The menin inhibitor ziftomenib (KO-539) synergizes with agents targeting chromatin regulation or apoptosis and sensitizes AML with *MLL* rearrangement or *NPM1* mutation to venetoclax.

J. RAUSCH[1], M.M. DZAMA[1], N. DOLGIKH[1], H. STILLER[1], S.R. BOHL[2], C. LAHRMANN[1], K. KUNZ[1], L. KESSLER[3], H. ECHCHANNAOUI[1], C. CHEN[4], T. KINDLER[5], F. BURROWS[3], M. THEOBALD[1], D. SASCA[1], S.A. ARMSTRONG[6,7], and M.W.M. KÜHN[1]

1. Department of Hematology and Medical Oncology, University Medical Center, Johannes Gutenberg-University, Mainz, Germany; 2. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 3. Kura Oncology, Inc., San Diego, CA 92130, USA; 4. Department of Systems Biology, Beckman Research Institute, City of Hope, Duarte, CA, USA ; 5. University Cancer Center Mainz, Mainz, Germany; 6. Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA; 7. Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, MA

uct | Universitäres Centrum für Tumorerkrankungen MAINZ

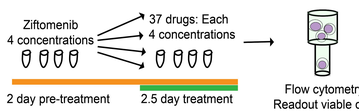
INTRODUCTION

NPM1 mutated (*NPM1*^{mut}) and *MLL*-rearranged Acute Myeloid Leukemia (AML) are dependent on the interaction of the methyltransferase MLL and its cofactor menin to express a particular leukemogenic transcriptional program. This includes the aberrant expression of the self-renewal associated *MEIS1*, *PBX3* and homeobox (*HOX*) transcription factor genes and their targets *FLT3* and *BCL2*[1-3]. Small-molecule inhibitors blocking the menin-MLL interaction reverse this gene expression program, induce differentiation, and have profound anti-leukemic activity against *NPM1*^{mut} and *MLL*-r leukemia models *in vivo* and *in vitro*[5-7]. Ziftomenib is one of five menin inhibitors currently assessed in a clinical phase I/II trial with reported explorative single-agent efficacy in relapsed or refractory AML[7].

AIMS & METHODS

To date, no single therapy has resulted in sustainable remission in AML[8]. We designed a synergy drug screen to identify effective combination partners to ziftomenib and aimed to characterize the highly synergistic effects with the BCL2 inhibitor venetoclax.

1. *In vitro* single-agent efficacy of ziftomenib was assessed by cell viability assays. To characterize treatment effects, differentiation was measured by CD11b surface expression, gene expression changes by RNA sequencing.
2. A drug synergy screen evaluated single and combined effects of ziftomenib and 37 targeted compounds with known preclinical or clinical efficacy in AML:



3. Dose-dependent killing and IC50 values of single and combined ziftomenib and venetoclax treatment was determined on various MLL-r and NPM1mut cell lines in vitro. Treatment-induced apoptosis was assessed by Annexin V staining and BH3 profiling.
4. The drug combination was then validated in *NPM1*^{mut} primary AML samples and *in vivo* in a *MLL*-r MV411 xenograft model.

RESULTS

1. Ziftomenib has profound and selective *in vitro* activity against *MLL*-r and *NPM1*^{mut} AML and induces transcriptional downregulation of *MEIS1, PBX3, FLT3*, and *BCL2*.

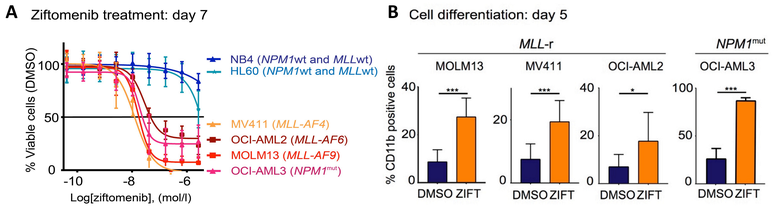


Figure 1: A Dose- response curves from cell viability assays in human AML cell lines after 7 days of treatment. **B** Surface CD11b expression after 5 days of treatment assessed by flow cytometry. **C** Volcano plots of RNA-seq data after 150nM ziftomenib treatment in OCI-AML3, MOLM13 (4 days) and MV411 (3 days). Negative log2 values represent downregulated genes with ziftomenib compared with DMSO.

