SIRT5 Is a Druggable Metabolic Vulnerability in Acute Myeloid Leukemia

Dongqing Yan1, Anca Franzini1, Anthony D. Pomicter1, Brayden J. Halverson1, Orlando Antelope1, Clinton C. Mason2, Jonathan M. Ahmann1, Anna V. Senina1, Nadeem A. Vellore1, Courtney L. Jones3, Matthew S. Zabriskie1, Hein Than4, Michael J. Xiao1, Alexandria van Scoyk1, Ami B. Patel5, Phillip M. Clair1, William L. Heaton1, Shawn C. Owen6, Joshua L. Andersen7, Christina M. Egbert7, Julie A. Reisz8, Angelo D’Alessandro3,8, James E. Cox3, Kevin C. Gantz1, Hannah M. Redwine1, Siddharth M. Iyer1, Jamshid S. Khorashad10, Nima Rajabi11, Christian A. Olsen11, Thomas O’Hare11, and Michael W. Deininger1,5
ABSTRACT

We discovered that the survival and growth of many primary acute myeloid leukemia (AML) samples and cell lines, but not normal CD34+ cells, are dependent on SIRT5, a lysine deacylase implicated in regulating multiple metabolic pathways. Dependence on SIRT5 is genotype agnostic and extends to RAS- and p53-mutated AML. Results were comparable between SIRT5 knockdown and SIRT5 inhibition using NRD167, a potent and selective SIRT5 inhibitor. Apoptosis induced by SIRT5 disruption is preceded by reductions in oxidative phosphorylation and glutamine utilization, and an increase in mitochondrial superoxide that is attenuated by ectopic superoxide dismutase 2. These data indicate that SIRT5 controls and coordinates several key metabolic pathways in AML and implicate SIRT5 as a vulnerability in AML.

SIGNIFICANCE: Reducing SIRT5 activity is detrimental to the survival of AML cells regardless of genotype, yet well tolerated by healthy hematopoietic cells. In mouse models, disrupting SIRT5 inhibits AML progression. SIRT5 controls several metabolic pathways that are required for leukemia cell survival. These results identify SIRT5 as a therapeutic target in AML.

See related commentary by Li and Melnick, p. 198.

INTRODUCTION

Acute myeloid leukemia (AML) is a genetically heterogeneous myeloid neoplasm with poor prognosis (1). With the exception of acute promyelocytic leukemia, standard therapy for AML remained unchanged for three decades, consisting of induction with anthracycline and cytarabine, followed by consolidation with high-dose cytarabine or bone marrow (BM) transplant (1–4). Despite high rates of complete response (CR) to initial chemotherapy, most patients relapse and eventually succumb to AML, suggesting that chemotherapy eliminates AML blasts, but not leukemia-initiating cells (LIC) that survive in the protective microenvironment of hematopoietic organs (5). Recently, therapy options have been enriched by inhibitors of FLT3 or mutant isocitrate dehydrogenase 1/2 (IDH1/2), validating the paradigm of genotype-directed therapy (6–9). Unfortunately, even with these new drugs, relapse is common and frequent due to selection of subclones with resistance mutations in the drug target (7, 10–12). Unlike FLT3 and IDH1/2 inhibitors, the BCL2 inhibitor venetoclax is active in multiple AML genotypes, indicating that targeting shared vulnerabilities in a genotype-agnostic manner can be effective (11, 13). However, many venetoclax-induced responses are not durable, as leukemia cells adapt by activating alternative antiapoptosis signaling or by reprogramming mitochondrial metabolism (14, 15). Thus, microenvironmental protection, intratumoral heterogeneity and metabolic flexibility limit the utility of current AML therapeutics.

Metabolic aberrancies are increasingly recognized as potential cancer therapy targets (16). One well-established example is elevated glycolytic flux despite adequate oxygen availability, which generates intermediates to sustain nucleic acid and protein biosynthesis in rapidly dividing cells, known as the Warburg effect (17, 18). Metabolic bottlenecks in AML include oxidative phosphorylation (OXPHOS), mitochondrial creatine kinase, branched-chain amino acid aminotransferase, glutaminase (GLS), the pentose phosphate pathway, and mitochondrial translation (19–26). While some dependencies are associated with specific genotypes such as GLS with FLT3 internal tandem duplications (ITD; refs. 24, 27), others are genotype agnostic or linked to cellular subsets such as quiescent LICs, suggesting that diverse genotypes can share metabolic vulnerabilities (28). The utility of targeting single metabolic pathways such as glycolysis (29) or GLS (30) is limited by the metabolic flexibility of cancer cells that allows for rapid adaptation and therapy escape (28, 31).

Sirtuins are NAD+-dependent lysine deacylases implicated in fundamental cellular processes such as senescence, DNA repair, and apoptosis (32). Posttranslational lysine acylation affects multiple protein classes, including metabolic enzymes (33). Among the seven mammalian sirtuins, sirtuin 5 (SIRT5) removes negatively charged acyl groups, specifically succinyl, malonyl, and glutaryl (34–36). Acylation is thought to occur nonenzymatically when relatively high pH and high concentration of acyl-CoA favor transfer of the acyl group to the deprotonated ε-amino group of lysines (34, 37). The
three SIRT5-dependent dec酰lations occur at overlapping as well as unique lysines within substrate proteins (38). SIRT5 regulates multiple metabolic pathways, including fatty acid β-oxidation, glycolysis, ketogenesis, OXPHOS, and urea cycle (34, 35, 37, 38). In several cases, decylation of specific lysine residues was shown to modulate enzymatic activity, mechanistically linking SIRT5 and metabolic programming (39, 40). At steady state, Sirt5^−/− mice exhibit only mild metabolic abnormalities, including reduced glycolysis and ketogenesis (37, 38, 41). More severe phenotypes are observed under stress, such as increased deampanergic neuronal damage upon challenge with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and cold intolerance upon caloric restriction (42–45). In several cases, phenotypes were attributable to SIRT5-dependent regulation of specific enzymes, such as uncoupling protein 1 in cold intolerance (43). These data indicate that SIRT5 orchestrates cellular responses to various stressors to reestablish tissue homeostasis.

We have discovered that knockdown (KD) or genetic absence of SIRT5 attenuates leukemia in multiple AML models and through several mechanisms, including oxidative stress. Inhibition of SIRT5 with NRD167, a cell-permeant SIRT5 inhibitor, impairs proliferation of AML, but not normal hematologic progenitors ex vivo, implicating SIRT5 as a therapy target in AML. Our data support the development of clinical SIRT5 inhibitors for the treatment of AML and other SIRT5-dependent cancers.

RESULTS

SIRT5 Is Essential for Survival of Primary AML Blasts Cultured on BM Stroma

To identify essential AML genes, we performed a cancer-focused shRNA screen on primary AML blasts freshly isolated from patients with newly diagnosed AML (83%-95% CD34^+; n = 12; Supplementary Table S1). As limited ex vivo growth and survival of primary AML cells hinder detection of relative shRNA abundance changes, most shRNA screens in AML have used cell lines. To enhance viability and growth, and mimic protective microenvironment effects, we cultured primary AML blasts on HS-5 human stromal cells (Supplementary Fig. S1A; ref. 46). We optimized the algorithm for identification of survival/growth-critical genes, prioritizing candidates based on at least two shRNAs targeting the same mRNA with a fold-reduction ranking in the top 2% of all scores, detected in at least two samples. Thirty-four genes met these criteria in at least two samples, and nine genes

AML Cells Are Selectively Dependent on SIRT5

Both tumor-promoting and tumor-suppressive roles have been attributed to SIRT5 in solid tumors, depending on the specific context, but its function in hematologic malignancies is unknown (39). We infected CD34^+ cells from additional AML patients (n = 25; Supplementary Table S1) and cord blood (CB; n = 5) with doxycycline-shSIRT5^2311/2312. SIRT5 KD was comparable in AML (median 52.4%; range 24.7%-89.6%) and CB (median 65.9%; range 37.5%-80.1%; P = 0.26). SIRT5 KD selectively reduced AML colony formation, with no effect on CB (Fig. 1A). For genetic validation, we infected BM cells from Sirt5^−/− and Sirt5^−/− mice with retrovirus for expression of the myeloid leukemia alleles FLT3-ITD, MLL-AF9, RUNX1-RUNX1T1/NRAS^G12D, BCR-ABL1, and MPL^W515L. Absence of Sirt5 reduced colony formation by approximately 50%, 60%, 25%, 50%, and 20% respectively, compared with Sirt5^+/+ controls (Fig. 1B). Routine hematopoietic parameters and myeloid colony formation were comparable between Sirt5^−/− mice and Sirt5^+/− littermates (Fig. 1C; Supplementary Table S3).

