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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Mediator Kinase/CDK8 Inhibition As a Strategy to Improve FLT3 Inhibitor Activity in Acute Myeloid Leukemia

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Acquired resistance to targeted FLT3 tyrosine kinase inhibitors (TKIs) remains a major barrier to durable clinical responses for patients with *FLT3*-mutant AML. Multiple resistance mechanisms to next generation FLT3 TKIs converge upon RAS/MAPK pathway activation, including protective cytokine stimuli of the bone marrow microenvironment or through secondary oncogenic *RAS* mutations. *RAS* mutations also decrease efficacy of the BCL2 inhibitor venetoclax now widely used in AML, suggesting an anti-apoptotic influence of MAPK signaling. These findings highlight a significant unmet need for novel therapeutic combinations to address MAPK-mediated resistance in AML.

We report results from a genome-wide CRISPR interference (CRISPRi) apoptosis screen using *FLT3*-ITD mutant MOLM-14 cells treated with the FLT3 TKI gilteritinib while cultured in HS5 conditioned media (CM) to mimic cell-extrinsic MAPK-driven TKI resistance. We identified RNA pol II and Mediator kinase/CDK8 ascritical nodes for AML cell survival in the setting of FLT3 inhibition. We further demonstrated that CDK8 inhibition with the small-molecule SEL120 re-sensitized multiple *FLT3*-mutant AML cell lines and primary samples to gilteritinib.

We also performed a second CRISPRi apoptosis screen to identify hits that sensitize to gilteritinib in a model of cell-intrinsic MAPK-mediated resistance using MOLM-14 cells that harbor secondary *NRAS*-G12C mutation.We used Enrichr to perform an integrated analysis and validated the Core Mediator Complex and CDK8 knockdown genes as cellular components significantly enriched among sensitizing hits in both screens. The Mediator subunit *MED12* was identified as a top gene level hit that strongly sensitized cells to gilteritinib in both screens, further emphasizing that Mediator kinase and its transcriptional regulation may be a central pathway engaged in MAPK-dependent survival in *FLT3*-mutant AML.

To better understand potential mechanisms of increased combinatorial CDK8i/FLT3i activity, we performed RNA-seq in MOLM-14 cells treated with gilteritinib, SEL120, or the combination in HS5 CM. Using GSEA we found that gilteritinib stimulated adaptive interferon/inflammatory gene signatures at 16h drug treatment, but this response was restrained by addition of SEL120. Additionally, we identified a striking upregulation of the lineage-controlling transcription factor *IRF8* and its gene expression signature with concomitant silencing of *SPI1* transcriptional output in cells treated with gilteritinib/SEL120. CDK8i treatment alone in AML has previously been shown to increase expression of super-enhancer (SE) associated genes, including *IRF8*. Interestingly, *SPI1*was also a significant gene-level hit in both CRISPRi screens. Using CRISPRi we repressed *IRF8* expression in MOLM-14, MOLM-14 *NRAS*-G12C, and MV411 (another *FLT3*-mutant line) cells and found this significantly blunted the combinatorial pro-apoptotic effect of SEL120, implying that induction of *IRF8* expression plays a significant role in driving response to combined gilteritinib/SEL120.

We finally assessed *in vivo* activity of gilteritinib combined with SEL120. We first performed pilot toxicity studies in NSG mice and determined that gilteritinib 30 mg/kg, SEL120 30 mg/kg, or the combination dosed 5 days/week by oral gavage (OG) were regimens well tolerated by mice. We then engrafted luciferase-tagged MOLM-14 and MOLM-14 *NRAS*-G12C cells into NSG mice and treated for 4-weeks with gilteritinib, SEL120, or the combination. We found gilteritinib/SEL120 extended survival in both MOLM-14 and MOLM-14 *NRAS*-G12C models. Similar efficacy studies are ongoing in *FLT3*-mutant patient-derived xenograft (PDX) models with NSGS mice and will be reported.

Our results indicate that *FLT3*-mutant AML cells employ an adaptive inflammatory response to evade FLT3i-induced apoptosis, and CDK8i represses this adaptation while concomitantly increasing the ratio of *IRF8* to *SPI1*-driven gene expression programs. We speculate this latter effect may in part alter an AML cell's differentiation state to sensitize cells to apoptosis, though further study to explore this hypothesis is needed. Our *in vitro* and *in vivo* efficacy data further validated combined FLT3i/CDK8i as a promising investigational strategy to pre-empt or overcome MAPK-mediated FLT3 TKI resistance.

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