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602.MYELOID ONCOGENESIS: BASIC

The LSD1 Inhibitor Ory-1001 (ladademstat) in Combination with Menin Inhibitor SNDX-5613 (revumenib) Has Synergistic in Vitro Activity in *KMT2A*-Rearranged AML Models

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Introduction: Menin is a scaffold protein that binds to gene promoters, enhancers and transcription factors, thereby influencing transcription. Leukemias with rearrangement of *KMT2A* known as mixed-lineage leukemia (MLL), as well as other abnormalities such as NPM1c, respond to Menin inhibition with promising early clinical results. MLL leukemias affect children and adults and are associated with high rates of resistance to conventional chemotherapy. In addition to these alterations, other leukemia subsets with similar transcriptional dependency could be targeted through Menin inhibition. LSD1 is an essential regulator of murine MLL-AF9 leukemia stem cells (LSCs) and maintains the LSC potential of MLL-AML cells through blocking of differentiation and apoptosis (*Harris et al, 2012)*. LSD1 acts at genomic loci that are bound by MLL-AF9 to maintain expression of the associated leukemogenic program, thus preventing differentiation and apoptosis. We investigated the preclinical efficacy of combined Menin and LSD1 inhibition in AML. PC4 and SF2 interacting protein 1 (PSIP1)/p75, also known as LEDGF, interacts with the N-terminus of MLL and Menin. This complex contributes to the association of MLL and MLL-fusion multiprotein complexes with the chromatin. Several studies have shown that both PSIP1 and Menin are required for efficient MLL-fusion-mediated leukemogenesis and for the expression of MLL-regulated genes. A proposed model of LSD1 interaction with KMT2A-Menin-PSIP1 suprcomplex is depicted in Fig. 1A.
Methods: To assess *in vitro* LSD1 inhibitor (ORY-1001) and Menin inhibitor (SNDX) potency, cell lines were plated and treated with the respective doses of ORY-1001, SNDX, and the combination of these drugs in 96-well plates. We performed synergy assays using increasing concentrations of SNDX and 3μM of ORY-1001. Cell viability and IC50 were measured after 4 days of treatment. The SynergyFinder 2.0 web application was used to calculate synergy scores and generate 2D synergy maps. Western blotting of LSD1, co-immunoprecipitation, and ChIP-qPCR studies were used to assess the depletion of the LSD1 from chromatin. The interaction network of LSD1 interactome proteins was assessed by mass spectrometry.
Results: We showed that LSD1 degrades with increasing concentrations of ORY-1001 after 48h of treatment (Fig.1 B). IC_{50} curves for ORY-1001, SNDX and synergy effect is shown with increasing concentrations of SNDX and 3μM ORY-1001 in MV4-11 cells (expressing MLL-AF4) (Fig.1 C). ORY-1001 alone shows an IC_{50} of ~0.5 μM, and SNDX alone shows an IC_{50} of ~10 nM. The combination of LSD1 and SNDX inhibitors synergistically reduced cell viability in MLL-rearranged MOLM-13 and MV4-11 cells (Fig.1 C). We also assayed the LSD1 endogenous protein interactome in MOLM-13 cells (expressing MLL-AF9) by mass spectrometry. Our LSD1 interactome analysis revealed an association of LSD1 with previously identified interactors such as RCOR1, HDAC1, HDAC2, CARM1 and HMG20B. We discovered an unexpected association between LSD1 and PSIP1. Interestingly, PSIP1 interact with MLL1-MEN1 complex and its crucial for assembling MLL-fusion proteins to their gene targets.
Conclusion: LSD1 Inhibitor ladademstat in Combination with Menin Inhibitor revumenib has synergistic effect in *KMT2A*-Rearranged AML models. Surprisingly, we also found that PSIP1, which is essential for inducing MLL-rearranged leukemia, interacts with LSD1. This suggests that PSIP1 may modulate gene expression through its ability to interact with both MLL1 and LSD1.
This study opens a new avenue to investigate the role of KMT2A-MEN1 in relation to other epigenetic modifiers, such as LSD1, and guide new potential combination therapies that may further potentiate differentiation and elimination of leukemia LSCs.

Disclosures Watts: *Rigel:* Consultancy; *BMS:* Consultancy; *Servier:* Consultancy; *Daiichi Sankyo:* Consultancy; *Reven Pharma:* Consultancy; *Rafael Pharma:* Consultancy; *Aptose:* Consultancy; *Takeda:* Consultancy, Research Funding; *Immune Systems Key:* Research Funding.

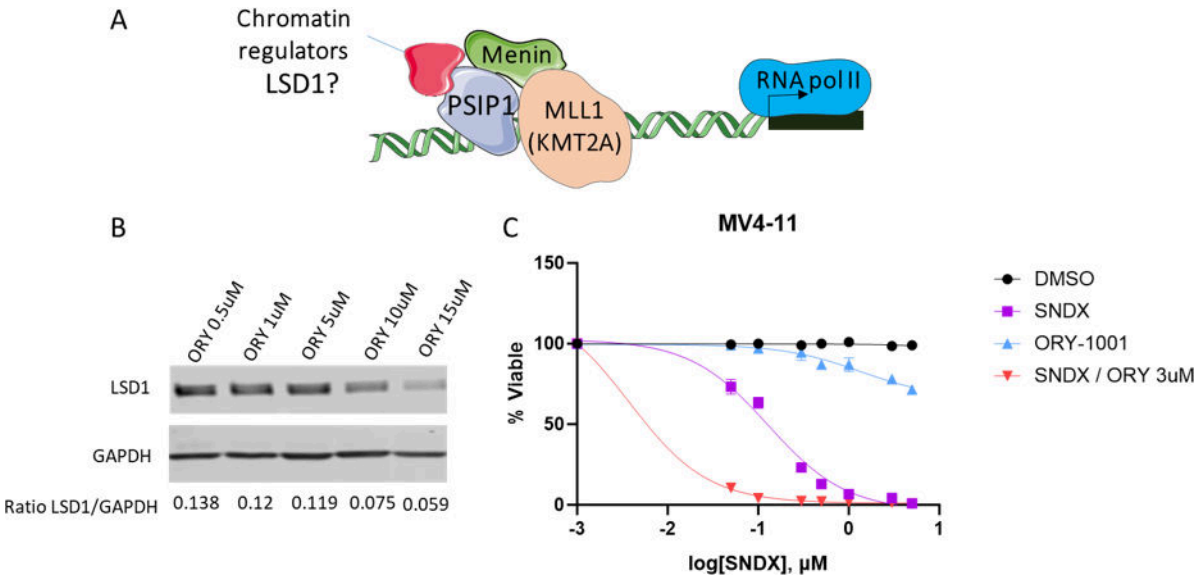

Figure 1

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