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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

CRISPR/Cas9 Screen Identifies CPX-351 and 7+3 Regimens Response Modulators with Distinct Sensitive and Resistant Profiles

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CPX-351 (dual-drug liposomal encapsulation of daunorubicin and cytarabine in a synergistic 1:5 molar ratio) is approved for newly diagnosed high-risk and secondary AML as an alternative to the widely used cytarabine and daunorubicin (7+3) regimen. Despite newly approved drugs, many AML patients still developed treatment resistance and relapse, leading to poor survival. Amid mounting research into the molecular mechanisms of AML therapies, treatment resistance remains a major concern. To overcome treatment resistance, it is necessary to examine the molecular mechanisms underlying the CPX-351 and 7+3 treatment response. We conducted custom CRISPR/Cas9 synthetic-lethal screens targeting 2440 genes (AML-relevant genes, cytarabine and daunorubicin pharmacology, and druggable genes) in six AML cell lines, profiled drug-sensitive/-resistant modulators, and identified novel targets for overcoming drug resistance in CPX-351 and 7+3 regimen. Six AML cell lines were transduced with a custom library and puromycin was selected before pool transduced cells were treated with DMSO, the IC30-IC50 of cytarabine, daunorubicin, or CPX-351 for 7 doubling-times at 500x coverage. All samples were processed with genomic DNA, sgRNAs were amplified by PCR, and Illumina NovaSeq 6000 SP 100SR sequencing was performed. The abundance of sgRNA was analyzed using MAGeCK-RRA to estimate the RRA drug response score (Drug vs. Control conditions at 7-doubling times) or RRA essential score (Control at 7-doubling time vs. Control at Day 0). Genes with an average RRA score of < -1 across six AML cell lines were defined as drug-resistant or essential genes (negative selection), whereas genes with an average RRA score of > 1 were categorized as sensitive or suppressor genes (positive selection). Next, comparisons of functional pathways with over-representation analysis were performed on resistant and sensitive genes across three drugs to determine differences in their functions.

At the average RRA response score cut-off, 61, 163, and 112 were identified as drug-resistant genes, while 46, 93, and 86 were sensitive genes to cytarabine, daunorubicin, or CPX-351 (provided by Jazz Pharmaceuticals), respectively. **Fig 1A** showed a Venn diagram of all significant resistant and sensitive genes across all three drugs with 14 common significant genes and 73, 174, and 118 unique genes to cytarabine, daunorubicin and CPX-351, respectively, along with their essential score. Among these significant common response genes, some notable genes included *ABCC1*, *SAMHD1* and *TP53*, where CRISPR knocking out screens led to depletion or enrichment of sgRNA when treated with drugs across all AML cell lines. *ABCC1* is a drug-resistant gene that encodes the ATP Binding Cassette C1-mediated drug efflux transporter; therefore, knocking out the efflux transporter causing drug resistance, would increase the lethality of AML cells . Our result also showed *ABCC1* isa tumor suppressor gene; because knocking out the *ABCC1* gene improves cell survival and growth . *SAMHD1* is another notable gene that encodes for deoxynucleoside triphosphate (dNTP) tri-phosphohydrolase that cleaves physiological dNTPs into deoxyribonucleosides and inorganic triphosphate. *SAMHD1* has been shown to cause resistance in cytarabine, but there is limited evidence of its response to other drugs. In this study, we showed that *SAMHD1* is a resistant gene for CPX-351 and a sensitive gene for daunorubicin, and it is a tumor suppressor gene. Lastly, *TP53* is one of the significant common sensitive genes to cytarabine, daunorubicin, and CPX-351. *TP53* encodes for the well-known tumor suppressor gene tumor protein 53, and mutations in TP53 have been shown to affect drug response. In this study, we demonstrated that knocking out *TP53* in

all AML cell lines contributed to cell survival and growth as a tumor suppressor gene and a drug-sensitive gene across the three drugs. Comparing significant resistant and sensitive genes across CPX-351, cytarabine, and daunorubicin, as shown in **Fig 1B**, revealed that resistant genes shared some common involvement in the cell cycle and cellular response to stress, but resistant genes for each drug displayed more distinct functional pathways. Moreover, each drug's sensitive genes revealed distinct functional enrichment pathways. Our current research is focused on identifying novel drug targets to combat drug resistance in the CPX-351 and 7+3 regimens.