We engineered 22 AML cell lines to express doxycycline-shSIRT5^2311/2312. In most cell lines, SIRT5 KD inhibited growth, reduced colony formation, and increased apoptosis, with the strongest effects on colony formation (Fig. 1D; Supplementary Fig. S2A–S2C). In seven lines (32%), colonies were reduced by >75%, in five lines (23%) by <25%, and by 25% to 75% in the remainder. The three lines most sensitive to SIRT5 KD (CMK, SKM-1, OCI-AML2; referred to as SIRT5-dependent) and three lines most resistant to SIRT5 KD (OCI-AML3, KG1a, Marimo; referred to as SIRT5-independent) were used in most subsequent studies (Fig. 1E and H). In view of the large range of sensitivity to SIRT5 KD, we assessed potential correlations between SIRT5 dependence and genotype. However, next-generation sequencing of 52 genes recurrently mutated in myeloid malignancies in patient samples (Fig. 1A;
A Critical Role for SIRT5 in AML Metabolism

**A** Primary cells

**D** Cell lines

**B** Colonies (% of WT)

**C** White blood cells (×10^9/L)

**E** OCI-AML2, CMK, SKM-1

**F** shSIRT5-Dox

**G** shSIRT5-Dox

**H** shSIRT5

**I** SKM-1 shSIRT5

**J** CMK, OCI-AML3

**K** SKM-1 shSIRT5

**L** Colony formation reduction (%)

**M** AML patient samples

**N** n = 25

**O** CB n = 5

**P** Association for Cancer Research.

**Q** DOI: https://doi.org/10.1158/2326-0242.CCR-18-0213

Downloaded from https://bloodcancerdiscover.aacrjournals.org by guest on September 17, 2021. Copyright 2021 American Association for Cancer Research.
Supplementary Table S1) and cell lines (Supplementary Table S4) failed to identify obvious correlations. Similarly, SIRT5 dependence was not correlated with SIRT5 protein expression (Supplementary Fig. S2D–S2F).

To rule out off-target shRNA effects, experiments were repeated with three doxycycline-inducible shRNAs and a non-inducible shRNA, each targeting different SIRT5 sequences, with consistent results (Fig. 1I; Supplementary Fig. S3A–S3C). Outgrowth after prolonged culture of SIRT5-dependent cells in doxycycline-containing media was associated with reexpression of SIRT5, suggesting cells are under selection pressure to reestablish SIRT5 expression (Supplementary Fig. S3D). Conditioned medium from HS-5 stromal cells can protect leukemia cells from cytotoxic and targeted therapies (47, 48), but did not protect AML cells from the effects of SIRT5 KD, consistent with the design of the initial screen (Fig. 1K; Supplementary Fig. S3E). Propidium iodide staining revealed increased sub-G1 cells upon SIRT5 KD in SIRT5-dependent cell lines, indicating increased apoptosis, but the proportion of cells in G0–1, S, and G2–M phases remained stable. SIRT5 KD induced neither markers nor morphologic signs of differentiation. We also confirmed that doxycycline-stable SIRT5 KD induced neither markers nor morphologic signs of differentiation. We also confirmed that doxycycline-shSIRT52311/2312 and luciferase, and randomized at 18 hours after injection of doxycycline-containing or regular water (control). Mice injected with CMK cells maintained on doxycycline showed no luminescence at week 3 (Fig. 2A, left), whereas mice injected with CMK cells in doxycycline-containing media did not show any luminescence (Fig. 2B, left and left middle panels). In the group started on regular water, mice switched to doxycycline-containing media at week 3 showed reduction of luminescence at week 4, cleared all luminescence at week 8, and survived until termination of the experiment, without evidence for leukemia at autopsy (Fig. 2B, right middle panel, and C). In contrast, mice continued on regular water died between weeks 4 and 5 with extensive leukemic involvement (Fig. 2A and B, right). Recipients of OCI-AML3 cells rapidly developed systemic disease (Fig. 2D), and died on day 31 (doxycycline) and 29 (control), with extensive leukemic involvement (Fig. 2E and F). SIRT5 KD was maintained in hCD45+ cells from doxycycline-treated mice, confirming that in vivo survival of OCI-AML3 cells was not due to reexpression of SIRT5 (Fig. 2G).

To assess the role of SIRT5 in a syngeneic context, we infected BM from Sirt5+/− and Sirt5−/− mice with MLL-AF9 retrovirus and injected 3 × 103 cells/mouse into lethally irradiated Sirt5−/− mice. In this aggressive AML model, median survival in recipients of MLL-AF9–transduced Sirt5−/− cells was 60 days versus 49 days for controls (P = 0.428; Fig. 3A), with similar GFP expression (86.4% ± 1.9% vs. 85.7 ± 4.7%; P = 0.9). We transplanted 5 × 103 GFP-LinKit−/− cells/mouse from primary leukemic mice into secondary Sirt5+/− recipients. Recipients of MLL-AF9–transduced Sirt5+/− BM developed leukocytosis, with mostly GFP+ cells, and showed massive splenomegaly at autopsy. White blood cells (WBC) in recipients of Sirt5+/− BM remained normal, with no or rare GFP+ cells, and no splenomegaly in the majority (Fig. 3B and C). Median survival in recipients of MLL-AF9/Sirt5+/− cells was 23 days but had not been reached in recipients of MLL-AF9/Sirt5−/− cells at 8 weeks (P < 0.0001; Fig. 3C). Similarly, we infected BM cells from S-fluorouracil (S-FU)–treated Sirt5+/− and Sirt5−/− mice with BCR-ABL1GFP retrovirus and injected 5,000 LSK cells/mouse into Sirt5+/− recipients. Median survival of mice receiving Sirt5+/− cells was 21 days, whereas 80% of mice receiving Sirt5−/− cells survived until day 47 (termination of experiment; P < 0.01; Fig. 3D). Finally, we probed the role of SIRT5 in a transgenic model of FLT3-ITD–positive myeloproliferative disease (MPD). The MPD in this model is mild, with modestly elevated WBCs and monocytosis at 4 to 6 months of age (51). FLT3-ITD transgenic mice were crossed with Sirt5+/− mice and bred to homozygosity, yielding...
A Critical Role for SIRT5 in AML Metabolism

**A**

NSG mice injected with CMK<sup>shSIRT5</sup> or OCI-AML3<sup>shSIRT5</sup> were treated with Dox or No dox.

**B**

Flow cytometry analysis of human CD45 expression in mouse CD45.

**C**

Survival rates for CMK<sup>shSIRT5</sup> groups.

**D**

Survival rates for OCI-AML3<sup>shSIRT5</sup> groups.

**E**

Survival rates for OCI-AML3<sup>shSIRT5</sup> groups.

**F**

Human CD45 expression in mouse CD45.

**G**

Western blot analysis of SIRT5 and α/β-tubulin.
SIRT5 Is Required for Oxidative Phosphorylation in SIRT5-Dependent AML Cells

SIRT5 desuccinylates multiple mitochondrial proteins, including electron chain components (34, 37, 38). To identify the mechanism by which SIRT5 KD kills SIRT5-dependent but not SIRT5-independent cells, we assessed mitochondrial protein succinylation in CMK cells (SIRT5-dependent) and KG1a cells (SIRT5-independent). Succinylation, malonylation, and acetylation increased in both upon SIRT5 KD, while glutarylation was low and unchanged, altogether suggesting that SIRT5 is active in SIRT5-dependent and SIRT5-independent cells (Fig. 4A; Supplementary Fig. S4F). AML cells depend on mitochondrial protein translation and OXPHOS, and exhibit reduced spare respiratory capacity (19, 25, 28). We measured the effect of SIRT5 KD on OXPHOS and glycolysis in AML cell lines upon SIRT5 KD. OXPHOS was consistently reduced in SIRT5-dependent cell lines, starting ~24 hours after doxycycline addition, but not in SIRT5-independent cell lines.

FLT3ITD/ITD/Sirt5+/+ and FLT3ITD/ITD/Sirt5−/− genotypes. At 5 months, WBCs, granulocytes, and monocytes were reduced in FLT3ITD/ITD/Sirt5−/− compared with FLT3ITD/ITD/Sirt5+/+ mice (Fig. 3E). Altogether these data show that SIRT5 is required for leukemogenesis by several myeloid oncogenes.

