A novel and potent EZH1/2 dual inhibitor, HM97662, demonstrates antitumor activity in malignant tumors

Seung Hyun Jung, DongJin Hong, Jiyoung Hwang, Somin Park, Jooyun Byun, Miyoung Lee, Kyounghwa Koo, Gunwoo Lee, Yu-Yon Kim, Yesol Bak, YoungGil Ahn , YoungHoon Kim, KweeHyun Suh

Hanmi Research Center, Hanmi Pharmaceutical Co. Ltd., Republic of Korea

Abstract #1142

Abstract

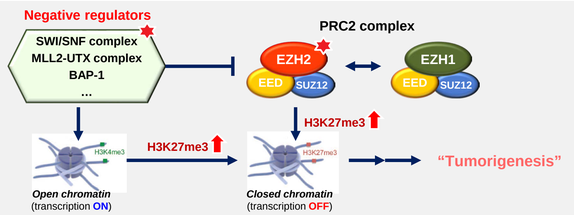
Either enhancer of zeste homolog 2 (EZH2) or its homolog EZH1, the enzymatic core subunit of polycomb repressive complex 2 (PRC2), acts an essential role for the maintenance of transcriptional repression by the methylation of histone H3 lysine 27 (H3K27). EZH2 gain-of-function (GOF) mutations (e.g. Y641, A677, and A687) or EZH2 overexpression lead to hyperinduce trimethylation of H3K27 (H3K27me3). Meanwhile, loss-of-function (LOF) mutation of major components (e.g. ARID1A, PBRM1, INI1) in chromatin remodeling complex, SWItch/Sucrose Non-Fermentable (SWI/SNF), results in a loss of its ability to oppose PRC2 and subsequent activation of EZH2. Accordingly, the dysfunctions of EZH2 or the alterations of regulatory SWI/SNF complex proteins have been reported to be associated with the development and progression of a variety of malignant tumors.

Although PRC2 is suppressed by EZH2 inhibition, the activity of EZH1 is complementarily increased to replace the role of EZH2. Consequently, dual inhibition of EZH1 and EZH2 could be more effective than EZH2 inhibition alone in blocking PRC2 function as an anti-cancer therapy. Thus, we have developed a novel EZH1/2 dual inhibitor, HM97662, which simultaneously inhibited the methyltransferase activity of wild-type EZH1 as well as wild-type and GOF mutant EZH2 at nanomolar concentrations.

Herein, we presented that HM97662, having an enhanced inhibition activity of EZH1 compared to other EZH2 inhibitors on clinical development, potently and dose-dependently decreased global trimethylation of H3K27 in various cancer cell lines. HM97662 showed broader and stronger anti-proliferative activities against various hematological cancer cell lines with EZH2 activating mutations as well as solid tumor cell lines with negatively mutated components of regulatory protein complexes. At this time, it was confirmed in the MCL cell line that HM97662, unlike the EZH2 selective inhibitor, did not cause the elevation of EZH1. Although EZH1 expression was elevated in Tazemetostat resistance clones of KARPAS-422 cells, HM97662 with enhanced inhibition activity of EZH1 overcame Tazemetostat-induced resistance. Moreover, HM97662 showed a longer residence time on EZH2 in TOV-112D cells compared to competitors. As a result, HM97662 inhibited tumor growth more effectively than the known EZH2 selective inhibitors without abnormal clinical signs at once daily dosing in xenograft mouse models with lymphoma cells bearing EZH2 GOF mutant and bladder cancer cells harboring a genetically mutated regulatory SWI/SNF complex protein (e.g. ARID1A), respectively.

Taken together, the present studies demonstrate that HM97662 is a novel and potent EZH1/2 dual inhibitor and has the promising potential for the treatment of patients with several types of cancers. Clinical trials to prove the effectiveness of HM97662 identified in preclinical studies need to be carried out immediately.

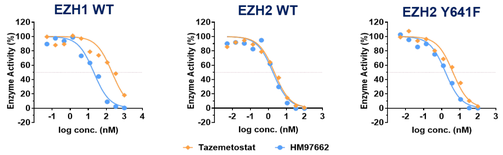
Role of EZH1/2 and Cancer Development



Target chromatin remodelers	Aberrations (representative types or components)
PRC2 complex	**EZH2 GOF mutation** (Y641/A677/A687)**, EZH2 overexpression**
Negative regulators	**SWI/SNF complex** (ARID1A, SMARCB1, SMARCA4/2, PBRM1…)**, MLL2-UTX complex** (KDM6A…)**, BAP1 LOF mutation**

In vitro Pharmacological Activity

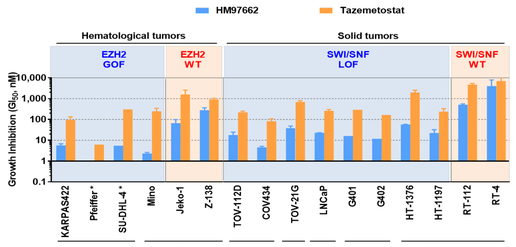
A. Inhibition of EZH1 and EZH2 catalytic activity



PRC2 complex	IC_{50} (nM)*	
	HM97662	Tazemetostat
EZH1 WT	23	240
EZH2 WT	2.2	2.7
EZH2 Y641F	2.0	3.6

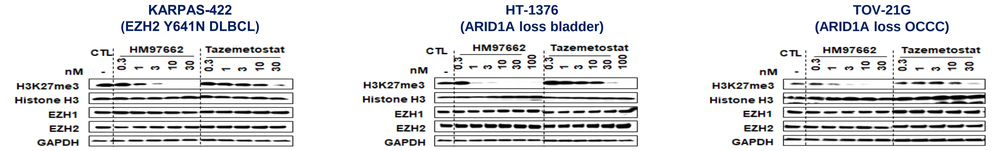
* Biochemical inhibition was conducted via external service.