Disclosures No relevant conflicts of interest to declare.

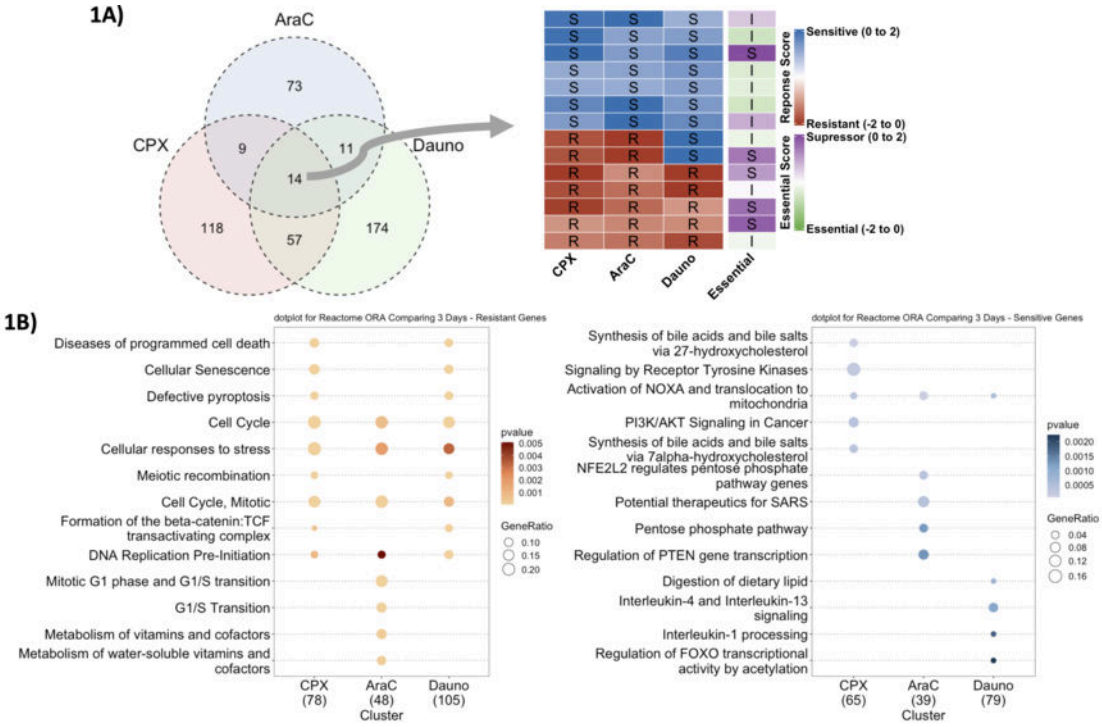

Figure 1: A) Significant genes hit from CPX-351 (CPX), cytarabine (AraC), and daunorubicin (Dauno) CRISPR screens. Venn diagram showed significant genes as either sensitive or resistant genes. Heatmap showed significant common genes across 3 CRISPR screens with each row as either drug-sensitive genes highlighted in blue (mean RRA score > 1) or drug-resistant genes highlighted in red (mean RRA score < -1) when comparing treatment vs control at the end of 7-doubling times for each drug. These significant common sensitive/resistant genes also had essentiality scores calculated and were defined as purple highlighted suppressor genes (S) or green highlighted essential genes (E) when comparing control at 7-doubling times vs. day 0. Genes deemed as I if not meeting CRISPR score significant. **B)** Comparison of functional pathways across three drugs, either resistant genes (left) or sensitive genes (right), via Clusterprofile using the Reactome database.

Figure 1

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