Figure 3. Absence of SIRT5 attenuates myeloid leukemia in syngeneic mouse models. A, BM from Sirt5+/+ versus Sirt5−/− C57Bl/6 mice was transduced with MLL-AF9 retrovirus and injected into irradiated Sirt5−/− recipients. Kaplan–Meier survival analysis of primary recipients (n = 6/group). KO, knockout; WT, wild-type. B, GFP-Lin Kit− BM cells were sorted from leukemic primary recipients and injected into secondary Sirt5−/− recipients. WBC counts and percent of GFP+ blood cells were quantified at week 3, and spleen weights were measured at the time of sacrifice (unpaired t test). C, Left, Kaplan–Meier survival analysis of secondary recipients of MLL-AF9-expressing cells. Right, representative blood smears at week 3. D, Kaplan–Meier survival analysis of primary recipients of BCR-ABL1−/− transduced Sirt5+/+ versus Sirt5−/− Lin−Sca− Kit− cells (n = 10/group). E, Left, WBC parameters in 5-month-old FLT3ITD/ITD/Sirt5+/+ (left; n = 2) compared with FLT3ITD/Sirt5−/− mice (n = 6; right; unpaired t test). Right, representative blood smears at 5 months. The log-rank (Mantel–Cox) test was used for comparisons of survival (*, P < 0.05; **, P < 0.01; ***, P < 0.001). Scale bars in C and E are 50 micrometers.
Figure 4. SIRT5 KD reduces OXPHOS in SIRT5-dependent, but not SIRT5-independent, cell lines. A, CMK (SIRT5-dependent) and KG1a (SIRT5-independent) cells expressing doxycycline (dox)-shSIRT5 were cultured ±100 ng/mL doxycycline for 48 hours. Mitochondria were isolated and proteins resolved by SDS-PAGE, followed by immunoblotting with anti–succinyl-lysine and anti-SIRT5 antibodies, with Ponceau S staining as loading control. B–E, CMK (SIRT5-dependent) and OCI-AML3 (SIRT5-independent) cells expressing doxycycline-shSIRT5 were cultured ±100 ng/mL doxycycline for up to 72 hours and subjected to metabolic profiling. B, SIRT5 expression was assessed by immunoblot. C, Oxygen consumption rate (OCR) was measured in CMK cells on an Agilent Seahorse XFe96 Analyzer under basal conditions and under stress (for details, see Methods). Top, Representative experiment. Bottom, Means of three independent experiments. D, OCI-AML3 cells were cultured ± doxycycline for 72 hours and OCR was measured as in B. Top, Representative experiment. Bottom, means of three independent experiments. E, ECAR as a proxy for glycolysis was measured upon SIRT5 KD in CMK cells under basal conditions and stress. Top, Representative experiment. Bottom, Means of three independent experiments. F, OCI-AML3 cells expressing doxycycline-shSIRT5 were cultured ± doxycycline for 72 hours, and ECAR was measured as in D. Data represent the mean ± SEM from three independent experiments. Comparisons were performed with multiple unpaired t tests (*, P < 0.05; **, P < 0.01;***, P < 0.001).
SIRT5 Regulates Glutamine Metabolism in SIRT5-Dependent AML Cells

To identify SIRT5-regulated metabolic pathways in AML cells, we performed untargeted metabolomics on SIRT5-dependent (CMK and SKM-1) and SIRT5-independent (OCI-AML and KG1a) cell lines expressing doxycycline-shSIRT531/312 at 36 and 48 hours after the addition of doxycycline. Steady-state levels of multiple metabolites were altered upon SIRT5 KD, often in opposite directions according to SIRT5 dependency (Supplementary Fig. S7A and S7B). Profound reductions in tri-carboxylic acid (TCA) cycle intermediates and amino acids were observed in SIRT5-dependent lines, while their concentrations were increased in SIRT5-independent cell lines. We used Ingenuity Pathway Analysis (IPA) to identify metabolic networks altered by SIRT5 KD and attribute metabolite alterations to pathways. We observed mostly consistent changes in TCA cycle metabolites, which were linked to SIRT5 (Supplementary Fig. S7C). IPA also identified potential upstream regulators, including GLS and α-ketoglutarate (Supplementary Fig. S7D).

SIRT5 KD increased superoxide in SIRT5-dependent cells, but not in SIRT5-independent cells (Fig. 5A–D). The superoxide increase preceded apoptosis and was partially rescued by vitamin E, but not other antioxidants (Fig. 5B and C; Supplementary Fig. S6E). SIRT5 KD increased superoxide in SIRT5-dependent cells, but not in SIRT5-independent cells (Fig. 5A–D). The superoxide increase preceded apoptosis and was partially rescued by vitamin E, but not other antioxidants (Fig. 5B and C; Supplementary Fig. S6E). SIRT5 KD increased superoxide in SIRT5-independent cell lines, while their concentrations were increased in SIRT5-independent cell lines. We used Ingenuity Pathway Analysis (IPA) to identify metabolic networks altered by SIRT5 KD and attribute metabolite alterations to pathways. We observed mostly consistent changes in TCA cycle metabolites, which were linked to SIRT5 (Supplementary Fig. S7C). IPA also identified potential upstream regulators, including GLS and α-ketoglutarate (Supplementary Fig. S7D).

At a global level, the pathways implicated by IPA were remarkably consistent, yet mostly regulated in opposite directions, in accord with the changes in total metabolite concentrations. Top IPA hits included RNA charging as well as alanine and serine metabolism (Supplementary Fig. S8).

GLS converts glutamine to glutamate, which is metabolized to α-ketoglutarate by glutamate dehydrogenase 1 (GLUD1) or aminotransaminases (GOT1/2, GPT2, and PSAT1). Recent studies have shown that SIRT5 promotes breast cancer by activating GLS and colorectal cancer by activating GLUD1 (54, 55). As previous work has shown that some AML cells are glutamine dependent and suggested a role for GLS in constraining de novo glutathione synthesis in AML, we hypothesized that SIRT5-dependent AML cell lines may require SIRT5 for regulating glutamine metabolism (24, 27, 56). To test this, we performed stable isotope metabolic tracing experiments using [13C5,15N2]-glutamine or [1,2,3-13C3]-glucose in SIRT5-dependent and SIRT5-independent cell lines (Fig. 6A; Supplementary Fig. S9A). To ensure the metabolite changes are due to SIRT5 KD, but not cell death, cells were analyzed after 36 hours in doxycycline, when cell viability was >90%. Initial time course experiments were performed in SKM-1 cells. [1,2,3-13C3]-glucose tracing did not show significant changes in glycolytic flux upon SIRT5 KD, consistent with Seahorse experiments showing reduced ECAR only at relatively late time points (Fig. 4E; Supplementary Fig. S5B). The glutamine/glutamate ratio was markedly increased in SIRT5-dependent cells and further elevated upon SIRT5 KD (Fig. 6B). Glutamine abundance was much higher in SIRT5-dependent cells at baseline and altered by SIRT5 KD only in SIRT5-dependent cell lines. In addition, only SIRT5-dependent cell lines showed altered abundances of TCA cycle intermediates upon SIRT5 KD (Fig. 6C; Supplementary Fig. S9B). In light of the results above, we hypothesized that SIRT5-dependent AML requires SIRT5 to regulate glutamine flux to sustain redox homeostasis and or anabolism. To test this, we cultured SIRT5-dependent and SIRT5-independent cells with or without glutamine, and measured apoptosis. SIRT5-dependent cell lines were more sensitive to glutamine deprivation than SIRT5-independent cell lines (Fig. 6D). Moreover, glutamine withdrawal and SIRT5 KD were synergistic (Fig. 6E).
Figure 5. SIRT5 KD induces mitochondrial superoxide in SIRT5-dependent, but not SIRT5-independent, cell lines. A-D, KG1a cells (SIRT5-independent) and CMK cells (SIRT5-dependent) expressing doxycycline (dox)-shSIRT5<sub>2311/2312</sub> were cultured for 96 hours ± 100 ng/mL doxycycline ± vitamin E (200 μmol/L). Following culture, cells were analyzed by FACS after staining with MitoSOX dye to measure mitochondrial superoxide, annexin V (An V) to identify apoptotic cells, and DAPI (DAP) to identify dead cells. Data represent three independent experiments. A, Gating strategy. B, Percent of KG1a and CMK cells positive for mitochondrial superoxide but negative for annexin V (multiple unpaired t tests). C, Percent CMK cells positive for annexin V. D, SIRT5 expression (immunoblot). E and F, CMK cells expressing doxycycline-shSIRT5<sub>2311/2312</sub> engineered to ectopically express SOD2 were cultured for 72 hours. E, Left, percent MitoSOX/annexin V-/DAPI<sup>-</sup> cells. Middle, percent annexin V<sup>-</sup> cells. Right, colony formation. Comparisons were performed with paired t test. F, SOD2 expression (immunoblot). G, SIRT5-independent (KG1a; Marimo; OCI-AML3) and SIRT5-dependent (CMK; OCI-AML2, SKM-1) cell lines expressing doxycycline-inducible shSOD2 were cultured in 100 ng/mL doxycycline. Left, viable cells (MTS assay) after 96 hours. Comparisons were performed with multiple paired t tests. Middle, apoptosis (annexin V staining) in OCI-AML2 cells. Right, apoptosis (annexin V staining) in KG1a cells. H, Immunoblot analysis of SOD2 expression shown for KG1a and OCI-AML2 cells. I, NAD<sup>+</sup>/NADH ratio was measured by GLO assay in SIRT5-dependent (OCI-AML3; KG1a) and SIRT5-independent (OCI-AML2; CMK; SKM-1) cell lines expressing doxycycline-shSIRT5<sub>2311/2312</sub> at 24 to 72 hours after addition of 100 ng/mL doxycycline. Data represent the mean ± SEM from three independent experiments. Comparisons were performed with multiple unpaired t tests (*, P < 0.05; **, P < 0.01; ***, P < 0.001).
A Small-Molecule SIRT5 Inhibitor Selectively Inhibits Cell Proliferation, Metabolism, and Colony Formation of AML Cells