2. Synergy drug screen of ziftomenib with 37 targeted drugs detects strong activity in *MLL*-r and *NPM1*^{mut} AML.

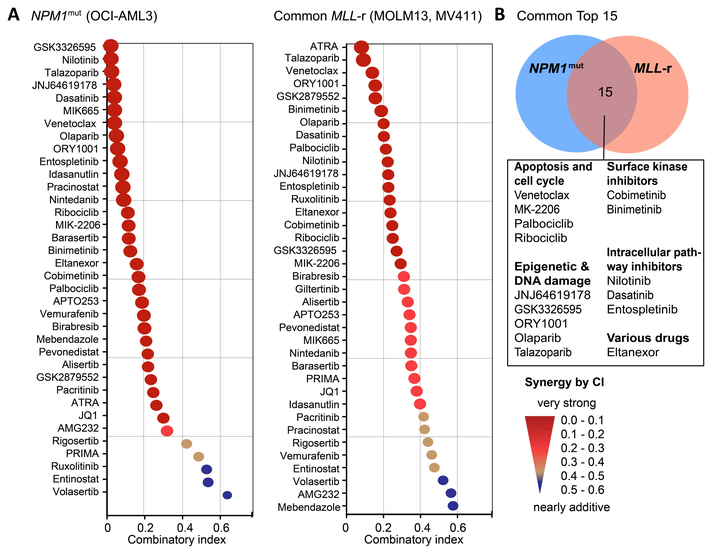


Figure 2: A Synergy screen of ziftomenib with 37 targeted drugs. Viable (DAPI-negative) cells were assessed by flow cytometry after 2 days pre-treatment with ziftomenib followed by 2.5 days combined treatment with small molecule inhibitors. Depicted are combination indices (CI) calculated using CompuSyn software and weighted for IC75, IC90 and IC95 values. For *MLL*-r cell lines, the mean CI of MV411 and MOLM13 was calculated. **B** Venn-Diagram of common top 15 synergistic drugs based on highest CI values.

3. The combination of ziftomenib and venetoclax leads to synergistic anti-proliferative activity and pronounced apoptosis *in vitro*.

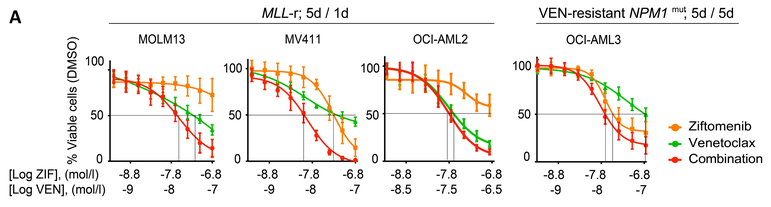


Figure 3: A Dose-response curves from cell-viability assays of MV411, MOLM13, and OCI-AML2 comparing ziftomenib (5 days), venetoclax (24h), and combinational treatment (4 days ziftomenib pretreatment, 24h venetoclax treatment). For OCI-AML3, treatment was 5 days / 5 days. **B** Percentage of apoptotic (Annexin V+) and dead (DAPI^{high}) cells after single and combinational treatment with ziftomenib (150nM) and venetoclax (100nM; OCI-AML3 500nM, OCI-AML2 25nM) **C** BH3 profiling of cells treated with 75nM ziftomenib for 48h before exposure to BH3-peptides (BIM 0,1µM, BAD 1µM, PUMA 0,3µM). Readout was flow cytometry based loss of cytochrome c as a surrogate for apoptotic priming.

4. Combined treatment with ziftomenib and venetoclax improves anti-leukemic effects in *NPM1*^{mut} primary samples and *MLL*-r leukemia *in vivo*.

