NGS-based Molecular Profiling Reveals Remarkable Anticancer Synergy of EZH1/2 Dual Inhibitor HM97662 with Standard-of-care on Small Cell Lung Cancer

#2405

Seungheon Baek*, Aran Park*, Gunwoo Lee, Jungwha Park, Seung Hyun Jung, Jooyun Byun, Young Gil Ahn, and In Young Choi
Hanmi Pharmaceutical Co., Ltd., Seoul, Republic of Korea

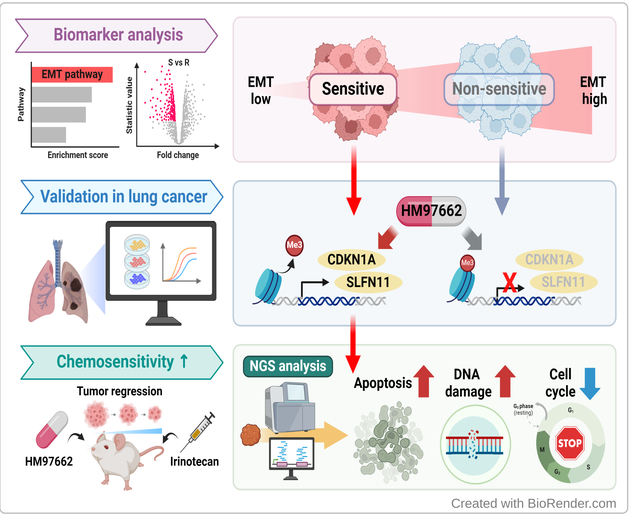
Introduction

The development of EZH1/2 inhibitors is crucial in targeting tumors due to their central role in epigenetic regulation. These EZH1/2 enzymes contribute to tumorigenesis by repressing transcriptional expression of tumor suppressor genes. Studies have shown that inhibiting EZH1/2 can suppress tumor growth and improve clinical outcomes, particularly in hematological malignancies[1]. However, the selective potency of EZH1/2 inhibitors in a limited range of solid tumors highlights the need for precise selection of indications based on molecular characteristics[2].

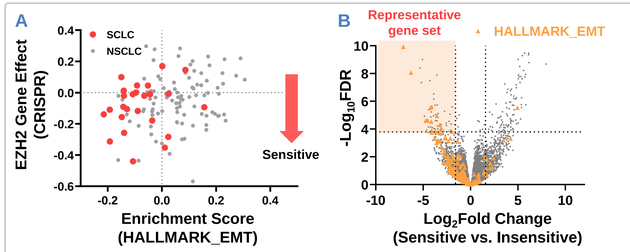
Recent research has shown that cancers with a small cell neuroendocrine (SCN) phenotype, characterized by high aggressiveness and limited treatment options, share molecular characteristics and drug susceptibility profiles with hematological malignancies[3]. Moreover, the role of EZH2 in cancers with SCN features, such as small cell lung cancer (SCLC) and neuroendocrine prostate cancer (NEPC), has been increasingly recognized[4]. Notably, EZH2 contributes to chemo-resistance in SCLC by repressing the transcription of Schlafen 11 (SLFN11), a biomarker of sensitivity to DNA-damaging chemotherapies. EZH2 inhibition upregulates SLFN11 expression and restores chemosensitivity in chemo-resistant SCLC models, supporting EZH2 blockade as a promising therapeutic strategy[5].

Here, using EZH2 CRISPR knock-out and RNA-seq datasets from the Cancer Dependency Map (DepMap), we identified a novel biomarker gene set predictive of sensitivity to HM97662, an EZH1/2 dual inhibitor. This gene set was validated in lung cancer cell lines with varying degrees of sensitivity to HM97662. We further compared the expression of EZH2 target genes in both sensitive and insensitive SCLC cell lines following HM97662 treatment. Finally, we demonstrated the antitumor efficacy and remarkable synergistic effect of HM97662 in combination with standard-of-care (SoC) therapies in SCLC xenograft models and elucidated its mechanism of action through NGS-based RNA-seq analysis.