To assess the potential of targeting SIRT5 catalytic activity in AML, we tested several published SIRT5 inhibitors in cell proliferation assays of AML cells and normal controls but found them indiscriminately toxic or inactive (52, 57). To overcome this limitation, we synthesized a prodrug of SIRT5 inhibitor 1 (SS11) named ethyl 3-[(3-((S)-6-((((S)-1-(cyclobutylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-5-((3-fluorophenyl)sulfonamido)-6-oxohexyl)thioureido)propanoate (NRE139; Fig. 7A; Supplementary Fig. S10). The parent inhibitor, SS11, inhibits SIRT5 with an IC50 of 110 nmol/L in a published...
biochemical assay (58) but exhibits no activity in cell-based assays. Presumably, S5I1 does not enter cells, partly due to a negatively charged carboxylate moiety. We modified S5I1 by masking the carboxylate as the corresponding ethyl ester NRE139, enabling cellular uptake. Inside the cell, NRE139 is converted into S5I1 by ubiquitous esterases. In addition, we included the structurally closely related prodrug NRD167 (Fig. 7A). To confirm that NRD167 is cell permeant and inhibits SIRT5 catalytic activity, HEK293 cells expressing FLAG-tagged SOD1 were treated with vehicle or NRD167 (10 μmol/L) for 18 hours. FLAG-tagged SOD1 was immunoprecipitated, followed by immunoblotting with an antibody raised against SOD1 succinyl-lysine 122, a documented SIRT5 target (59, 60). Succinylation of SOD1 at lysine 122 was increased in the presence of NRD167 (Fig. 7B).

The effect of S5I1, NRE139, and NRD167 was compared against SIRT5-dependent (OCI-AML2; SKM-1) and SIRT5-independent (KG1a; Marimo) cell lines were treated with graded concentrations of SIRT5 inhibitor 1, NRE139, and NRD167 for 72 hours. Cell proliferation and induced apoptosis in SIRT5-dependent cells, with minimal effects on SIRT5-independent cells. NRD167 was more...
potent than NRE139, inhibiting proliferation of SIRT5-dependent cell lines with an IC_{50} of 5 to 8 µmol/L and inducing >80% apoptosis at 5 to 10 µmol/L (Fig. 7C and D), and was chosen for further evaluation. Globally NRD167’s effects on OXPHOS and ECAR, as assessed by Seahorse, resembled those of SIRT5 KD (Fig. 7E), suggesting that inhibition of SIRT5 catalytic activity is sufficient to reproduce the metabolic consequences of SIRT5 KD, although subtle differences were noted such as a decrease in ECAR in ECAR with NRD167 in KG1a cells and preserved spare oxidative capacity in SKM-1 cells. Next, NRD167 was tested against CD34+ cells from patients with AML and CB CD34+ cells in colony assays (n = 3 each; Fig. 7F). Reduction of colony formation was significantly more pronounced in AML versus CB samples (57x ± 8.6% vs. 102% ± 4.4%, P = 0.03; 12.5 µmol/L: 62% ± 6.7% vs. 92% ± 7.9%, P = 0.044). Cells from sample 17-268 were treated ex vivo with NRD167 for 72 hours and then injected into NSG-SGM3 mice. The survival of mice injected with NRD167-treated cells trended longer compared with controls (Fig. 7G). These data implicate SIRT5 catalytic activity as a potential therapeutic target in AML.

DISCUSSION

We discovered that SIRT5 KD reduced colony formation by >25% to 100% in primary AML samples and 77% of AML cell lines, while colony formation by normal CD34+ cells was unaffected. Genetic absence of SIRT5 impairs transformation of primary hematopoietic cells by AML-associated oncogenes in vitro, and absence of SIRT5 attenuates myeloid leukemia in vivo. Similar to BCL2, SIRT5 dependence is not associated with particular genotypes, and extends to p53- and N/KRAS-mutated cells, suggesting that targeting SIRT5 may be effective in these challenging cases.

Through a combination of steady-state measurements and metabolic tracing with stable isotope-labeled substrates, we identified a role for SIRT5 in regulating glutaminolysis in multiple SIRT5-dependent cell lines. Decreased glutaminolysis upon SIRT5 KD in SIRT5-dependent, but not -independent, cells, was accompanied by an increase in mitochondrial superoxide that preceded apoptosis, and was partially rescued by vitamin E or ectopic SOD2. Consistent with a critical role for this pathway, SIRT5-dependent AML cell lines were highly sensitive to SOD2 KD. In striatonigral neurons of Sirt5<sup>−/−</sup> mice challenged with the reactive oxygen species (ROS) inducer MTPT, SOD2 expression decreases much more than in Sirt5<sup>+/+</sup> controls, although the mechanism for this is unknown (42). In contrast, SIRT5 KD had no effect on SOD2 expression in AML cells. While SIRT3 has been shown to positively regulate SOD2 by lysine deacetylation (61), there is no evidence that deacetylation of any SOD2 lysines is controlled by SIRT5 (34, 35, 37, 38). Consistent with this, SOD2 activity was unaffected by SIRT5 KD in OCI-AML2 cells, suggesting that the increase in superoxide is caused by an alternative mechanism. Regardless, our data implicate glutamine metabolism in maintaining redox homeostasis in AML cells, adding to previous evidence pointing to glutamine as an AML vulnerability (23, 24, 26, 27, 56). We have now identified SIRT5 as a master regulator of this process in AML.

SIRT5 may regulate glutamine metabolism at several levels and in a cell type-specific manner. In colorectal cancer, SIRT5 was shown to activate GLUD1 through deglutaryl-lation, thereby increasing α-ketoglutarate concentrations (54). In contrast, SIRT5-dependent desuccinylation of GLS K158 in breast cancer protects GLS from ubiquitination at K158 and subsequent degradation (55). Similar to the latter study, we find that SIRT5 KD increases the glutamine/glutamate ratio in SIRT5-dependent, but not SIRT5-independent, AML cell lines, consistent with positive regulation of GLS by SIRT5. Strikingly, [13C<sub>6</sub>]glutamine at steady state is much higher in SIRT5-dependent compared with SIRT5-independent cell lines, suggesting fundamental differences in glutamine uptake. However, RNA sequencing shows comparable expression of the main glutamine transporter SLC1A5, and additional studies are needed to clarify the underlying
A Critical Role for SIRT5 in AML Metabolism

Sirt5 may participate in glutationylation of succinate dehydrogenase (SDH; complex II), which was previously associated with increases in OXPHOS in leukemia stem cells from patients with AML (63). It remains possible that SIRT5 directly regulates OXPHOS, corresponding to the presence of SIRT5-regulated lysines in respiratory chain complex proteins (34, 37). In complex V, 12 lysine succinylation sites are identified, including K97 with >300-fold increased stoichiometry in Sirt5−/− liver mitochondria (37). SIRT5 enhances OXPHOS in HEK293T cells and Sirt5−/− murine embryonic fibroblasts (MEF) exhibit reduced OXPHOS and spare reserve capacity (64, 65). In contrast, Park and colleagues report that SIRT5 inhibits SDH (complex II) in MEFs through desuccinylation of lysines in the SDHA/B subunits (34). Our data implicate SIRT5 as a positive regulator of OXPHOS in SIRT5-dependent AML cells, although precisely elucidating the mechanism will require additional studies. The fact that OXPHOS is regulated by SIRT5 in SIRT5-dependent, but not SIRT5-independent, AML cells lines aligns well with our inability to generate p0 derivatives from SIRT5-dependent cell lines. Mitochondria from some AML cases are increased in size, but reduced in number, and have lower activity of mitochondrially encoded respiratory chain complexes III, IV, and V; while mitochondrial mass is increased, spare reserve capacity is reduced, and cells are dependent on OXPHOS (19). These features are also exhibited by AML leukemia-initiating cells, which, unlike hematopoietic stem cells, use OXPHOS as their main energy source, with limited glycolysis capacity in anaerobic conditions (13, 28). In line with this, knockout of genes driving mitochondrial processes, including OXPHOS, sensitizes AML cells to venetoclax (14, 15).

