Abstract Title: Imetelstat-Mediated Alterations in Lipid Metabolism to Induce Ferroptosis As Therapeutic Strategy for Acute Myeloid Leukemia

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Abstract: Imetelstat is a first-in-class telomerase inhibitor with clinical efficacy in myelofibrosis and myelodysplastic syndromes (Steensma et al. 2021; Mascarenhas et al. 2021). We have previously demonstrated that imetelstat is active in AML patient-derived xenografts (PDX). Here, we performed functional genetics combined with lipidomics to identify the key mediators of imetelstat efficacy.

We performed genome-wide CRISPR knockout screening using the Brunello library to identify gene knockouts that confer resistance to imetelstat. Cas9 expressing NB4 cells transduced with the Brunello library or untransduced controls were cultured in the presence of imetelstat concentrations that resulted in significant cell death (IC98) of the untransduced control cultures but allowed the enrichment of imetelstat-resistant cells. Combined RIGER and STARS gene-ranking algorithms on guide RNA sequencing data from imetelstat-resistant cultures identified seven significant hits: fatty acid desaturase 2 (FADS2), acyl-CoA synthetase long chain family member 4 (ACSL4), translocase of inner mitochondrial membrane 17A (TIMM17A), myosin regulatory light chain interacting protein (MYLIP), and late endosomal/lysosomal adaptor, MAPK and MTOR activator 1-3 (LAMTOR1, LAMTOR2, LAMTOR3). Single guide RNA-mediated editing in multiple AML cell lines (n=4) confirmed that loss-of-function editing of ACSL4 or FADS2 confers competitive growth advantage under imetelstat pressure.

ACSL4 and FADS2 encode key enzymes regulating polyunsaturated fatty acid (PUFA)-containing phospholipid synthesis. Targeted lipidomics on 593 lipid species and their desaturation levels demonstrated clear effects of imetelstat treatment and FADS2 editing on the cellular lipidome. Imetelstat significantly increased the levels of phospholipids with triglycerides and reduced the levels of phospholipids containing cholesteryl esters and ceramides when compared to vehicle control. Furthermore, the levels of phospholipids containing fatty acids with three unsaturated bonds were significantly increased in imetelstat-treated compared to vehicle control-treated NB4 cells, and this increase in lipid desaturation was diminished by FADS2 editing. These data demonstrate imetelstat-induced PUFA phospholipid synthesis in an FADS2-dependent manner.

ACSL4 has previously been identified as key regulator of ferroptosis, an iron-dependent non-apoptotic type of regulatory cell death that is driven by excessive PUFA-phospholipid peroxidation. Imetelstat significantly induced lipid peroxidation and lipid ROS production in an ACSL4 and FADS2-dependent manner, and the lipid ROS scavengers ferrostatin-1 and liproxstatin-1 completely prevented imetelstat-induced cell death in all AML cell lines tested (n = 8). In vivo AML PDX efficacy of imetelstat was partially diminished by liproxstatin-1 treatment. This proposed mechanism of action of imetelstat-induced ferroptosis led to the hypothesis that oxidative stress induction can sensitize to imetelstat therapy.

Anthracycline/cytarabine chemotherapy significantly increased ROS levels in a dose-dependent manner in all AML cell lines tested. As proof-of-concept in vivo, we sequenced oxidative stress-inducing standard induction chemotherapy prior to administration of imetelstat in a PDX cohort from 20 individual AML patient samples (each performed in n=6 replicates per treatment group; n = 480 NRGS recipients in total). Combination therapy significantly prolonged survival when compared to imetelstat monotherapy (median 158 days vs. 139 days; $p = 0.0328$), induction chemotherapy alone (158 days vs. 139 days, $p = 0.0100$), or vehicle control (158 days vs. 104 days, $p < 0.0001$). Moreover, AML burden was significantly reduced in the combination therapy group when compared to either monotherapy or vehicle treated control groups.

In summary, we have identified ferroptosis inducers as key mediators of imetelstat efficacy. Imetelstat promotes the formation of PUFA-containing phospholipids, causing excessive levels of lipid peroxidation and oxidative stress. Pharmacological inhibition of ferroptosis diminishes imetelstat efficacy. These mechanistic insights may be leveraged to develop an optimized therapeutic strategy using oxidative stress-inducing chemotherapy to sensitize leukemia cells to imetelstat providing significantly improved disease control for AML.

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Author note: The oral presentation that was given at ASH 2022 will be made available on the Geron website immediately following publication of this work in a peer-reviewed journal.