Overcoming immune checkpoint blockade resistance *via* EZH1/2 dual inhibition by HM97662 in KRAS/LKB1 mutated NSCLC

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Introduction

Recently, immune checkpoint inhibitors (ICIs) have been proposed as promising treatment options for KRAS-mutated non-small cell lung cancer (NSCLC). However, in NSCLC patients with both KRAS mutation and LKB1 loss (hereafter KL), ICIs show low therapeutic effects.[1)] It has been reported that the decreased responsiveness of ICIs in KRAS mutant lung cancers accompanied by LKB1 loss is because LKB1 loss induces STING silencing *via* EZH2 activation and evades immunity.[2)] Epigenetic regulation of EZH2 can enhance the anticancer immunity of the innate immune response by increasing the expression of STING and inducing type I interferon.[3)]

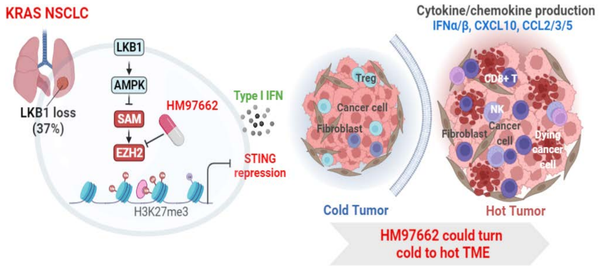
In this presentation, we report that HM97662, a novel EZH1/2 dual inhibitor,[4)] induces STING expression and increases anti-cancer immunity in KL NSCLC cell lines, furthermore, enhances the anticancer effect of anti-PD-(L)1 agents.

As a result, HM97662 potently restored STING expression along with significant decreases of global H3K27me3 in human KL NSCLC cell lines such as A549 and H460 as well as KRAS mutated and LKB1 knockout LL/2 murine lung carcinoma cell line. In addition, HM97662 dose-dependently increased not only the mRNA expression of CXCL10, IFN-α and IFN-β, but also the secretion of immune-activating cytokines such as CXCL10, IFN-α, CCL2, CCL3, and CCL5 in KL H460 cells. On the other hand, the production of immune suppressive cytokine such as IL-8 was decreased in a dose dependent manner. Furthermore, HM97662 in combination with ICIs showed potent anti-proliferative activity against KL lung cancer cells. For example, the combination of HM97662 with anti-PD-(L)1 mAbs such as pembrolizumab or avelumab potently increased anti-proliferative activity against KL H460 and LL/2 cells in a medium co-cultured with human PBMC and mouse splenocytes, respectively.

These results mean that EZH1/2 inhibitor HM97662 increases anti-cancer immunity by changing tumor microenvironment from cold tumor to hot tumor. Currently, studies are underway in various animal models to verify *in vivo* antitumor activity (results be reported later).

EZH2 as a Potential Target for the Treatment of KRAS Mutation and LKB1 Loss Lung Cancers

Loss of LKB1 in KRAS mutation cancer drives the tumor immune escape against immune checkpoint inhibitors



STING Induction *via* EZH1 and EZH2 Dual Inhibition

A. Inhibition of EZH1 and EZH2 catalytic activity

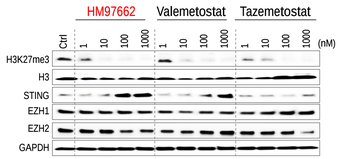
PRC2 complex	IC_{50} (nM)*		
	HM97662	Valemetostat	Tazemetostat
EZH1 WT	16	30	188
EZH2 WT	2.1	1.2	2.8
EZH2 Y641F	1.4	1.1	2.7

* Biochemical inhibition was conducted *via* external service.

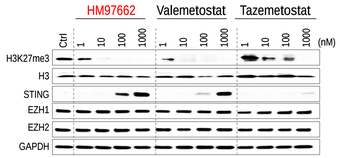
B. H3K27me3 inhibition and STING expression in KL lung cancer cells (no increment of STING in KP-mutated H358 cell, *data not shown*)

KL: KRAS mutation & LKB1 loss,
KP: KRAS mutation & TP53 loss.

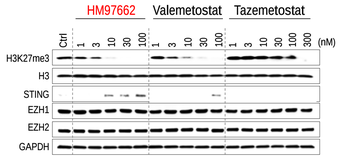
H460
KL human NSCLC cell
KRAS Q61H & STING **low**



A549
KL human NSCLC cell
KRAS G12S & STING absent



LL/2*
KL murine lung carcinoma
KRAS G12C & STING low

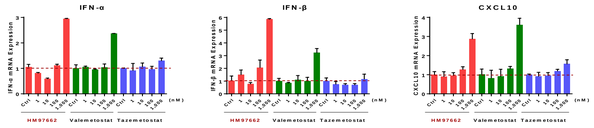


Activity	H3K27me3 inhibition, IC_{50} (nM)			STING induction, EC_{50} (nM)		
Cell lines	hNSCLC		Murine lung	hNSCLC		Murine lung
	H460	A549	LL/2* (LKB1 KO)	H460	A549	LL/2* (LKB1 KO)
HM97662	8.5	1.9	1.3	72	109	26
Valemetostat	26	1.6	3.1	618	252	~100
Tazemetostat	29	413	130	>1,000	>1,000	>300

