A Novel and Potent EZH1/2 Dual Inhibitor, HM97662 Demonstrates Antitumor Activity in T-cell Lymphoma

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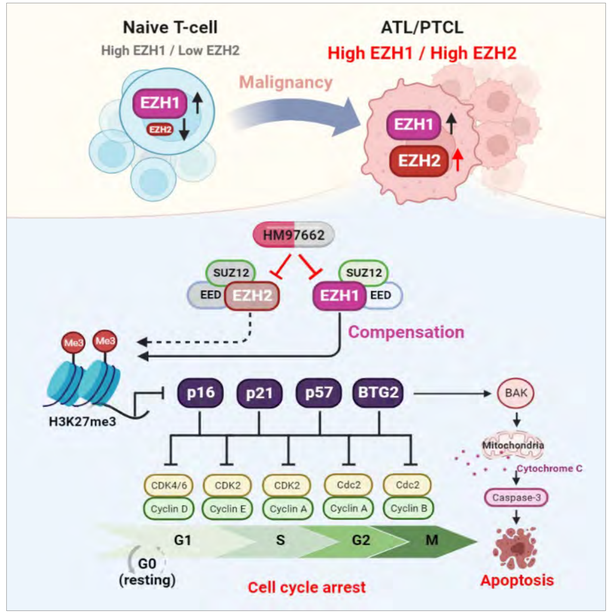
Introduction

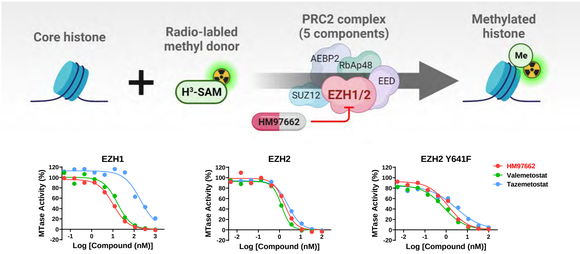
Chromatin remodeling is a crucial process for transcriptional regulation, of which dysregulation is often observed in various human cancers[1]. The enhancer of zeste homolog 2 (EZH2) and its homolog EZH1 are catalytic components of polycomb repressive complex 2 (PRC2), which tri-methylates histone H3 at lysine 27 (H3K27me3) to repress transcription of its target genes[2]. Although methyltransferase activity of PRC2 is mainly contributed by EZH2, EZH1 also conducts a compensatory role to maintain tri-methylation of H3K27. Moreover, EZH1 directly binds to chromatin and modulates its condensation[3].

Recent studies have indeed suggested that EZH1 as well as EZH2 played a critical role in T-cell lymphomas such as ATL/L and PTCL, which had high innate EZH1 and increased EZH2 expression upon acquisition of their malignancy[4]. Consequently, dual inhibition of EZH1/2 might induce higher expression of their downstream tumor suppressor genes than blocking EZH2 alone, expecting greater anti-tumor activity as an anti-cancer therapy.

Role of EZH1/2 in Cell Cycle and Apoptosis of TCL[5]



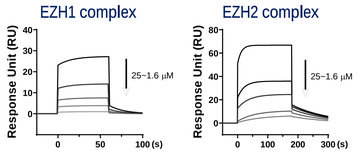
Methyltransferase Assay of HM97662



Complexes	Methyltransferase activity (IC_{50}, nM)		
	HM97662	Valemetostat	Tazemetostat
EZH1	16	30	188
EZH2	2.1	1.2	2.8
EZH2 Y641F	1.4	1.1	2.7

Methyltransferase assay was conducted using PRC2 complex with 5 components; EZH, EED, SUZ12, RbAp48, AEBP2.

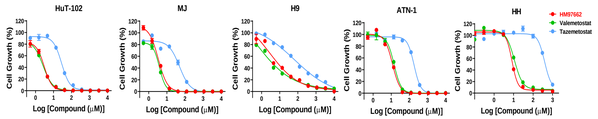
SPR Analysis of HM97662



Complexes	Binding affinity (K_D, μM)
EZH1-EED-SUZ12	15
EZH2-EED-SUZ12	3.6

Binding affinity to EZH1 or EZH2-EED-SUZ12 complex was evaluated with *Biacore T200*.