B. Cellular activity in target-associated cancer cell lines



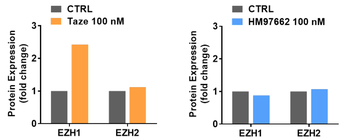
* Marked cell growth inhibition assay were conducted via external service.

C. EZH2-dependent epigenetic modulation of H3K27 in target-associated cancer cell lines

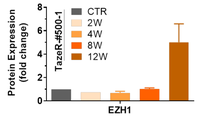


Differentiation Points: Overcoming EZH2i-Resistance and Prolonged Drug Residence

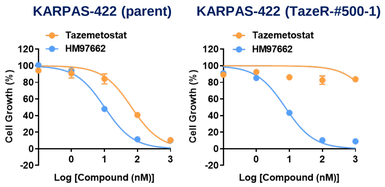
A. Elevation of EZH1 by EZH2i, but not by EZH1/2i in Mino MCL cell line



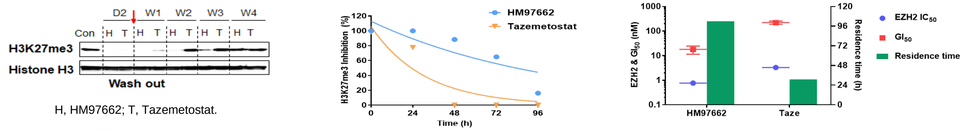
B. Induction of clone resistant to EZH2i, but HM97662 is active against Tazemetostat resistant clone



*** Construction of resistant cell line:** collection of surviving clones after prolonged treatment with Tazemetostat in EZH2 Y641N mutation KARPAS-422 DLBCL cell line

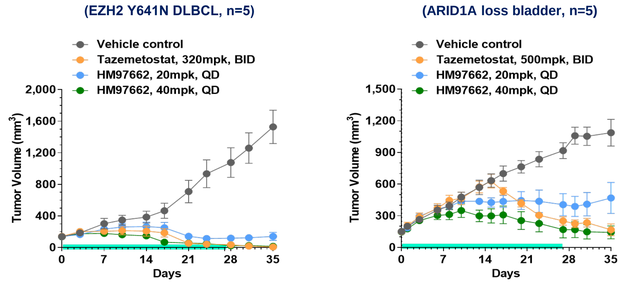


C. Prolonged drug residence time in washout assay of TOV-112D (SMARCA4/2 loss SCCOHT) cells

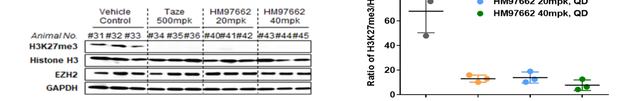


H, HM97662; T, Tazemetostat.

Antitumor Efficacy in Malignant Tumors



H3K27me3 (PD marker) inhibition after treatment for 14days (n=3) in HT-1376 model



* HM97662 was rally administered once daily for 28days in mice subcutaneously transplanted with KARPAS-422 lymphoma (on NOD SCID mice) and HT-1376 bladder cancer (on BALB/c nude mice) cell lines and resulted in significant antitumor efficacy (p<0.01 or p<0.001 *vs*. vehicle, ANOVA or Kruskal-Wallis).

Conclusion

- HM97662 is a next generation EZH2 inhibitor with an enhanced inhibition activity of EZH1 (dual inhibition), showing the potential on EZH1/2 dual inhibition for overcoming the resistance by EZH2 inhibition through the effective target modulation such as the inhibition of H3K27me3.
- HM97662 potently inhibited the growth of a variety of cancer cell lines associated with the activation of PRC2 complex due to mutant eZH2 or altered negative regulatory proteins such as ARID1A, SMARCA4/2 or SMARCB1.
- Also, significant antitumor activity of HM97662 was demonstrated from typical target-associated malignant tumor models.
- GLP-toxicity studies are currently in progress, and a first-in-human study is planned to be conducted early next year.

References

1. Kim KH, *et al.* Targeting EZH2 in cancer. *Nat. Med.* **2016**, *22*, 128.
2. Kuntson SK, *et al*. Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in *EZH2*-mutant non-Hodgkin lymphoma. *Mol. Cancer Ther.* **2014**, *13*, 842.
3. Honma D, *et al*. Novel orally bioavailable EZH1/2 dual inhibitors with greater antitumor efficacy than an EZH2 selective inhibitor. *Cancer Sci.* **2017**, *108*, 2069.
4. Pyziak K, *et al.* Potential of enhancer of zeste homolog 2 inhibitors for the treatment of SWI/SNF mutant cancers and tumor microenvironment. *Drug Dev. Res.* **2021**, *1*.

Hanmi Pharm. Co., Ltd. (http://www.hanmipharm.com)