cells when in combination with a BCL2 inhibitor, FLT3 inhibitor, azacytidine and CDK4/6 inhibitor, indicating its multiple combination potentials in leukemia treatment.**
- **HMPL-506 displayed low risk of cardiac toxicity and acceptable safety properties.**
- **Phase 1 clinical study of HMPL-506 will start in 2024 Q2.**

References

1. Issa GC. et al. Nature. 2023;615(7954):920-924.
2. Harry P. et al. Blood. 2022; 140 (Supplement 1): 153–156.

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