An integrated approach reveals monocyte chemoattractant protein 1 (MCP-1) modulates trametinib resistance in Acute Myeloid Leukemia

Modak, R.,[1] Gosline S.J. [3], Damnernsawad, A.,[1] Laderas, T.,[2] Wu, G.,[2] Nestor M.,[3] Piehowski P., [3] Tyner, J.,[1] Rodland, K.,[3, 4] McWeeney, S.,[2] Agarwal, A[1, 4]

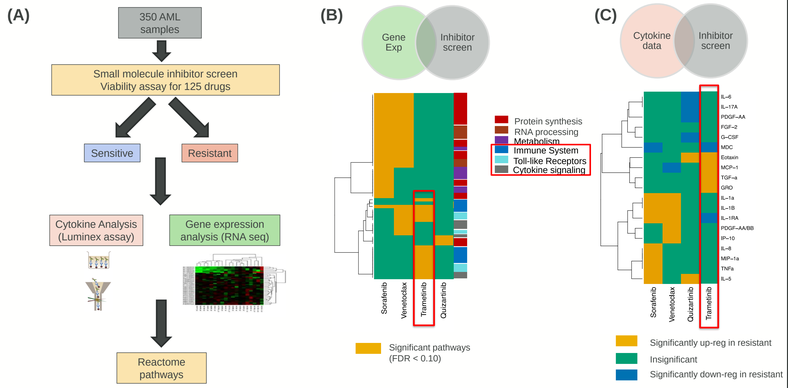
[1]Division of Hematology and Medical Oncology, The Knight Cancer Institute, Oregon Health & Science University, Portland, OR 97239, [2]Department of Medical Informatics and Clinical Epidemiology, The Knight Cancer Institute, Oregon Health & Science University, Portland, OR 97239, [3]Biological Sciences Division, Pacific Northwest National Laboratory, Richland WA 99352 [4]Department of Cell, Development and Cancer Biology, School of Medicine, Oregon Health & Science University, Portland, OR 97239

Introduction

- Acute myeloid leukemia (AML) is characterized by an aberrant expansion of myeloid progenitors in the bone marrow and peripheral blood.
- AML is a heterogeneous disease with a poor five-year survival rate, <30% even after treatment with conventional chemotherapy.
- Current AML therapies have provided little improvement in achieving complete remission. This is attributed to:
  - Development of drug resistance due to novel mutations.
  - Extrinsic factors from the microenvironment that promote AML progression.
- Cytokines and growth factors from the AML microenvironment promote leukemogenesis, AML progression and drug resistance by modulating cell survival, proliferation and differentiation of cells.

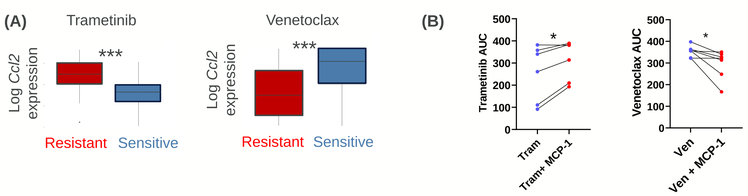
Methods and Results

Figure 1: Data integration reveals that drug resistance correlates with cytokine regulation pathways



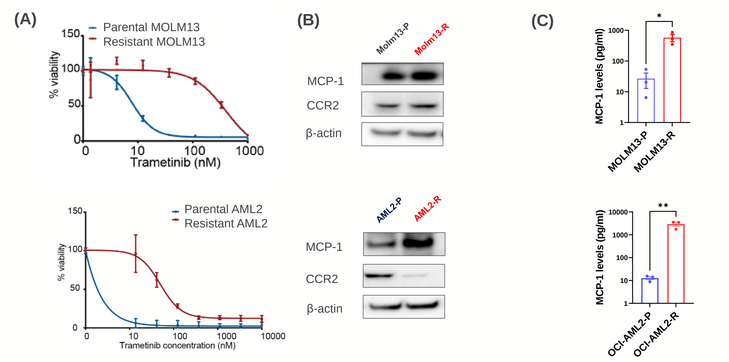
(A) Schematic of data integration components- Inhibitor screen from 350 AML patient samples classified patients into sensitive and resistant categories. RNA sequencing and cytokine screen were integrated with the sensitive and resistant patients to identify resistance signatures. (B) Heat-map of pathway analysis upon integrating RNA-seq data with inhibitor response profile. Red box highlights cytokine pathways altered with trametinib-resistance. (C) Heat-map integrating cytokine expression data with inhibitor response profile. Red box highlights increased MCP-1 levels (x axis) intersecting with trametinib-resistance (y axis).