In aggregate, our data indicate that SIRT5 is critical for metabolic adaptability in a large subset of primary AML cases and cell lines, similar to its role in healthy tissues under stress (42, 43, 45, 66). Because SIRT5 regulates several important metabolic pathways (Fig. 7G), it is conceivable that inhibiting SIRT5 would avoid the rapid adaptation that limits the efficacy of targeting single metabolic pathways in cancer. From a clinical perspective, it would be important to identify a biomarker of SIRT5 dependence, but we found no obvious correlation between SIRT5 dependence and AML genotype or SIRT5 expression. Similarly, correlations between BCL2 expression or specific somatic mutations and AML responses to venetoclax are weak (67, 68). The effects of venetoclax on AML stem cells are mediated through the inhibition of OXPHOS (13, 69), indicating that metabolic vulnerabilities in AML can be genotype agnostic and involve critical regulators that are neither mutated nor aberrantly expressed. The sensitivity to SIRT5 disruption may therefore reflect a specific metabolic phenotype generated by multiple alternative mechanisms that turn SIRT5 into a bottleneck, similar to SIRT3’s role in diffuse large B-cell lymphoma (70). For instance, reactive acyl-CoA species may create “carbon stress” due to nonenzymatic protein lysine acylation and cells with high steady-state concentrations of acyl-CoA species may be particularly dependent on SIRT5 for the maintenance of proper protein function (71). Ongoing work in our lab is seeking to identify metabolic markers of SIRT5 dependence.

Although the SIRT5 catalytic pocket exhibits several unique features, published compounds either lack potency or selectivity over other sirtuins, or are peptide based with insufficient cell permeability (39). To the best of our knowledge, no clinical SIRT5 inhibitors have been reported to date. We have identified NRD167 as a potent, cell-permeant SIRT5 inhibitor that overcomes several of the limitations of previous SIRT5 inhibitors. While NRD167’s limited bioavailability precludes in vivo studies, inhibition of SIRT5 catalytic activity with NRD167 mirrors the phenotypic consequences of SIRT5 KD, selectively inhibiting AML over CB CD34+ cells and SIRT5-dependent over SIRT5-independent AML cell lines. These data provide a strong rationale for the development of clinical SIRT5 inhibitors to treat AML.

METHODS

Patient Samples

This study was approved by the University of Utah Institutional Review Board, based on the recommendations of the Belmont Report, and all patients provided written informed consent to protocol 45880 before donating samples. Mononuclear cells (MNC) or CD34+ cells were isolated from blood or BM of patients with newly diagnosed (N = 48), relapsed/refractory AML (N = 5), and one unknown using Ficoll-Paque Premium (GE Healthcare) and an AutoMACS Pro Separator (Miltenyi Biotec). Twelve samples (unique patients) were used in the initial shRNA screen and 51 in subsequent experiments. For clinical and molecular diagnostic details, see Supplementary Table S1.

shRNA Library Screen

Lentiviral shRNA Library. A custom pooled shRNA library targeting leukemia-related genes was provided by Cellecta (http://www.cellecta.com). This library contains 10,091 shRNAs targeting 1,287 genes associated with leukemia, as well as positive and negative control shRNAs (Supplementary Table S5). The shRNA library was purchased from Cellecta (http://www.cellecta.com). This library contains 10,091 shRNAs targeting 1,287 genes associated with leukemia, as well as positive and negative control shRNAs (Supplementary Table S5). The shRNA constructs were designed in a pRS16 Clonal Barcode vector containing a puromycin-resistant gene (Puro+) and a red fluorescent protein (RFP) marker (TagRFP). Each shRNA was linked to a unique 8-bp barcode identifiable by sequencing. The pooled shRNA library was received as packed lentiviral particles. The titer of the leukemia library stocks was determined by the manufacturer as 1.56 × 10^7 TU/mL.

Infection of Primary AML Cells. MNCs were cultured at up to 5 × 10^6 cells/mL in RPMI supplemented with 10% FBS (Sigma-Aldrich), 2 mmol/L L-glutamine, 100 U/mL penicillin/streptomycin plus CC100 (StemCell Technologies), GM-CSF (10 ng/mL), and G-CSF (10 ng/mL; PeproTech). At 48 hours, cells were diluted to 10^6/mL and distributed into 6-well plates at 2 mL/well. shRNA lentiviral particles were added, targeting a multiplicity of infection (MOI) of 0.3, followed by the addition of polybrene (2 μg/mL) and HEPES buffer (Invitrogen; 10 mM). The cells were centrifuged at 1,800 rpm for 90 minutes at 32°C. Following centrifugation, the plates were kept in a humidified incubator at 5% CO2 and 37°C. The medium was changed daily until the knockdown efficiency was determined.

Downloaded from https://bloodcancerdiscov.aacrjournals.org by guest on September 17, 2021. Copyright 2021 American Association for Cancer Research.
replaced with fresh medium at 12 to 18 hours after transduction. At 72 hours after transduction, the cells were pooled and RFP+ cells quantified by flow cytometry [Becton-Dickinson (BD) FACS Aria II].

**Culture and Selection.** At 48 hours after the infection of the AML cells, HS-5 human stromal cells (a kind gift of Dr. Beverly Torok-Storb, University of Washington, Seattle, WA; ref. 46) were plated in 6-well plates at 3 × 10^5 cells/well and cultured for 24 hours in RPMI1640 medium supplemented with 10% FBS. At this point, the infected AML cells were layered on top of the adherent HS-5 cells, without additional cytokines. The medium was changed every 24 hours. To avoid depleting the cytokines produced by HS-5 cells, half of the medium was removed from each well and pooled. The AML cells were spun down, and the supernatant was removed. Next, the cells were resuspended in fresh medium, and an equal volume of the previously pooled medium was redistributed to each well. After 7 to 9 days of coculture, the AML cells growing in suspension were removed and pooled. To quantitatively collect all AML cells attached to HS-5 cells, the cells were trypsinized and sorted for RFP+ on a BD FACS Aria II. The sorted RFP+ cells were combined with the remaining AML cells, pelleted, and stored for subsequent DNA extraction.

**DNA Extraction and Sequencing of Barcodes.** The cell pellets were resuspended in Qiagen Buffer P1 supplemented with 100 μg/ml RNase A (Qiagen) at a concentration of 10^5 cells/mL and mixed with 1/20 volume of 10% SDS followed by incubation for 5 minutes at room temperature. Next, cells were sheared by passing through a 22-gauge syringe needle. DNA was extracted with phenol/chloroform as reported previously (72). Sequence reads from an Illumina HiSeq were converted into barcode counts and deconvoluted by Cellecta, with subsequent merging of gene-target information. The entire final set of sequences consisted of 10,091 uniquely barcoded shRNA targeting 1,287 loci. Most genes (1,110) were targeted by eight barcodes, and the remainder by five to seven barcodes.

**Bioinformatics Analysis.** We analyzed the read reduction for all shRNA probes across all samples compared with the total reads observed for each shRNA in a simultaneously run plasmid sample (control). We initially analyzed the abundance of barcodes corresponding to survival-critical genes (as identified previously by Cellecta across multiple and diverse cell lines) included as positive controls but found the fold changes of these genes to be subtle, consistent with a general trend across the entire library. On the basis of this, we conjectured that giving greater weight to frequently recurring, highly ranked shRNAs (ranked by fold reduction compared with plasmid) across samples would increase the likelihood for identifying genes critical for leukemic cell survival and/or proliferation due to their recurrence across multiple samples. Barcode counts for each sample were normalized to the number of reads in a simultaneously assessed plasmid sample, and the relative barcode depletion was estimated by calculating the ratio of measured shRNA plasmid input with barcode output counts, adding 20 to numerator and denominator to mitigate high ratios resulting from low read counts. As such, each gene’s depletion, also referred to as fold change, is estimated as (plasmid_input gene + 20)/(barcode_count gene + 20). Degrees of depletion were rank partitioned into percentiles for each sample. Candidate genes were prioritized by requiring that at least two of their shRNAs were ranked in the top two-percentile depletion in at least two samples among the set of 12. Sequence reads that did not correspond to known barcodes from the shRNA library were not counted toward the tally for any gene locus. Finally, genes were tallied and ranked for the total number of AML samples within this remaining set. Additional statistical calculations, including ranking of fold reductions, were performed in SAS ver. 9.1 (SAS Institute, Inc.).

