Bioinformatics-based Mechanistic Insights into SLFN11-Mediated Chemosensitivity by EZH1/2 Dual Inhibitor HM97662

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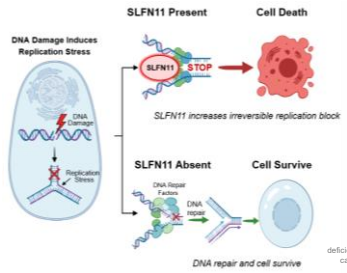
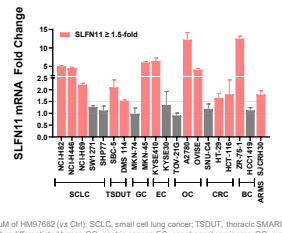
Introduction

EZH2 is the catalytic subunit of the polycomb repressive complex 2 (PRC2) and mediates trimethylation of histone H3 at lysine 27 (H3K27me3), leading to transcriptional silencing of critical tumor suppressor genes [1]. This mechanism has been implicated in the progression of various cancers, where EZH2 overexpression is frequently associated with tumor growth, metastasis, and drug resistance [2]. HM97662, a dual EZH1/2 inhibitor, significantly induces Schlafen 11 (SLFN11) expression, a key regulator of the replication stress response that promotes replication block and impairs DNA damage repair under genotoxic stress, thereby enhancing tumor susceptibility to DNA-damaging agents (DDA) and improving therapeutic response to DDA-based treatments[3]. On this basis, HM97662-induced SLFN11 expression was anticipated to confer vulnerability to replication stress-inducing agents including TOP1 inhibitors. To elucidate the underlying mechanism, integrative bioinformatics analyses incorporating *in vivo* tumor NGS, pathway enrichment, and network propagation were performed to define the molecular link between HM97662-mediated EZH1/2 inhibition, SLFN11 activation, and enhanced chemosensitivity.

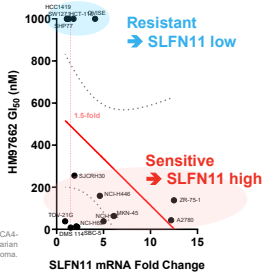
HM97662 Induces SLFN11 Expression Across Solid Cancers

EZH1/2 dual inhibitor, HM97662 regulates SLFN11 expression across multiple solid cancers



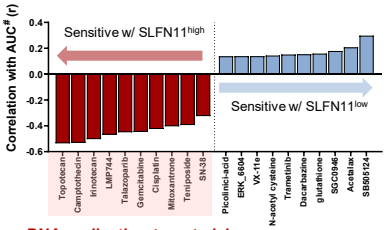


1μM of HM97662 (vs Ctrl); SCLC, small cell lung cancer; TSDUT, thoracic SMARCA4-deficient undifferentiated tumor; GC, gastric cancer; EC, esophageal carcinoma; OC, ovarian cancer; CRC, colorectal cancer; BC, breast cancer; ARMS, alveolar rhabdomyosarcoma.



SLFN11 Correlates with DDA Sensitivity

SLFN11-associated drug sensitivity is enriched for DNA damage agents including topoisomerase I

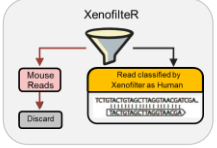


GDSC-based correlation analysis using AUC as a drug sensitivity metric

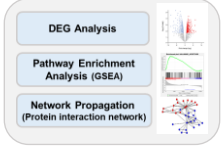
Bioinformatics Workflow

① Tumor RNA profiling from xenograft models

② Obtaining human-specific transcripts using XenofilteR

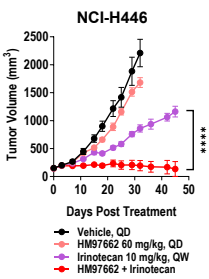


③ Integrative analysis to identify key pathways

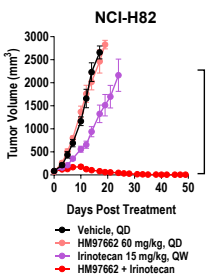


HM97662 Enhances TOP1 Inhibitors Efficacy via SLFN11 Induction *in vivo*

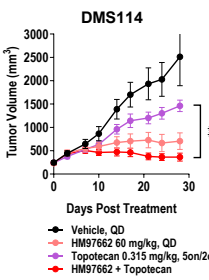
HM97662 enhances the anti-tumor efficacy of topoisomerase I Inhibitors with concurrent SLFN11 induction *in vivo*



