NOVEL COMBINATION OF CLINICAL MENIN INHIBITOR ZIFTOMENIB AND THE NUCLEAR EXPORT INHIBITOR SELINEXOR SYNERGISTICALLY INHIBIT MLL-R AML

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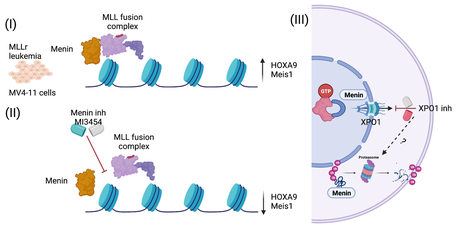
INTRODUCTION

- **Menin is a scaffold protein that tethers histone-lysine-N-methyltransferase MLL1 (KMT2A) to chromatin by binding to the menin binding domain (MBD) of KMT2A.**
- **The N-terminus of the KMT2A gene recombines with a range of genes to form more than 80 fusion proteins (FP) in MLL-rearranged (MLL-r) AML.**
- **In myeloid stem progenitor cells, the menin-KMT2A complex modulates the expression of the leukemogenic homeoboxA9 (HOXA9) gene, as well as its co-factor, MEIS1.**



- **Menin inhibitors evict menin from the chromatin and reduce KMT2A and KMT2A-FP binding to their targets in MLL-r leukemia, breaking the differentiation block and inducing tumor regression.**
- **Aberrant nuclear export is common in cancer, resulting in anomalous localization of various proteins, including tumor suppressor proteins (TSPs).**
- **Blocking XPO1/CRM1 using a selective inhibitor of nuclear export (SINE) compound selinexor has been shown to have robust anti-leukemia activity.**
- **In the present study, we hypothesized that inhibition of menin and nuclear export would synergistically suppress AML cell proliferation and survival.**
- **We have used the clinical-stage menin inhibitor ziftomenib and selinexor to simultaneously target menin-KMT2A protein-protein interactions and nuclear export.**

RESULTS

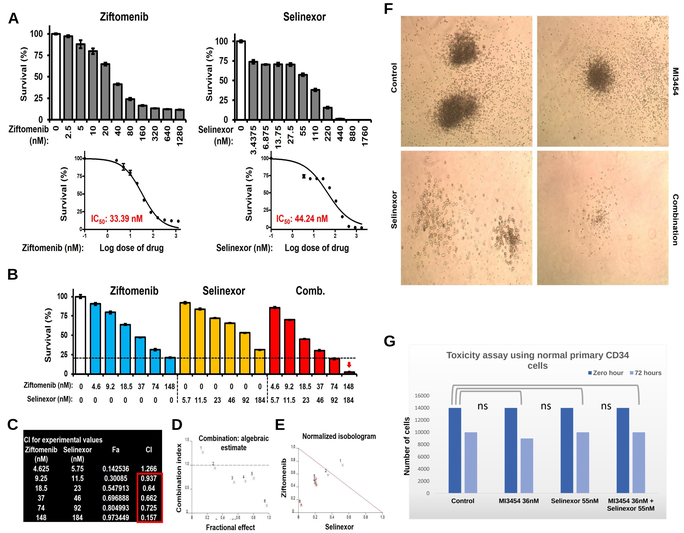


Figure 1: Menin and nuclear export (XPO1) inhibitors synergistically inhibit the growth of MLL-r AML. [A] MV4;11 cells were treated with varying concentrations of ziftomenib or selinexor for 72 hrs (upper panel). Cell titer glo assay was performed to determine the growth inhibition. Lower panel is the dose-response curve generated from GraphPad Prism software, showing IC50 doses. [B] Synergistic combination of ziftomenib and selinexor. [C-D] The combination indexes (CI) and [E] isobologram were generated using Calcusyn 2.1 software. [F] The combination of menin and XPO1 inhibitors in the suppression colony formation of CD34+ MLL-r progenitor cells derived from primary patient samples. [G] The effect of menin and XPO1 inhibitors on the growth of normal CD34+ human hematopoietic progenitor stem cells (HPSCs).