**IPA**

Pathway-associated gene analyses were generated using commercially available software (Qiagen; https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa/). For pathway analysis, metabolite lists were uploaded as exp.log ratio [Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB, identification numbers] into the IPA server. The IPA function Core Analysis/Metabolomics Analysis was used for the enriched pathway identification. In addition, IPA includes an upstream regulator analysis to determine whether the observed metabolic perturbations are associated with a particular upstream regulator. P values, calculated from the right-tailed Fisher exact test, reflect whether the number of overlapping metabolites associated with a particular pathway or upstream regulator is greater than expected by chance. For upstream regulator analysis, both direct and indirect relationships between metabolites and their targets were considered. The overall activation/inhibition status of the upstream regulator was determined from the level of consistency in the observed up or down changes of the metabolites. The strength of evidence was statistically represented by z-score.

**Statistical Methods**

Prism 8 (GraphPad) was used to perform all statistical analysis. For the comparisons of two groups, data were presented as mean ± SEM and analyzed by t test. For the comparisons of median survival, Kaplan-Meier curves were analyzed by the log-rank (Mantel-Cox) test. Cellular IC_{50} values were calculated by nonlinear regression analysis. P < 0.05 was considered to be statistically significant.

**Doxycycline-Inducible shRNA Constructs**

For initial validation, the two SIRT3-targeting shRNAs with the highest depletion during the screening (shSIRT3^{721–23} and shSIRT3^{721+17}) were inserted into a doxycycline-inducible vector (pRS1616-U6-Tet-shCMV-TetR-ZF-FRP-2A-Puro, Cellecta) containing the wild-type tetracycline repressor (tetR), which blocks transcription unless 100 ng/mL doxycycline is added. Constructs were packaged and used individually for infection. Cells were sorted for RFP-positive cells. For primary cells, the shRNA-induced suppression of SIRT3 was assessed by measuring mRNA transcripts using SYBR Green– and SIRT3-specific primers (Supplementary Table S6) in a CFX96 Real-Time System (Bio-Rad). For cell lines, the KD of SIRT3 was assessed by immunoblotting.

**CRISPR**

**SIRT3 Guide RNA/Cas9 Expression Vector Construction.** The Lenti-CRISPR-v2-PURO plasmid was purchased from Addgene (#52961, a gift from Feng Zhang, Broad Institute, Cambridge, MA; http://n2t.net/addgene:52961; RRID:Addgene_52961; ref. 73). The puromycin-resistant gene was removed and replaced with ZsGreen1 using restriction-enzyme cloning to produce Lenti-CRISPR-v2-ZsGreen. Three 20-bp guide sequences (gRNA-A: 5′-CATCGATGAGCTGACCAGCA-3′, gRNA-B: 5′-CTCTTCTTCTGGATGGACG-3′, and gRNA-C: 5′-AAAGCATAGTCATCATCTC-3′) targeting SIRT3 were selected from a published database of predicted high-specificity protospacer adjacent motif (PAM) target sites for human SIRT3. In addition, a nontargeting oligo was cloned (5′-TGCATCAATTATATAC-3′). Complementary oligos were designed with 5′- and 3′-overhangs for cloning and purchased from Invitrogen. Each pair of oligonucleotides was annealed at a concentration of 10 μmol/L in annealing buffer. The Lenti-CRISPR-v2-ZsGreen plasmid was digested with BsmBI (New England BioLabs) to remove the filler and gel purified. Each pair of oligonucleotides (790 pg) was ligated in the Lenti-CRISPR-v2-ZsGreen vector (50 ng) using QuickLigase and transformed in OneShot chemically competent Stbl3 cells (Invitrogen) using the manufacturer’s protocol. Clones were expanded in liquid culture, and sequencing confirmed the correct CRISPR guide RNA (gRNA) sequence.
and the extracted DNA plasmids (Qiagen) were verified by Sanger sequencing. The four plasmids created in this fashion were as follows: Lentiviruses Production. Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviruses were produced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with psPAX2 (15 μg) and pVSV-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.
1.5 × 10^7 cells in 400 μL of cold D-PBS. The cell suspension was probe sonicated and centrifuged according to the manufacturer’s instructions. Potassium cyanide was added at 2 mmol/L to inactivate SOD1 and SOD3. Results were interpolated from a standard curve in GraphPad Prism and normalized to the results of a Bicinchoninic Acid Protein Assay (Pierce, Thermo Fisher Scientific) run on the same lysate.

**Transgenic Mice**

C57BL/6 FLTR3-ITD mice were purchased from The Jackson Laboratory (stock number 01112) and were homozygous for the FLTR3-ITD mutation. C57BL/6 SIRT5+/+ mice were kindly given by Dr. Johan Auwerx (Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland). SIRT5−/− mice were first crossed with wild-type C57BL/6 mice to generate SIRT5−/− mice, which subsequently were bred with homozygous FLTR3-ITD mice to generate final experimental mice: FLTR3-ITD/Sirt5−/− and FLTR3-ITD/Sirt5+/+. The experimental mice were monitored daily and blood was analyzed monthly on a HemaTrue Veterinary Hematology Analyzer (Heska). All animal studies were approved by the Institutional Animal Care and Use Committee of the University of Utah (Salt Lake City, UT).

**Cell Line Xenografts**

NOD.Cg-Prkdcscid Il2rtgm1Wjl/SzJ (NSG) mice (8 weeks old, male, Jackson) were irradiated (200 rad, RadSource 2000, RadSource Technologies), and then injected intravenously with 5 × 10^5 – 1 × 10^10 OCI-AML3 cells or 2 × 10^6 CMK cells engineered to express doxycycline-inducible shSIRT5 (231–232) and luciferase. Doxycycline (2 g/L, with 5% sucrose, Gold Biotechnology) was prepared in 18 MΩ water, filter sterilized, and then delivered as drinking water 18 hours after cell injection and replaced twice weekly. All mice received sulfadimethoxine (0.125 mg/mL, Vet One) in the drinking water throughout the study. Mice were imaged with an IVIS Spectrum in vivo imaging system every week, and images were analyzed with Living Image 4.5.4 (PerkinElmer). Luciferin (Gold Biotechnology) was injected intraperitoneally at 150 mg/kg 15 minutes prior to imaging. For CMK cells, mice were randomized between doxycycline and regular water and then maintained on that regimen for 3 weeks. Half of each group was then randomized to cross over to the other regimen, as shown in Fig. 3A. For OCI-AML3 cells, six mice were injected with 0.5 – 1 × 10^5 cells/mouse. At 18 hours, mice were randomized between doxycycline and regular water. Mice were considered moribund and euthanized in case of weight loss ≥2 g maintained over consecutive days and in case of other signs of poor health, including minimal responsiveness, hind limb paralysis, or hunched appearance. BM and spleen cells were analyzed for FPD human CD45-APC (Clone H130), and mouse CD45-FITC (Clone 30-F11; Tonbo Biosciences) on a BD Biosciences LSRFortessa flow cytometer. Mouse procedures were carried out according to guidelines approved by the Institutional Animal Care and Use Committee at the Utah State University. For secondary transplants, 5,000 GFP-Lin Kit+ cells sorted from the BM of primary recipients were injected into secondary Sirt5+/− recipients, with 3 × 10^4 untransduced BM cells from Sirt5+/− or Sirt5−/− donors, respectively, for support. Mice were followed as described above for BCR-ABL1Δ240.

**Quantification of Mitochondrial DNA Copy Number**

Analysis of mitochondrial DNA copy number was performed by comparing expression of the mitochondrial encoded gene NADH Dehydrogenase 1 (ND1) with the nuclear encoded gene Hemoglobin (HGB) as described previously (19). Expression data were generated by performing qPCR with 5 ng genomic DNA per reaction, 500 μmol/L target-specific primers for ND1 and HGB (for sequences see Supplementary Table S6), and SsoFast EvaGreen Supermix (Bio-Rad) on a C1000 Thermal Cycler and CFX96 Real-Time System (Bio-Rad). The reaction consisted of 10-minute activation at 95°C, followed by 40 cycles at 95°C for 15 seconds and 95°C for 1 minute. Reactions were set up in triplicate. Relative expression of ND1 was then calculated from the ΔCt (HGB Ct – ND1 Ct) and expressed as 2^(-ΔΔCt).