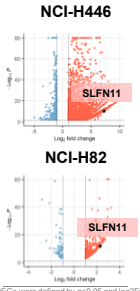
Mean ± SEM / ****p<0.0001, Two-way ANOVA on Day 45



Mean ± SEM / ****p<0.0001, Mixed-effects Analysis on Day 24



Mean ± SEM / **p<0.01, Two-way ANOVA on Day 28

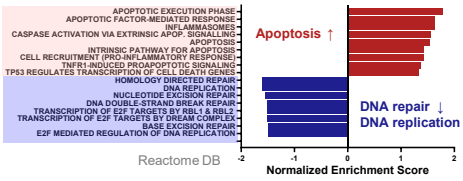


DEGs were defined by p<0.05 and log2FC > 1

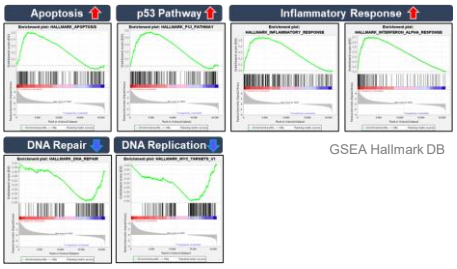
HM97662 Induces Replication Stress and DNA Damage Response Pathways Associated with SLFN11 Activation

HM97662 → SLFN11 activation → DNA replication stress ↑ / repair ↓ → apoptosis in NCI-H446 xenograft mouse model

Enrichment analysis (GSEA)

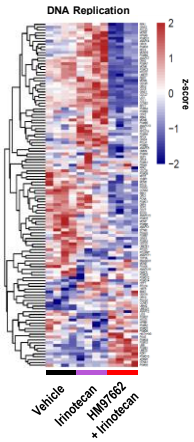


Reactome DB

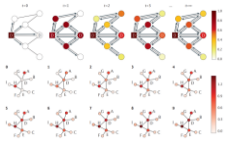


GSEA Hallmark DB

DEG analysis



Network propagation



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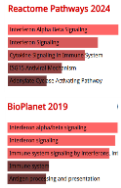
DEG analysis

Gene	Log₂FC
IFI27	10.21
COL6A2	9.37
GABRA5	9.35
MYO6	9.23
NPAS2	9.20
BACE2	9.17
SERPING1	9.15
HLA-C	9.14
FAM189A1	8.98
POTED	8.92
⋮	

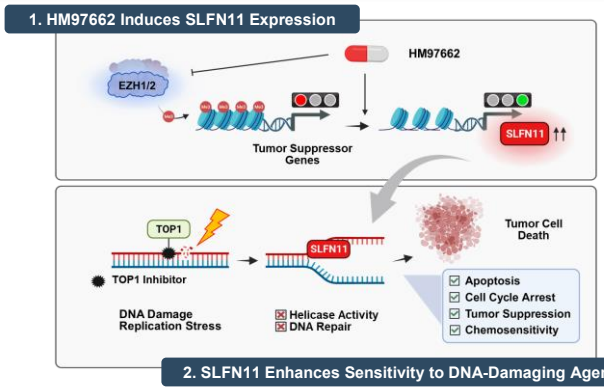
NP analysis

Gene	Log₂FC
IFI27	7.73
HLA-C	7.62
GNAL	7.42
EGFR	7.26
MYO6	7.00
HLA-B	6.97
NPAS2	6.88
COL6A2	6.80
FAM189A1	6.71
SERPING1	6.67
⋮	



Network propagation analysis of the diffusion of DEGs across a biological interaction network

Graph Summary



Conclusion

- HM97662 induces SLFN11 expression across multiple solid cancers, and elevated SLFN11 expression is strongly associated with sensitivity to DNA damage-inducing agents.
- In vivo, HM97662 increased SLFN11 expression in tumors, and combination therapy with TOP1 inhibitors synergistically enhanced anti-tumor efficacy compared with TOP1 inhibitor monotherapy.
- Integrative bioinformatics analyses further identified activation of replication stress- and DNA damage response-related pathways accompanying SLFN11 induction.
- Collectively, HM97662 induces SLFN11 and increases vulnerability to replication stress, thereby potentiating TOP1 inhibitor response.
- A first-in-human phase 1 dose-escalation and dose-ranging study of HM97662 in patients with advanced or metastatic solid tumors is currently underway in Korea and Australia (NCT05598151).

References

1) Mabe, Nathaniel W et al. *Nature cancer*, vol. 5,6 (**2024**): 844-865
2) Comet, Itys et al. *Nature reviews cancer*, vol. 16,12 (**2016**): 803-810
3) Gardner, Eric E et al. *Cancer cell*, vol. 31,2 (**2017**): 286-299.