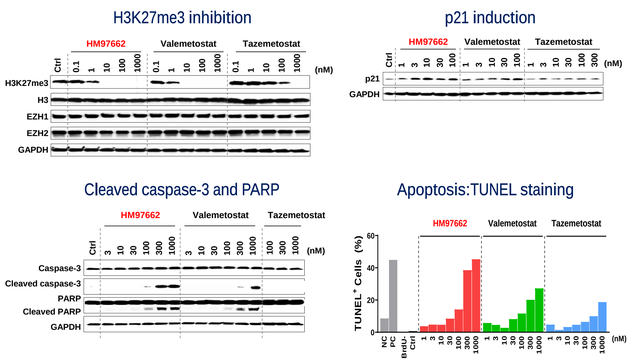
Growth Inhibition in Various PTCL Cell Lines



Cell lines	Genetic alteration	Cell viability (GI_{50}, nM)		
		HM97662	Valemetostat	Tazemetostat
HuT-102	-	2.5	2.1	26
MJ	KMT2A(MLL) deletion	3.8	3.3	39
H9	NRAS Q61K	7.0	4.0	90
ATN-1	-	12	14	175
HH	FOXK2-TP63	12	22	387

Target Modulation and Pharmacological Effect

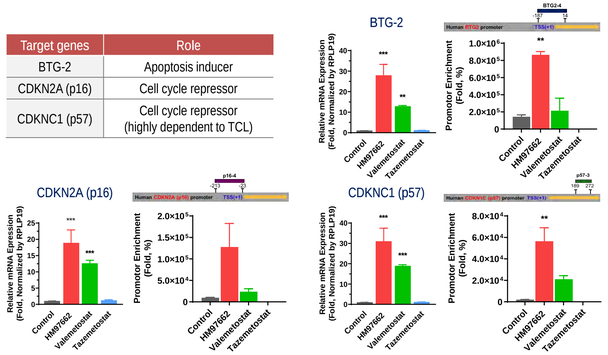
A. Regulation of target proteins and apoptosis in HH cell line



Compounds	On-target modulation		Apoptosis induction (EC_{50}, nM)		
	H3K27me3 inh. (IC_{50}, nM)	p21 activation (EC_{50}, nM)	Cleaved caspase-3	Cleaved PARP	TUNEL+
HM97662	1.0	2.9	215	145	155
Valemetostat	1.2	33	914	296	458
Tazemetostat	101	> 100	> 1,000	> 1,000	~ 1,000

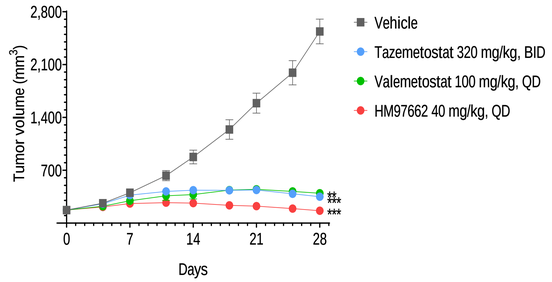
B. Target gene expression and chromatin accessibility in HH cell line

Target genes	Role
BTG-2	Apoptosis inducer
CDKN2A (p16)	Cell cycle repressor
CDKNC1 (p57)	Cell cycle repressor (highly dependent to TCL)



Gene expression (left), chromatin accessibility (right) at 300 nM treatment (*p<0.05, **p<0.01, ***p<0.001 *vs.* Control, ANOVA).

Antitumor Efficacy of HM97662 in HuT-102 Xenograft Model



HM97662 was orally administered once daily for 28 days to NCG mice subcutaneously inoculated with HuT-102 lymphoma cell line, and it significantly inhibited tumor growth at 40 mg/kg

(**p<0.01, ***p<0.001 *vs.* vehicle, Kruskal-Wallis).

Concluding Remarks

- HM97662 is a next generation EZH2 inhibitor with an enhanced profile on EZH1 inhibition (EZH1/2 dual inhibitor), which shows broad and strong anti-proliferative activity against various T-cell lymphoma cell lines.
- HM97662 increased mRNA expression of several target genes, CDKN2A, CDKNC1, and BTG-2, which induce cell cycle arrest and apoptosis in HH cells.
- HM97662 showed effective anti-tumor activities in the subcutaneous HuT-102 xenograft mouse model at lower dose than those of competitors.
- Taken together, the present studies demonstrate that HM97662 has promising prospective for the treatment of patients with T-cell lymphoma.
- Currently, a first-in-human phase 1 dose escalation study of HM97662 in advanced or metastatic solid tumors is underway in KR/AU (NCT05598151).

References

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2) Raphael M., et al., *Mol. Cell.* 2008, *32*, 503;
3) Soo Hyun L., et al., *BMB Rep.* 2022, *55*, 595;
4) Yamagishi M., et al., *Cell Rep.* 2019, *29*, 2321;
5) Schematic illustration was created with BioRender.com.

Acknowledgements

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