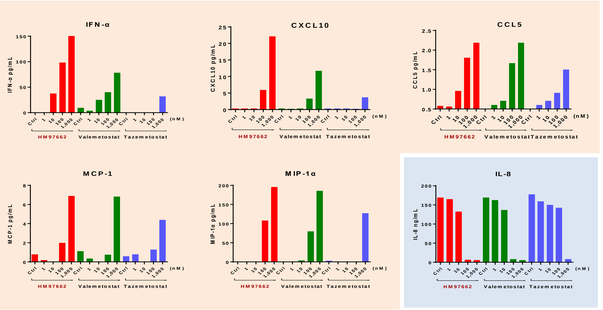
* LL/2 LKB1 KO cells (3C2 clone) were engineered by CRISPR-Cas9;
** LL/2 parental cells ($KRAS^{G12C}$/$LKB1^{WT}$) were not affected on STING expression (data not shown).

Regulation of Immune Related Cytokines & Chemokines in H460 KL NSCLC Cells

A. Induction of mRNA expression levels



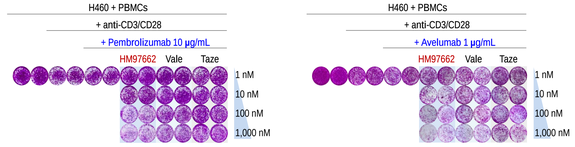
B. Regulation of pro/anti-inflammatory cytokines and chemokines



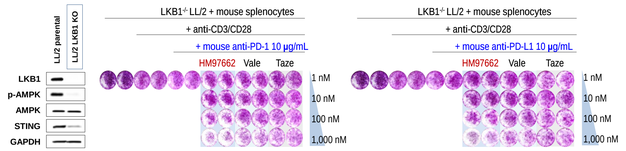
Induction of immune activating cytokines/chemokines
Inhibition of immune suppressive cytokines/chemokines

Anti-proliferative Activity in Combination with Immune Checkpoint Inhibitors in the Presence of Activated T-cells

Human PBMC co-cultured to H460 cells (KL, STING low hNSCLC)



Mouse splenocytes co-cultured to LL/2 LKB KO cells (KL, STING low 3C2 clone)



Test cells	H460 cell, GI_{50} (nM)			LL/2* cell, GI_{50} (nM)		
ICI combination	EZH2 inhibitor alone	Pembrolizumab (anti-PD-1)	Avelumab (anti-PD-L1)	EZH2 inhibitor alone	anti-PD-1 (mouse)	anti-PD-L1 (mouse)
HM97662	111(P), 439(A)	32	12	136	16	35
Valemetostat	>1,000	142	150	228	100	95
Tazemetostat	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000

Concluding Remarks

- HM97662 is a next generation EZH2 inhibitor with an enhanced inhibition activity of EZH1 (dual inhibition).[4)]
- HM97662 dose-dependently induced STING expression in human NSCLC and mouse lung carcinoma cell lines accompanied by KRAS mutation and LKB1 loss.
- Also, HM97662 dose-dependently increased not only the mRNA expression of CXCL10, IFN-α and IFN-β, but also the secretion of immune-activating cytokines such as CXCL10, IFN-α, CCL2 , CCL3, and CCL5, changing tumor microenvironment from cold tumor to hot tumor.
- When combined with anti-PD-(L)1 mAb in a medium of human PBMCs and mouse splenocytes, HM97662 potently inhibited proliferation of lung cancer cells with KRAS mutation and LKB1 loss.
- Taken together, HM97662 can increase the sensitivity of immune checkpoint inhibitors (ICIs) through restoration of STING expression and immune activating cytokine/chemokines production in KL NSCLC (most effective among EZH2 inhibitors tested). That is, HM97662 can be a good combinational options to improve the therapeutic effect of ICIs in KL NSCLC patients.

References

1. Skoulidis F, Goldberg ME, Greenawalt DM, *et al.* STK11/LKB1 Mutations and PD-1 Inhibitor Resistance in KRAS-Mutant Lung Adenocarcinoma. *Cancer Discov*. 2018;8(7):822-835.
2. Della Corte CM, Byers LA, *et al.* Evading the STING: LKB1 Loss Leads to STING Silencing and Immune Escape in KRAS-Mutant Lung Cancers. *Cancer Discov*. 2019;9(1):16-18.
3. Mograbi B, Heeke S, Hofman P, *et al.* The Importance of STK11/LKB1 Assessment in Non-Small Cell Lung Carcinomas. *Diagnostics (Basel)*. 2021;11(2):196.
4. First-in-human phase 1 clinical trial of HM97662 in multiple cancers will be initiated in the first half of this year. Some pharmacological results previously reported (refer AACR 2021, poster presentation, abs# 1142).

Acknowledgements

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