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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Cebpβ/IL1/TNFα Positive Feedback Loop Drives Drug Resistance of BCL2 and MDM2 Inhibitors in Monocytic Leukemia Cells

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Introduction:Ventoclax-based combinations have emerged as a new standard of care for patients with acute myeloid leukemia (AML) who are not suitable for intense chemotherapy. Not all patients respond to these treatments, and those who do may relapse. MDM2 inhibitors are promising therapeutics for treating TP53 wild-type tumors, including most de novo AML cases, with numerous compounds currently in pre-clinical and clinical evaluation. However, clinical trials of MDM2 inhibitors have shown modest and variable clinical activity. Functional genomic data showed that monocytic leukemia (FAB M4/M5) is resistant to venetoclax-based therapies and MDM2 inhibitors. Notably, venetoclax and an MDM2 inhibitor, idasanutlin, demonstrated a strong positive correlation in the Beat AML cohort.

We hypothesize that upregulation of certain myeloid transcription factors in monocytic leukemia may confer intrinsic resistance to BCL2 and MDM2 inhibitors, while certain environmental cues upregulated in these cells may confer extrinsic drug resistance. Additionally, there may be crosstalk between these intrinsic and extrinsic mechanisms.

Methods: We performed differential expression gene (DEG) analysis of transcription factors (TFs) between M4/M5 and M0/M1 samples in the Beat AML and TCGA AML cohort. We overexpressed seven myeloid TFs (SPI1, CEBPB, CEBPD, JUNB, IRF8, KLF4, and MAFB) that are upregulated in M4/M5 and performed competitive drug assays. We treated M4/M5 and M0/M1 samples with venetoclax and idasanutlin, in the presence of a panel of myeloid cytokines and measured cell viabilities. A Luminex assay on monocyte supernatants from M4/M5 and M0/M1 AML samples were conducted to identify dysregulated cytokines. Furthermore, RNAseq DEG and immunoblot analysis were performed on CEBPB-overexpressing cell lines and IL-1-treated AML samples to elucidate the underlying mechanisms.

Results: CEBPB overexpression conferred drug resistance to a broad range of BH3 mimetics, venetoclax combinations, and MDM2 inhibitors. RNA-seq and immunoblot analyses demonstrated that CEBPB overexpression downregulated CASP3, CASP6, BCL2, and TP53 pathway targets (CDKN1A, PMAIP1, BBC3, BMF, TP53), while upregulated MCL1, BCL2A1, and the NF-κB/IL-1/TNF pathway at transcription and/or translation levels. Phenotyping analysis showed that CEBPB overexpression drives myelo/monocytic differentiation. In accordance, CEBPB expression in primary AML correlates with drug responses of idasanutlin, venetoclax, and many venetoclax combinations. Additionally, primary monocytic leukemia expresses significantly higher levels of IL-1/TNF family genes, BCL2A1, and reduced CASP3, CASP6, and BCL2.

Abnormal monocytes, but not granulocytes, T cells, or blasts from M4/M5 leukemia, extrinsically protect leukemia blasts from venetoclax and MDM2 inhibition by secreting elevated IL-1 and TNFα. IL-1β/TNFα treatment drove myelo/monocytic differentiation and up-regulated inflammatory cytokines, including an autoregulatory loop, as well as a number of cytokine receptors, such as IL1R1, TNFRSF1R, TNFRSF2R, and CSF2RB. Remarkably, IL-1α/IL-1β and TNFα uniquely upregulated CEBPB

expression in M4/M5 cells and protected them from apoptosis induced by venetoclax and MDM2 inhibitors. Conversely, TNFα treatment induced augmented extrinsic apoptosis in M0/M1 leukemia cells.
Interestingly, treatment with venetoclax and idasanutlin led to a feedback upregulation of CEBPB, IL-1β, and/or TNFα, along with their respective receptors, and promoted myelomonocytic differentiation.
IL-1/TNFα antagonists or an IRAK inhibitor alone did not kill leukemia cells, but they showed synergistic cytotoxicity when combined with venetoclax and idasanutlin.
Conclusions: In summary, we have described a positive feedback loop between CEBPB, IL-1/TNFα, and monocytic differentiation in monocytic leukemia that contributes to intrinsic and extrinsic drug resistance against BCL2 and MDM2 inhibitors. This crosstalk and the consequent drug resistance are further reinforced by venetoclax/MDM2 inhibition treatment. Combining venetoclax or idasanutlin with IL-1/TNFα antagonists or an IRAK inhibitor can abrogate the feedback loop and induce synergistic cytotoxic effects, offering promising therapeutic strategies to enhance the treatment efficacy of venetoclax and MDM2 inhibitors for monocytic leukemia.

