ELSEVIER

Blood 142 (2023) 5747–5748

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Variable Response of MLL-Rearranged Leukemia Cell Lines to Combinations of Menin, CDK9 and DOT1L Inhibitors

Aysenur Esen, MD[1], *Roger Luo, PhD*[2], *Joseph Kaberlein*[2], *Michael J Thirman, MD*[3]

[1] Department of Pediatrics, Section of Hematology/Oncology, University of Chicago, Chicago, IL
[2] Department of Medicine, Section of Hematology/Oncology, University of Chicago, Chicago, IL
[3] Department of Medicine, Section of Hematology/Oncology, University of Chicago, Chicago, IL

Background: MLL (Mixed Lineage Leukemia) rearrangements occur in approximately 10% of leukemias. Infant leukemia has a higher frequency of MLL rearrangement (MLL-r) than leukemia in other age groups. Despite intensified treatment in infant leukemia, outcomes remain poor. MLL-r leukemia depends on the interaction of MLL fusions with menin, DOT1L, and CDK9. Single-agent treatment with menin, DOT1L, and CDK9 inhibitors has shown promising results in preclinical studies, but their clinical efficacy has been limited by primary and acquired resistance. We hypothesized that combination therapy targeting multiple regulators, including menin, DOT1L, and CDK9, would provide a more effective treatment of MLL-r leukemia.
Methods: We performed cell viability experiments on leukemia cell lines (MV4;11, MOLM13, RS4;11, THP1, SEM, KOPN8, NOMO1, HL60, ML2) with single, two and three drug combinations using a menin inhibitor (VTP50469), a DOT1L inhibitor (EPZ5676), and a CDK9 inhibitor (AZD4573). The single drug treatment durations with VTP50469, EPZ5676, and AZD4573 were 10 days, 14 days, and 3 days, respectively. The number of viable cells was determined by trypan blue exclusion staining. After the combination treatment, cells were washed with PBS, placed in fresh media for recovery and counted on day 4 or 6. The effectiveness of the combination treatments was determined using SynergyFinder 3.0 web application. Annexin V/PI and CD11b staining were performed using flow cytometry. To demonstrate MLL-menin binding inhibition, we generated an N-terminal FLAG-tagged MLL expression construct in leukemia cell lines and performed co-immunoprecipitation and western blot analysis.
Results: We first determined theIC50 values for each inhibitor separately. Based on the IC50 values of VTP50469 (menin inhibitor), we categorized MLL-r cell lines into three groups: very sensitive (MOLM13 and MV4;11), moderately sensitive (RS4;11 and SEM) and resistant (THP1 and ML2) [Fig. A]. Sensitivity followed a similar pattern (MOLM13 and MV4;11 -sensitive, THP1-resistant) when MLL-r cell lines were treated with EPZ5676 (DOT1L Inhibitor). In contrast, AZD4573 demonstrated consistent efficacy across all MLL-r leukemia cell lines, with THP1 and MOLM13 being particularly sensitive with IC50 values in the low nanomolar (nM) range. After identifying the IC50 values of each inhibitor, we performed viability experiments using two and three drug combinations. The combination of VTP50469 (menin inhibitor) and EPZ5676 (DOT1L inhibitor) was superior in decreasing cell viability in MLL-r leukemia cell lines sensitive to VTP50469 (MV4;11, MOLM13 and RS4;11) but not effective in THP1 cells which are resistant to VTP50469 and EPZ5676 [Fig. B]. Synergy scores analyzed using the ZIP model showed additive effects in MOLM13 and a synergistic response in MV4;11 and RS4;11. After 14-17 days of treatment with EPZ5676 combined with VTP50469, MV4;11, MOLM13 and RS4;11 cells exhibited no recovery from treatment, indicating a profound and persistent antileukemia effect of the combination treatment. Conversely, the combination of VTP5069 (menin inhibitor) and AZD4573 (CDK9 inhibitor) showed a synergistic inhibitory effect on cell viability only in THP1 [Fig. B]. Compared to the single-drug treatments, we observed an increased rate of apoptosis and differentiation in THP1 cells treated with the combination of VTP50469 and AZD4573. The three-drug combination of EPZ5676, VTP50469, and AZD4573 was not superior to the two-drug combinations in any of the MLL-r cell lines examined. To characterize VTP50469 resistance, we analyzed the inhibition of MLL-menin binding using an MLL expression construct in VTP50469 sensitive and resistant cell lines treated with VTP50469 doses ranging between 5-500 nM. Following treatment with VTP50469, the level of expression of the MLL construct correlated with the sensitivity of MLL-r leukemia cell lines to the menin inhibitor.
Conclusions: Based on our preclinical in vitro findings, the co-inhibition of menin-DOT1L or menin-CDK9 is a promising approach for the treatment of MLL-r leukemia. The varying responses of different MLL-r leukemia cell lines to drug combination treatment indicate that not all cases of MLL-r leukemia will respond uniformly to treatment combinations. Taken together, our data provide a strategy to determine optimal treatment combinations for personalized treatment of MLL-r leukemia.