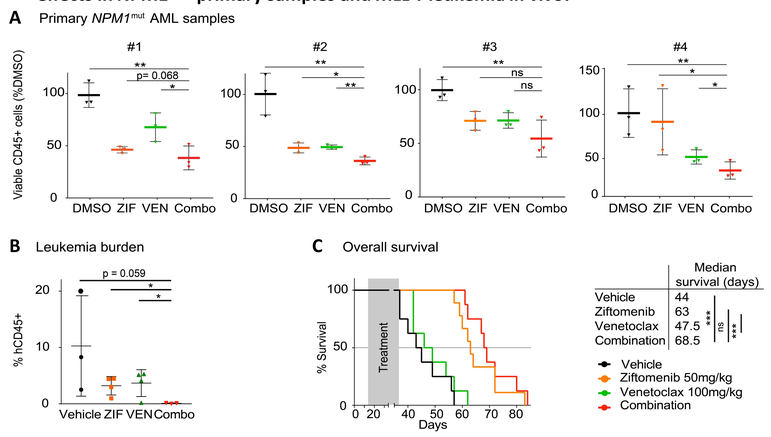


Figure 4: A Viability of 4 independent *NPM1*^{mut} de novo AML samples treated in stromal cell co-culture for 5 days with DMSO, ziftomenib (75nM, 5 days), venetoclax (10nM, 24h) or combination (5 days / 24h). **B** Assessment of leukemic burden as CD45+ cells in the bone marrow of MV411-derived leukemic xenograft mice (n=3 and 4 mice/group) after treatment with drug vehicles, ziftomenib (50 mg/kg; PO; once daily (QD)), venetoclax (100 mg/kg; PO; QD), or combined treatment. **C** Kaplan-Meier estimates of MV411-derived leukemic xenograft mice (n=8 mice/group) after treatment with drug vehicles, ziftomenib (d 12-35, 50 mg/kg; PO; QD), venetoclax (d 16-35, 100 mg/kg; PO; QD) or both.

CONCLUSIONS

- Ziftomenib has significant activity against *NPM1*^{mut} and *MLL*-r AML, suppresses specific leukemogenic gene expression, and induces differentiation.
- Ziftomenib exhibits synergistic leukemia cell killing in combination with drugs from various classes, e.g. targeting chromatin regulation & DNA damage (LSD1, PRMT5, PARP) and apoptosis & cell cycle (BCL2, AKT, CDK4/6).
- The combination with venetoclax has profound anti-proliferative activity. Ziftomenib-induced apoptotic priming may contribute to the synergistic effects.
- The combination of venetoclax and ziftomenib exhibits synergistic anti-leukemic activity, and these data support clinic evaluation of the combination in the treatment of acute leukemias.

REFERENCES

1. Krivtsov AV et al. A Menin-MLL Inhibitor Induces Specific Chromatin Changes and Eradicates Disease in Models of MLL-Rearranged Leukemia. Cancer Cell. Elsevier Inc; 2019 Dec 9;36(6):660–673.e11.
2. Spencer DH et al. Epigenomic analysis of the HOX gene loci reveals mechanisms that may control canonical expression patterns in AML and normal hematopoietic cells. Leukemia. 2015;29(6):1279.
3. Kühn MWM et al. Targeting Chromatin Regulators Inhibits Leukemogenic Gene Expression in NPM1 Mutant Leukemia. Cancer Discovery. 2016 Oct 2;6(10):1166–81.
4. Uckelmann HJ et al. Therapeutic targeting of preleukemia cells in a mouse model of NPM1 mutant acute myeloid leukemia. Science. 2020 Jan 31;367(6477):586–90.
5. Klossowski S et al. Menin inhibitor MI-3454 induces remission in MLL1-rearranged and NPM1-mutated models of leukemia. J Clin Invest. 2020 Feb 3;130(2):981–97.
6. Borkin D et al. Pharmacologic Inhibition of the Menin-MLL Interaction Blocks Progression of MLL Leukemia In Vivo. Cancer Cell. 2015 Apr; 27(4):589–602.
7. Issa GC et al. Therapeutic implications of menin inhibition in acute leukemias. Leukemia. Nature Publishing Group; 2021 Sep; 35(9):2482–95.
8. Engert A, et al. EHA Roadmap for European Hematology Research. a consensus document. Haematologica. 2016 Feb; 101(2):115-208

CONTACT

Johanna Rausch, MD
johrausc@uni-mainz.de
Department of Hematology and Oncology,
University Medical Center Mainz, Germany

This work was supported by grants from the German Research Foundation (DFG): KU-2688/2-2 and SFB1292/2 TP12

Emmy Noether-Programm DFG