Figure 2: Cytokines modulate resistance and sensitivity in AML patient samples



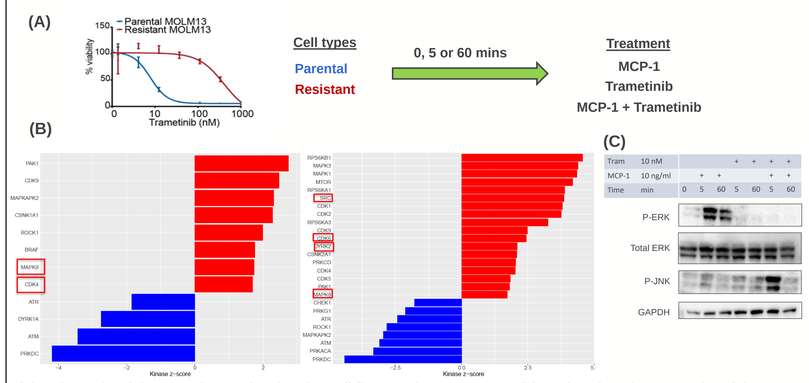
(A) MCP-1 mRNA levels from AML patient samples that are resistant and sensitive to Trametinib and Venetoclax. (B) Primary cells from AML patients were cultured for 72 hours in the presence of MCP-1 and the indicated drug. Viability was determined using a standard MTS assay. Area under the curve (AUC) of primary cells treated with MCP-1 in the presence of trametinib (Tram) and venetoclax (Ven).

Figure 3: Trametinib resistance in AML cell line models correlates with MCP-1 expression



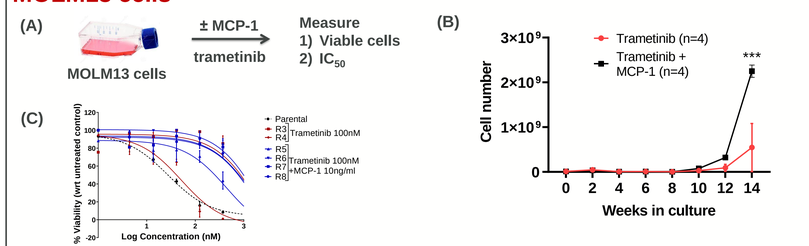
(A) Trametinib resistant cells were generated from AML cell lines MOLM13, and OCI-AML2 by culturing in increasing concentrations of trametinib over 4 months. (B) Western blots from parental and resistant MOLM13 and OCI-AML2. Cell lines were probed for MCP-1 and its receptor CCR2. (C) MCP-1 protein expression measured by an ELISA from the supernatant of parental and resistant MOLM13 and OCI-AML2 cell lines.

Figure 4: MCP-1 stimulation leads to activation of distinct phosphorylation signatures in MOLM13 parental cells



