Acute myeloid leukemia (AML) is a cancer of the blood and bone marrow with a low two-year survival rate. Standard chemotherapy achieves complete remission in 60-80% of patients, but only 20-30% survive two years due to relapse. This is a result of leukemia stem cells (LSCs) that can self-renew and recapitulate disease. AML with TP53 alterations has a particularly poor prognosis with lower survival rates of 0-10% at one year. Broad, in vitro drug screens have identified drugs which have some activity in AML with TP53 alterations. These therapies include Crizotinib, Elesclomol, AZD1480, GW2580, Entrectinib, and Venetoclax, which are signaling pathway inhibitors. The overall goal of this research is to understand the molecular mechanisms of self-renewal and therapeutic vulnerabilities in LSCs of AML with TP53 alterations. Specifically, we aim to identify effective drugs that target leukemia-initiating cells in this treatment-refractory cancer.

1. Determining Starting Inhibitor Concentrations
   • We reviewed literature to determine drug concentrations (IC50 values) for AML samples with TP53 alterations.
   • The mean IC50 values of AML samples with TP53 alterations were derived from the literature and used as starting concentrations in our studies.

2. In Vitro Viability Assays
   • Human AML samples with TP53 alterations were treated, in vitro, with drug (or vehicle control).
   • Viability was assessed at 24 and 48 hours by Trypan Blue exclusion dye.

3. In Vitro Colony Forming Assays (CFAs)
   • Cells were treated with drug (or vehicle control) and plated in semi-solid media.
   • Colony formation was scored at 7-14 days.

4. Mass Cytometry (CyTOF)
   • CyTOF was used to quantitatively assess cellular surface and intracellular signaling proteins at the single-cell level. These experiments are used to assess the effects of each inhibitor on signaling within leukemia subpopulations.

Viability Assays: Crizotinib reduces in vitro viability of AML with TP53 Alterations

Colony Forming Assays (CFAs): AZD1480, Crizotinib, Elesclomol and Venetoclax at higher concentrations reduce colony formation in primary AML with TP53 Alterations

Mass cytometry: Crizotinib induces a loss of CD34, CD38, CD11B and NFKB-high cells in primary AML with TP53 Alterations.

SUMMARY & CONCLUSIONS
• Our data suggests that Crizotinib inhibits in vitro viability and colony formation of primary human AML with TP53 alterations. These data suggest that Crizotinib may be an effective therapy for patients with this disease.
• Higher doses of Elesclomol reduced in vitro viability in one AML sample suggesting that this agent may be of therapeutic benefit as well.

FUTURE DIRECTIONS
• The effect of these panel of drugs will be tested at a range of concentrations in a wider panel of TKI samples to assess the generality of these findings.

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