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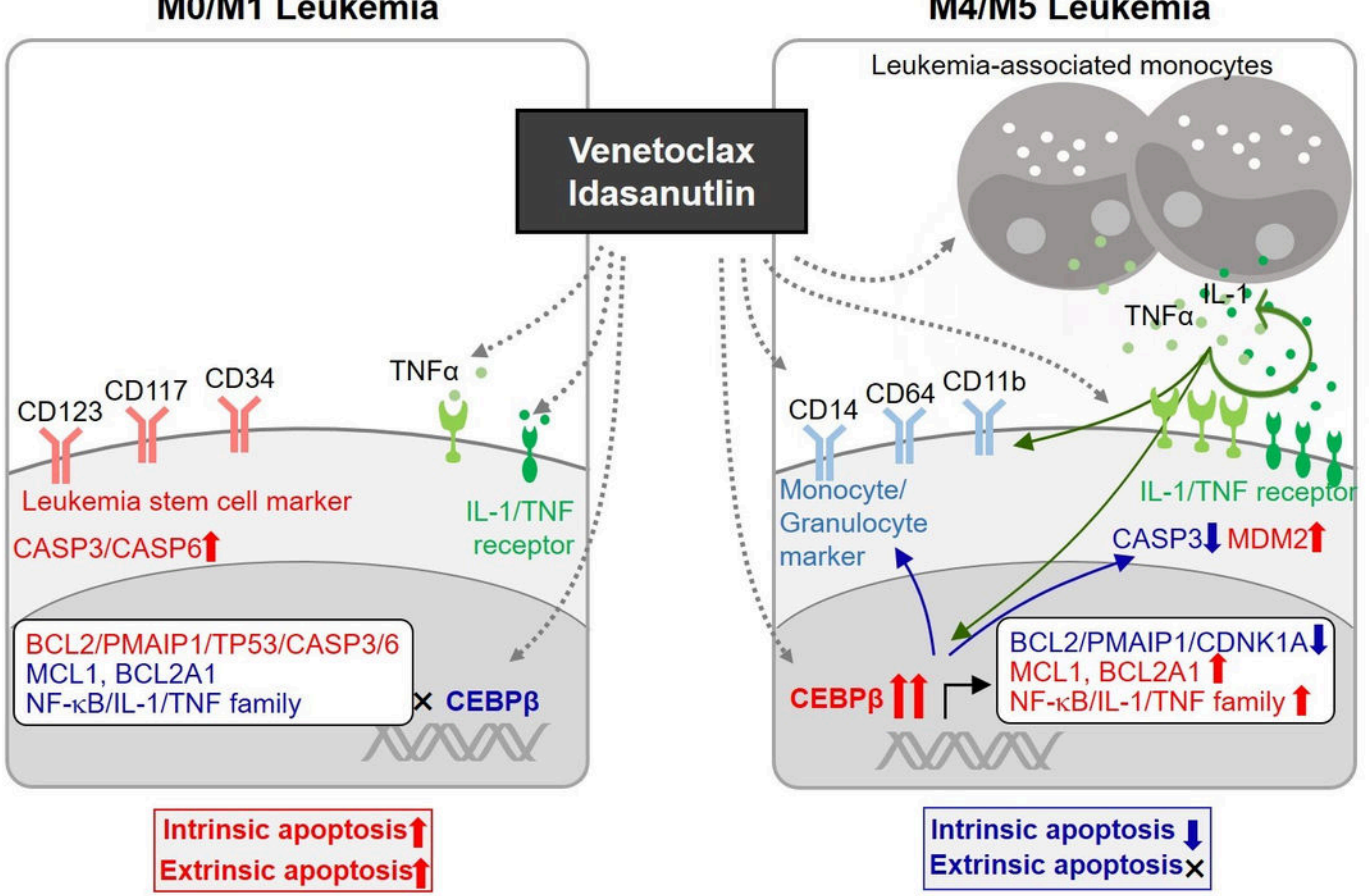

Figure Legend: Monocytic leukemia (M4/M5) intrinsically upregulates CEBPB and IL-1/TNF family receptors. Overexpression of CEBPB downregulates CASP3, CASP6, BCL2, and p53 downstream targets and upregulates MCL1, MDM2, and NF-κB/IL-1/TNF pathway at transcription and/or translation levels. Abnormal monocytes in monocytic leukemia secrete increased levels of IL-1 and TNFα, which induce a marked increase of CEBPB in M4/M5, but not in M0/M1 cells. Treatment with CEBPB and IL-1β/TNFα drives myelo/monocytic differentiation and up-regulates inflammatory cytokines, including an autoregulatory loop, as well as a number of cytokine receptors, such as IL1R1, TNFRSF1R, TNFRSF2R, and CSF2RB. Venetoclax or idasanutlin treatment leads to a feedback upregulation of CEBPB, IL-1β, and/or TNFα, along with their respective receptors, and promotes myelomonocytic differentiation. IL-1α/IL-1β and TNFα uniquely protected M4/M5 cells from venetoclax and MDM2 inhibitors, but not M0/M1. IL-1β and TNFα treatment protects M4/M5 leukemia cells from BCL2 and MCL1 inhibition. Conversely, TNFα treatment leads to increased extrinsic apoptosis in M0/M1 leukemia cells. In summary, a positive feedback loop between CEBPB, IL-1/TNFα, and monocytic differentiation in monocytic leukemia leads to reduced intrinsic apoptosis and extrinsic apoptosis anergy, which ultimately leads to drug resistance of BCL2 and MDM2 inhibitors.

M0/M1 leukemia expresses high levels of BCL2, CASP3, and CASP6, and low levels of MCL1, MDM2, and NF-κB/IL-1/TNF pathway genes. Venetoclax and idasanutlin treatment leads to the activation of both intrinsic and extrinsic apoptosis pathways in these cells.

Figure 1