Schematic Illustration of Biomarkers for HM97662

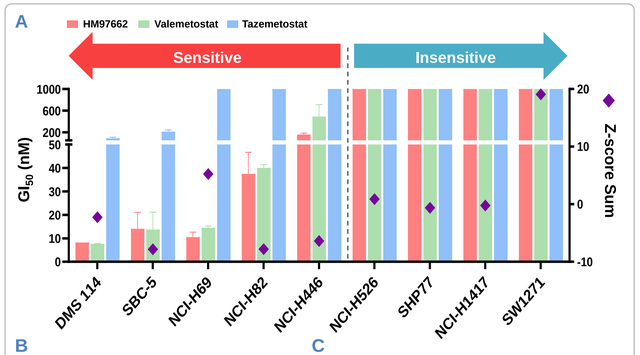


Prediction of Sensitivity on EZH2 Inhibition



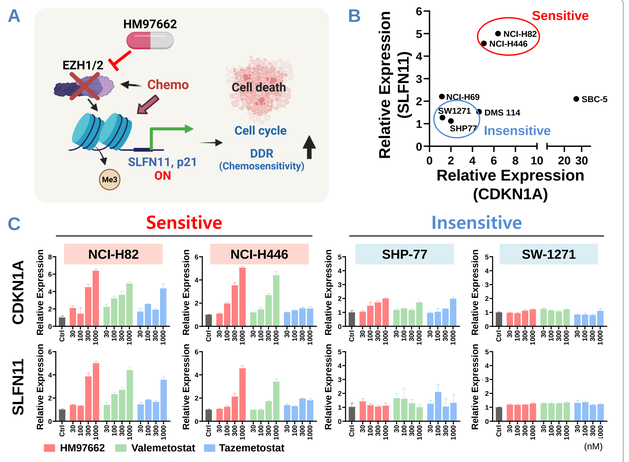
(A) Using DepMap datasets, the enrichment score of the HALLMARK EMT gene set was plotted against the EZH2 CRISPR gene effect across lung cancer cell lines.
(B) A representative biomarker gene set was identified in lung cancer cell lines to predict sensitivity to EZH2 inhibition.

Demonstrating HM97662 Sensitivity on Lung Cancer



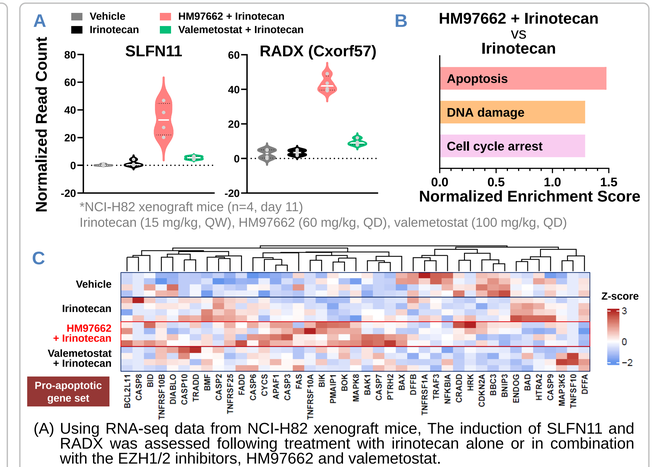
(A) The sum of Z-scores for the biomarker gene set and the sensitivity to HM97662 were assessed in lung cancer cell lines.
(B) ROC-AUC analysis of the biomarker gene set was performed to predict sensitivity to HM97662 treatment.
(C) Gene set enrichment analysis (GSEA) was performed to compare sensitive and insensitive cell lines, focusing on the HALLMARK_EMT pathway (FDR q-value <0.05, gene set permutation).