**Transmission Electron Microscopy**

Mitochondrial morphology was assessed by transmission electron microscopy at the University of Utah Electron Microscopy Core Laboratory (Salt Lake City, UT) following standard procedures.

**Measurement of ROS**

Following treatment with doxycycline and/or antioxidant compounds, cells were washed with PBS and incubated with 5 μmol/L MitoSOX Red (Thermo Fisher Scientific) for 60 minutes at 37°C. Cells were washed in PBS and suspended in annexin V binding buffer containing 10 mmol/L HEPES/NaOH (pH 7.4), 140 mmol/L NaCl, 1.5 mmol/L CaCl2, and 4% paraformaldehyde, and then analyzed by flow cytometry.

**Figure 3A**

For OCI-AML3 cells, six mice were injected with 0.5 – 1 × 10^5 cells/mouse. At 18 hours, mice were randomized between doxycycline and regular water and then maintained on that regimen for 3 weeks. Half of each group was then randomized to cross over to the other regimen, as shown in Fig. 3A. For OCI-AML3 cells, six mice were injected with 0.5 – 1 × 10^5 cells/mouse. At 18 hours, mice were randomized between doxycycline and regular water. Mice were considered moribund and euthanized in case of weight loss ≥2 g maintained over consecutive days and in case of other signs of poor health, including minimal responsiveness, hind limb paralysis, or hunched appearance. BM and spleen cells were analyzed for FPD human CD45-APC (Clone H130), and mouse CD45-FITC (Clone 30-F11; Tonbo Biosciences) on a BD Biosciences LSRFortessa flow cytometer. Mouse procedures were carried out according to guidelines approved by the Institutional Animal Care and Use Committee at the University of Utah (Salt Lake City, UT), protocols 16-11002 and 19-11010 (principal investigator: M.W. Deininger).
and 2.5 mmol/L CaCl₂ and incubated with annexin V-APC (BD Biosciences). After 15 minutes, 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) was added to 2 μg/mL and fluorescence was measured on a LSR Fortessa (BD Biosciences) flow cytometer and analyzed with FlowJo analysis software (Treestar; ref. 62).

GSH Measurement

Cells were seeded in duplicate in white, opaque-walled 96-well plates (8 × 10³ cells/well) ± doxycycline (100 ng/mL) for 24, 48, and 72 hours. NAD/NADH and GSH were measured using luminescence-based assays according to the manufacturer’s instructions (NAD+/NADH-Glo, and GSH-Glo, Promega) on a Synergy HT microplate spectrophotometer (BioTek Instruments).

Global Metabolic Profiling

Oxygen consumption and glycolysis of intact cells were measured using XF Cell Mito Stress Test Kit and Glycolysis Stress Test Kit on a Seahorse XFe96 Analyzer (Agilent) at the Metabolic Profiling Core Facility at the University of Utah (Salt Lake City, UT) according to the manufacturer’s instructions. Briefly, Seahorse XF96 microplates were first coated with 20 μL/well of Cell-TAK adhesive (Corning) at 22.4 μg/mL in 0.1 mol/L NaHCO₃ (pH 8.0) for 20 minutes at room temperature to increase adherence of AML cells. Next 1–1.5 × 10⁵ cells/well were attached to the plate by centrifugation at 200 × g for 3 minutes without brakes. After spinning, cells were rested at 37°C for 30 to 40 minutes in a CO₂-free incubator before analyzing. XF Base medium (DMEM without sodium bicarbonate; Agilent) was used for all experiments. Depending on the experiment, XF base medium was supplemented with either 2 mmol/L glutamine (for glycolytic stress test) or pyruvate (1 mmol/L), glucose (for mitochondrial stress test), and antimycin A (for oxidative phosphorylation). Altered metabolites were identified, and statistical analysis was performed using a combination of an in-house metabolite library developed using pure purchased standards and the commercially available NIST library.

LC-MS Analysis

Prior to analysis, samples and process blanks were transferred to PTFE autosampler vials. Quality control samples were tested in four technical replicates, and the order of actual samples was randomized. An Agilent 6550 UPLC-QToF (Agilent Technologies, Inc.) run in both positive and negative modes was used for analysis. Separation was achieved using a Sequest zic-pHILIC 2.1 × 100 mm column (Millipore Sigma) with Phenomenex Krudkatcher (Phenomenex). An initial concentration of 90% ACN (buffer A) and 10% 10 mmol/L ammonium carbonate in ddH₂O (buffer B) was held for 1 minute at a flow rate of 0.3 μL/min. A was decreased to 20% over 20 minutes and held for 2 minutes. A was returned to starting conditions over 2 minutes, and the system was allowed to re-equilibrate for 6 minutes between runs at a flow rate of 0.4 μL/minute. Data were collected using MassHunter 7.0 software (Agilent). Molecular features were identified using MassHunter Profinder 8.0, and their peak area was recorded using QuanLynx. These data were transferred to an Excel spreadsheet (Microsoft). Metabolite identity was established using a combination of an in-house metabolite library developed using pure purchased standards and the commercially available NIST library.

Steady-state Metabolomics

Cell Culture and Sample Preparation. SIRT5-dependent cell lines (CMK and SKM-1) and SIRT5-independent cell lines (OCI-AML3 and KG1a) expressing doxycycline-shSIRT5 constructs were harvested after culturing ± doxycycline (100 ng/mL) for 36 and 48 hours, and washed twice in cold PBS. Samples were aliquoted into five 1.5-mL tubes (replicates) with 8–12 × 10⁶ cells/tube, and snap-frozen in liquid nitrogen. Cell numbers were equal for each cell line at each time point ± doxycycline. For metabolite extraction, 450 μL of cold 90% methanol solution containing an appropriate amount of internal standard (1 μg of d₄-succinic acid) was added to each sample tube. Each tube was vortexed thoroughly, sonicated for 3 minutes, and incubated at –20°C for 1 hour. After incubation, the sample tubes were centrifuged at 20,000 × g for 5 minutes at 4°C. The supernatant was transferred from each sample tube into labeled microcentrifuge tubes. A quality control sample was created by removing approximately 15% volume of the collected supernatant from each sample and mixing it in a single tube. Simultaneous to the extraction of samples, process blanks were prepared in the same manner as real sample but substituting deionized water for sample.

Gas Chromatography–Mass Spectrometry Analysis. All gas chromatography–mass spectrometry (GC-MS) analysis was performed with a Waters GCT Premier mass spectrometer fitted with an Agilent 6890 gas chromatograph and a Gerstel MPS2 autosampler. Dried samples were suspended in 40 μL of a 40 mg/mL O-methoxysalmine hydrochloride (MOX) in pyridine and incubated for 1 hour at 30°C. To autosampler vials was added 25 μL of this solution; 40 μL of N-methyl-N-trimethylsilyltrifluoroacetamide was added automatically via the autosampler and incubated for 60 minutes at 37°C with shaking. After incubation, 3 μL of a fatty acid methyl ester standard (FAMES) solution was added via the autosampler and then 1 μL of the prepared sample was injected to the gas chromatograph inlet in the split mode with the inlet temperature held at 250°C. A 10:1 split ratio was used for analysis. The gas chromatograph had an initial temperature of 95°C for 1 minute, followed by a 40°C/minute ramp to 110°C and a hold time of 2 minutes. This was followed by a second 5°C/minute ramp to 250°C, a third ramp to 350°C, and then a final hold time of 3 minutes. A 30 m Phenex ZB-5 MS column with a 0.25 μm long guard column was employed for chromatographic separation. Helium was used as the carrier gas at 1 mL/minute. Because of the high amounts of several metabolites, the samples were analyzed once more at a 10-fold dilution. Data were collected using MassLynx 4.1 software (Waters). Metabolites were identified, and their peak area was recorded using QuanLynx. These data were transferred to an Excel spreadsheet (Microsoft). Metabolite identity was established using a combination of an in-house metabolite library developed using pure purchased standards and the commercially available NIST library.