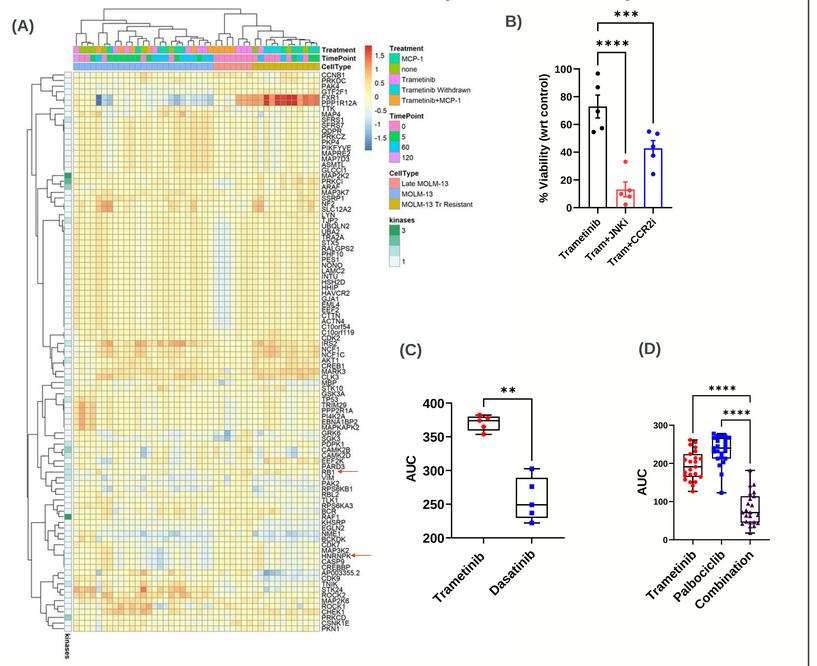
(A) Schematic of the experiment showing the cell lines and treatment conditions for Phosphoproteomics. (B) z scores from the KSEA analysis of MOLM13 cell lines treated with a combination of trametinib (10nM) and MCP-1 (10ng/ml) for 5 minutes (left) and 60 minutes (right). (C) Western blot from MOLM13 parental cell lines stimulated with MCP-1 for 0, 5 and 60 minutes with or without trametinib. Expression of phosphorylated ERK, and JNK were measured.

Figure 5: MCP-1 confers growth advantage in long term cultures of MOLM13 cells



(A) MOLM13 AML cell lines were treated with trametinib (0.1-1μM) only or trametinib (0.1-1 μM) + MCP-1 (10ng/ml) for 4 months (n=4). (B) Viability in the presence of trametinib, determined using the MTS assay for MCP-1 treated cells. (C) Cell numbers over 14 weeks of treatment with trametinib or combination of trametinib and MCP-1.

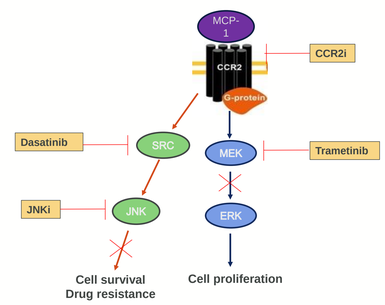
Figure 6: Phosphoproteomics reveals novel targetable pathways in trametinib resistant MOLM13 cell lines (JNK, SRC, CDK)



(A) Heatmap of substrate activity of MOLM13 sensitive and resistant cell lines plotted using log fold change. Substates of MAPK8, SRC and CDK4/6 are denoted with arrows. (B) MOLM13 trametinib resistant cell lines were cultured in trametinib alone or in combination with JNK inhibitor SP600125 or CCR2 inhibitor BMS-CCR2 for 3 days and viability was measured by the MTS assay. (C) MOLM13 trametinib resistant cell lines were cultured in trametinib alone Src/ Abl inhibitor Dasatinib. Viability represented as Area Under the Curve (AUC). (D) AUC of AML patient samples resistant to trametinib, and palbocyclib and combination.

Conclusion and Perspectives

- Data integration revealed specific cytokines levels correlated with resistance to therapy.
- MCP-1 correlated with trametinib resistance in AML patients.
- Trametinib resistant cell lines upregulate MCP-1.
- Phosphoproteomics revealed novel pathways in trametinib resistance such as JNK, SRC, and cell cycle regulation are dependent on CDK4/6.
- Combination therapy using trametinib with JNK, SRC, CDK4 and CDK6 inhibitors mitigates AML cell survival.



Future Directions

- Experiments are underway to investigate the source of MCP-1.
- CRISPR screening is in progress to determine the mediator(s) for trametinib resistance in cells.
- *In vivo* experiments using patient derived xenograpfts will be performed to test the efficacy of combination therapy on trametinib resistant samples.

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Contact: Rucha V. Modak, modak@ohsu.edu, Anupriya Agarwal, agarwala@ohsu.edu)