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ORAL ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Synthetic Lethal Interactions with IRAK4 Inhibition in Myeloid Malignancies

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Overexpression of immune-related genes is widely reported in acute myeloid leukemia (AML). Research from our lab and others has demonstrated that AML leukemic stem and progenitor cells (LSPC) rely on innate immune signaling through IRAK4 (reviewed in Bennett et al., Blood 2023). Moreover, AML LSPCs express a hypermorphic isoform of IRAK4 (IRAK4-L), due to U2AF1- and SF3B1-dependent mis-splicing. Building on this preclinical evidence, several IRAK4 inhibitors are now undergoing clinical trials in AML patients. In Phase-1 findings with a selective IRAK4 inhibitor (CA-4948; Curis Therapeutics), it was found that MDS and AML patients with splicing factor mutations responded best to monotherapy IRAK4 inhibition, although the overall response rate with monotherapy was modest. These findings suggest that IRAK4 is a relevant target in myeloid malignancies, but the identification of synthetic lethal dependencies may be crucial in revealing effective combination therapies.

In this study, we sought to identify synthetic lethal interactions upon IRAK4 inhibition in AML. For this, we first generated isogenic AML cells that are proficient (WT THP1) or deficient for IRAK4 (IRAK4 ^{KO} THP1) using site-directed mutagenesis with CRISPR-Cas9. Deletion of IRAK4 resulted in a modest (~50%) reduction in leukemic colony formation and negligible differences in growth potential. We used the isogenic WT and IRAK4 ^{KO} AML cells to screen for synthetic lethal interactions by performing a 2400+ drug in vitro screen. Hits were prioritized for potency in IRAK4 ^{KO} cells vs WT AML cells, degree of fitness, and overall effect. Among these hits, the Cereblon E3 ligase modulator (CELMoD) CC-885 emerged as the top target. Independent validation confirmed that CC-885 significantly decreased proliferation and viability of isogenic IRAK4 ^{KO} MDS (MDSL) and AML (THP1) cells by approximately 3-4-fold compared to WT MDS or AML cells. Colony formation in methylcellulose after treatment with CC-885 was also significantly reduced in IRAK4 ^{KO} compared to WT MDS and AML cells. Based on Caspase-3 activation and AnnexinV staining, CC-885 induced significantly more apoptosis of IRAK4 ^{KO} cells as compared to WT AML cells. We extended our synthetic lethal analysis to a clinical-stage IRAK4 inhibitor (CA-4948). MDS and AML cells were treated with CA-4948 concurrently with CC-885 or pretreated for 7 days prior to treatment with CC-885. Unexpectedly, we found that only prior exposure of the AML cells to the IRAK4 inhibitors resulted in sensitivity to CC-885. These findings indicate that IRAK4 inhibition reprograms AML cells so that they acquire a synthetic lethal interaction with CC-885.

CC-885 is known to target multiple substrates for degradation by the ubiquitin proteasome, including IKZF1, IKZF3, CK1a, and GSPT1. We compared the effect of CC-885 to CC-90009, a CELMoD with GSPT1 specific activity in the isogenic IRAK4 ^{KO} and WT AML cell lines. CC-90009 did not result in complete cell death up to concentrations of 10mM in both WT and IRAK4 ^{KO}, suggesting that the selective sensitivity of IRAK4 ^{KO} AML cells to CC-885 is not due to inhibition of GSPT1. In support of these interpretations, IRAK4 deficient AML cells exhibit increased protein expression of IKZF2 and IKZF3, but not GSPT1 or CK1a. Given the unique responses of IRAK4 ^{KO} cells to CELMoDs, we posited that IRAK4 inhibition alters the neosubstrate profile of CELMoDs in AML cells. Total proteome characterization of CC-885 treated WT and IRAK4 ^{KO} THP1 cells by mass spectrometry identified a significant decrease in 90 protein substrates in IRAK4 ^{KO} cells treated with CC-885 as compared to WT cells treated with CC-885. These findings suggest that IRAK4 inhibition alters the pool of neosubstrates in AML cells for certain CELMoDs. Overall, our study demonstrates that IRAK4 is a therapeutic target in AML, but that combination therapies, such as with certain CELMoDs, will be necessary to achieve better clinical responses.

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