Target Gene Regulation upon EZH1/2i Sensitivity



(A) The regulation of EZH2 target genes by HM97662 was illustrated in SCLC cell lines.
(B) Changes in mRNA expression of SLFN11 & CDKN1A following treatment with 1,000 nM HM97662 were assessed in four sensitive & two insensitive cell lines.
(C) The mRNA expression levels in response to dose-dependent EZH1/2 inhibitor treatment were evaluated in both sensitive and insensitive cell lines.

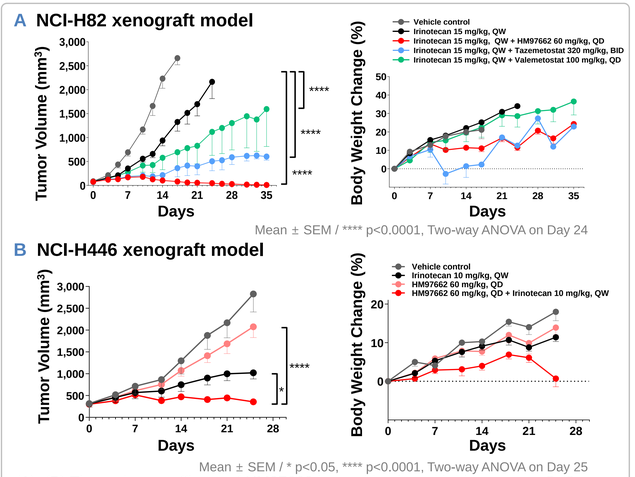
NGS-based Gene Profiling with SoC Combination



*NCI-H82 xenograft mice (n=4, day 11)
Irinotecan (15 mg/kg, QW), HM97662 (60 mg/kg, QD), valemetostat (100 mg/kg, QD)

(A) Using RNA-seq data from NCI-H82 xenograft mice, The induction of SLFN11 and RADX was assessed following treatment with irinotecan alone or in combination with the EZH1/2 inhibitors, HM97662 and valemetostat.
(B) Gene set enrichment analysis (GSEA) was conducted to compare irinotecan monotherapy versus the combination of HM97662 with irinotecan (FDR q-value <0.25, phenotype permutation; BioPlanet 2019 and WikiPathways 2023).
(C) A heatmap of pro-apoptotic genes was generated to visualize distinct expression profiles between the treatment groups.

Efficacy & Synergy with SoC in SCLC Xenografts



Mean ± SEM / **** p<0.0001, Two-way ANOVA on Day 24
Mean ± SEM / * p<0.05, **** p<0.0001, Two-way ANOVA on Day 25

(A), (B) The antitumor efficacy of HM97662 in combination with irinotecan, a SoC agent for SCLC, was evaluated in comparison to irinotecan alone in NCI-H82 (A) and NCI-H446 (B) xenograft models.

Concluding Remarks

- Representative genes from the EMT gene set demonstrated strong predictive power for sensitivity to HM97662, a dual EZH1/2 inhibitor, in lung cancer cell lines.
- HM97662 dose-dependently increased the mRNA expression of CDKN1A and SLFN11 in sensitive SCLC cell lines, but not in insensitive ones.
- HM97662 exhibited strong synergistic effects with irinotecan, a SoC agent for SCLC, outperforming other competitors in xenograft mouse models.
- In combination with irinotecan, HM97662 more effectively induced SLFN11 and RADX, genes that enhance chemosensitivity, compared to valemetostat.
- The combination of HM97662 and irinotecan activated tumor cell death-related pathways and exhibited a distinct pro-apoptotic gene signature compared to the valemetostat combination.
- Taken together, these preclinical findings suggest that HM97662 holds promising therapeutic potential for SCLC when combined with SoC treatment.
- Currently, a first-in-human phase 1 dose escalation study of HM97662 in advanced or metastatic solid tumors in underway in KR/AU (NCT05598151).

References

*These authors contributed equally to this work.

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