Disclosures Thirman: *AbbVie:* Honoraria; *Merck:* Research Funding; *AbbVie:* Research Funding; *Nurix:* Research Funding; *Syndax:* Research Funding.

OffLabel Disclosure: VTP50469-Leukemia Treatment. EPZ5676-Leukemia Treatment. AZD4573-Leukemia Treatment

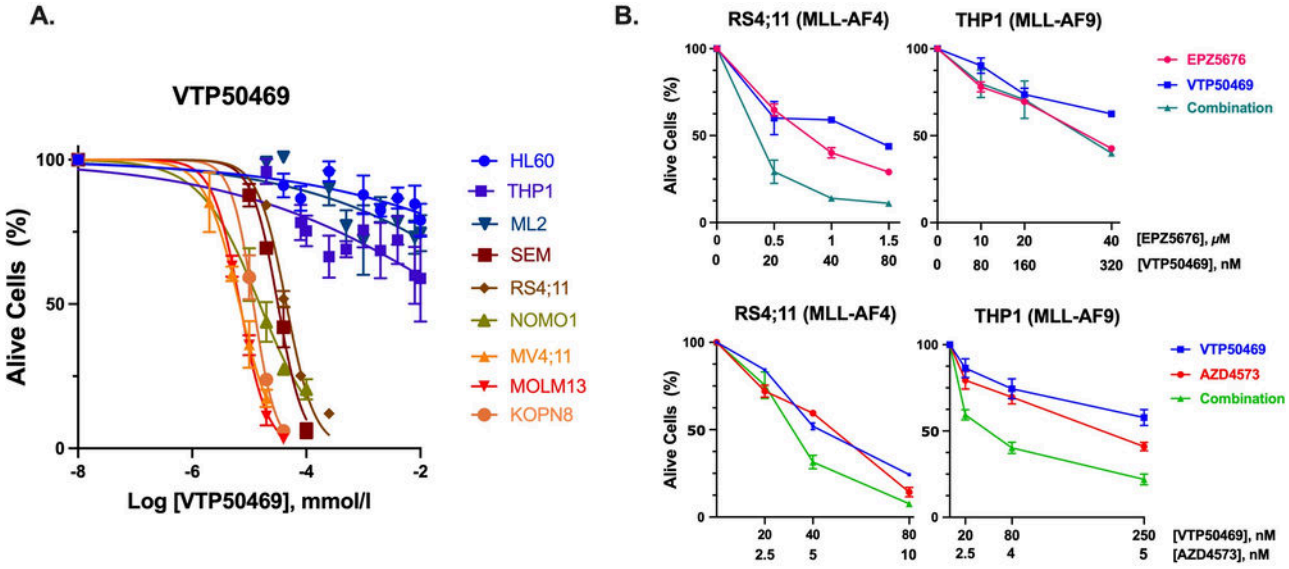

Figure 1

https://doi.org/10.1182/blood-2023-177700