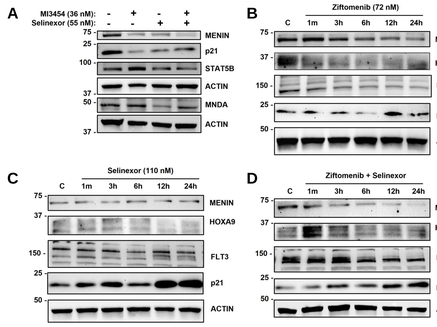


Figure 2: Western blot analysis of menin and associated proteins. [A] Cells were treated with ziftomenib and selinexor either alone or in combination for 72 hrs. DMSO used as vehicle. [B-D] Time dependent effect of ziftomenib and selinexor on the expression of menin and related proteins. Treatment doses are indicated in parenthesis. ACTIN or GAPDH was detected as loading control. Cell lysate was prepared in RIPA buffer with protease and phosphatase inhibitors. A total of 30 ug of total protein was separated in SDS-PAGE.

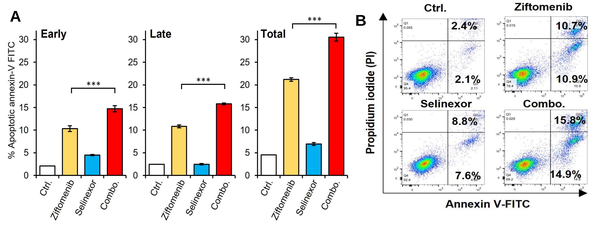


Figure 3: Apoptotic cell death with ziftomenib and selinexor in MV4;11 AML cells. Apoptosis was determined by the flow cytometric detection of annexin-V and propidium iodide (PI). [A] Early, late and total apoptotic cell deaths from ziftomenib and selinexor treatment alone or in combination. [B] Representative flow cytometric image of different treated groups as shown in Fig. A. ***, $p < .001$.

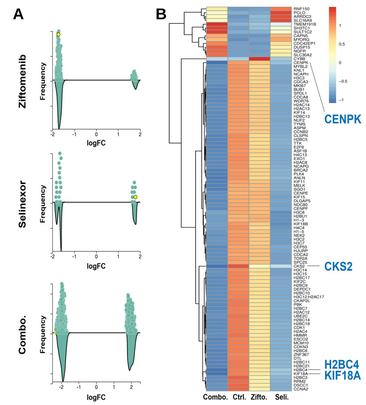


Figure 4: Differential expression of mRNAs from total RNA sequencing of ziftomenib and selinexor treated MV4;11 AML cells. Cells were treated with single or inhibitor combinations for 24 hours. RNA was extracted with Qiagen RNA extraction kit and total RNA with RIN no more than 9 utilized for sequencing. [A] Global expression of mRNAs in different treatment groups. [B] Heatmap showing differentially expressed genes including CENPK, CKS2, KIF18A, and H2BC4.

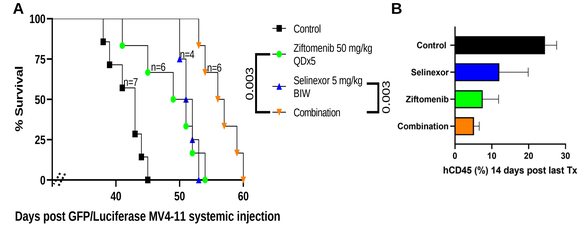


Figure 5: Xenografts in NSG mice using GFP/Luciferase expressing MV4;11 cells. About 2 million cells were injected through tail vein. Mice were randomized based on the luciferase intensity on bioluminescent imaging on Day 8 and received treatment from Day 9-22.
[A] Survival of vehicle or inhibitors treated mice. [B] Percentage of human CD45 positive cells in the peripheral blood of mice groups two weeks post last treatment (3 mice/group).

METHODS

- **To assess growth inhibition, an ATP-based cell proliferation assay was used.**
- **Combination synergy was determined using CalcuSyn Version 2.0 synergy software.**
- **Stem-like progenitor cells were isolated using the StemSpan CD34+ expansion kit (StemCell Tech).**
- **Colony formation efficiency was determined using a Methocult assay (StemCell Tech).**
- **Gene and protein expression and cell death were detected using quantitative real-time PCR, western blotting, and annexin-V/PI fluorescence-activated cell sorting, respectively.**

CONCLUSIONS

- **The clinical candidate ziftomenib, in combination with selinexor, synergistically inhibited the growth of MLL-r AML cells (MV4;11,MOLM13, and SEM; CI < 1) without inducing toxicity to normal cells.**
- **Enhanced suppression of menin expression by the combination of ziftomenib and selinexor suggests molecular basis for the synergy between these two compounds.**
- **The topmost upregulated gene TMEM191B and downregulated gene CENPK warrant further investigation for their role in the synergy.**
- **These preclinical findings demonstrate that simultaneous inhibition of the menin-KMT2A interaction and nuclear export could be a viable strategy for the treatment of MLL-r AML.**

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