Metabolic Flux

SKM-1 cells expressing doxycycline-shSIRT5 constructs were cultured in RPMI medium ± 100 ng/mL doxycycline. At 28, 32, or 35 hours after doxycycline addition, cells were spun down and resuspended in either RPMI medium (no -glutamine, 21870–076, Gibco) supplemented with [13C₅-15N₂]+-glutamine (final concentration: 2 mmol/L; CNLM-1275-H-0.1, Cambridge Isotope) or RPMI medium (no glucose, 11879–020, Gibco) supplemented with 1,2,3,4,5-[13C5]-glucose (final concentration: 11 mmol/L; 720127; Sigma-Aldrich) ± doxycycline for 1, 4, and 8 hours, for a total time of 36 hours ± doxycycline. Cells were then harvested and washed three times with cold PBS, counted and aliquoted into 1.5-mL tubes with 1–2 × 10⁶ cells/tube, four replicates/sample/time point. Cells were then snap-frozen and stored in liquid nitrogen and shipped on dry ice to the University of Colorado (Aurora, CO). Metabolites were extracted from frozen cell pellets at 2 × 10⁶ cells/mL of ice-cold lysis/extraction buffer (5:3:2 MeOH:MeCN:H₂O) as described previously (69, 74). Following
centrifugation, samples were randomized, and 10 μL of supernatants were injected into a Thermo Vanquish UHPLC coupled online to a Thermo Q Exactive mass spectrometer operating in positive and negative ion modes (separate runs). Metabolites were separated on a 5-minute C18 gradient as described previously (74). Instrument stability was assessed via injections of technical replicates samples every 10 runs and ensuring a peak area coefficient of variation of <10%. Metabolite assignments and peak area integrations were performed using Maven (Princeton University, Princeton, NJ) in conjunction with the KEGG database following conversion of raw files to the .mzXML file type using RawConverter. Stable isotope tracing data was plotted in Prism 8 (GraphPad).

**Experimental Methods for Organic Synthesis**

All reagents and solvents were of analytic grade and used without further purification as obtained from commercial suppliers. Anhydrous solvents were obtained from a PureSolv-system. Reactions were conducted under an atmosphere of argon or nitrogen whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel-coated plates (analytic SiO2–60, F-254). TLC plates were visualized under UV light and by dipping in either (i) a solution of potassium permanganate (analytic SiO2–60, F-254). TLC plates were visualized under UV light and by thin-layer chromatography (TLC) using silica gel–coated plates were conducted under an atmosphere of argon or nitrogen when-further purification as obtained from commercial suppliers. Anhydrous CH2Cl2 (8.0 mL) was added dropwise (5 minutes) to a solution of S2 (Supplementary Fig. S10; 87 mg, 0.15 mmol) in anhydrous CH3Cl (4.0 mL) at 0°C. The reaction mixture was stirred at 0°C for 10 minutes and was then concentrated under reduced pressure. The crude residue and the TFA salt of the Boc-protected derivative of compound S1 (98 mg, 0.15 mmol) were dissolved in anhydrous DMF (4.0 mL). Then, IrPrNEt was added (129 μL, 0.56 mmol), and the reaction mixture was stirred overnight at ambient temperature and was then concentrated under reduced pressure. Preparative reverse-phase HPLC purification of the crude residue afforded the desired ester NRE139 (supplementary Fig. S10) (37% from S1) as a colorless fluffy material after lyophilization.

**NRE139**

The Boc group in S1 (Supplementary Fig. S10) was removed by IrPrSiH (0.3 mL, 1.5 mmol) and TFA (5 mL, 65.3 mmol) added to a solution of S1 (870 mg, 1.35 mmol) in CH2Cl2 (7.5 mL). The reaction mixture was stirred at ambient temperature for 1.5 hours and was then concentrated in vacuo. Excess TFA was removed by coevapo-
ration with CH2Cl2/toluene (1:1, 2 × 100 mL), CH3Cl/Heptane/CH3OH (1:10.0:6, 2 × 100 mL), and CH2Cl2/CH3OH (1:0.06, 100 mL), affording an off-white solid, tentatively assigned as the resulting TFA salt. UPLC-MS tR 120 minutes, m/z 544.2; [M+H]+, C23H35N5O4SF2Caled 544.2, which was used without further purification. A solution of HClp-alanine tbutyl ester (50 mg, 0.32 mmol) and IrPrNEt (85 μL, 0.49 mmol) in anhydrous CH2Cl2 (8.0 mL) was added dropwise (5 minutes) to a solution of S2 (Supplementary Fig. S10; 87 mg, 0.31 mmol) in anhydrous CH2Cl2 (4.0 mL) at 0°C. The reaction mixture was stirred at 0°C for 10 minutes and was then concentrated under reduced pressure. The crude residue and the TFA salt of the Boc-protected derivative of compound S1 (98 mg, 0.15 mmol) were dissolved in anhydrous DMF (4.0 mL). Then, IrPrNEt was added (129 μL, 0.56 mmol), and the reaction mixture was stirred overnight at ambient temperature and was then concentrated under reduced pressure. Preparative reverse-phase HPLC purification of the crude residue afforded the desired ester NRE139 (supplementary Fig. S10) (37% from S1) as a colorless fluffy material after lyophilization.

| H NMR (600 MHz, DMSO-d6) Δ 10.80 (d, J = 2.4 Hz, 1H, NHdiazole), 8.09 (d, J = 8.6 Hz, 1H, NH4), 8.05 (d, J = 7.9 Hz, 1H, Hβ), 8.00 (d, J = 7.8 Hz, 1H, CO2NH), 7.53 (dr, J = 8.5, 2.1 Hz, 1H, H2phenyl), 7.51–7.47 (m, 2H, H4phenyl, H6phenyl), 7.43–7.39 (m, 3H, H3phenyl, H5phenyl, NH(CH2)2CO2Et), 7.31 (dd, J = 8.1, 0.9 Hz, 1H, H4phenyl), 7.05 (dd, J = 8.1, 7.0, 1.1 Hz, 1H, H6phenyl), 7.01 (d, J = 2.3 Hz, 1H, H5phenyl), 6.96 (dd, J = 8.0, 6.9, 1.0 Hz, 1H, H6phenyl), 4.26–4.21 (m, 1H, H3phenyl), 4.13–4.02 (m, 3H, CH2CH2CH2, H1cyclobutyl), 3.76 (td, J = 8.6, 5.5 Hz, 1H, Hβ), 3.59 (br s, 2H, CH2CH2CO2Et), 3.20 (br s, 2H, H3phenyl), 2.90 (m, J = 14.4, 6.9 Hz, H1cyclobutyl), 2.72 (m, J = 14.4, 6.8 Hz, 1H, Hβ), 2.54 (t, J = 6.7 Hz, 2H, CH2CO2Et), 2.13–2.03 (m, 3H, H2phenyl), 2.02–1.93 (m, 1H, H2cyclobutyl), 1.84–1.74 (m, 1H, H3phenyl), 1.70–1.68 (m, 1H, H4phenyl), 1.49 (m, 2H, H5phenyl), 1.49–1.27 (m, 4H, H3phenyl, H2phenyl), 1.23–1.13 (m, 4H, H3phenyl, H2phenyl, CH2CH2CO2Et), 1.05 (dd, J = 17.0, 12.9, 4.0 Hz, 1H, H4phenyl). 13C NMR (151 MHz, DMSO) Δ 171.5 (CO2Et), 170.1 (C7phenyl), 169.6 (CO2phenyl), 161.5 (d, J = 247.7 Hz, C3phenyl), 158.2 (q, J = 36.9 Hz, CO2phenyl, residual TFA), 143.0 (d, J = 6.6 Hz, C1phenyl), 135.9 (C7diazole), 131.1 (d, J = 7.9 Hz, H5phenyl), 127.4 (C3diazole), 123.4 (C2diazole), 122.6 (d, J = 3.0 Hz, C6phenyl), 120.8 (C1diazole), 119.3 (d, J = 21.1 Hz, C4phenyl), 118.4 (C4diazole), 118.1 (C5diazole), 115.4 (d, J = 290.8 Hz, CF3TFA residual TFA), 113.6 (d, J = 24.2 Hz, C2phenyl), 111.1 (C7diazole), 109.6 (C3phenyl), 59.9 (CH2CH2), 56.2 (C1cyclobutyl), 53.2 (C6phenyl), 43.8 (C1cyclobutyl), 43.3 (C5phenyl), 38.7 (CH2CH2CO2Et), 33.6 (CH2CO2Et), 32.6 (C3phenyl), 30.0 (C2phenyl), 29.9 (C8cyclobutyl), 28.1 (C1phenyl), 27.7 (C5cyclobutyl), 22.5 (C4phenyl), 14.6 (C2cyclobutyl), 14.1 (CH2CH2), 13.4 (CF3). The peak for C = S was not visible in 13C NMR, presumably due to fast quadrupolar relaxation via the nearby 14N-nuclei. UPLC-MS tR 1.93 minutes, m/z 703.4 ([M+H]+, C23H40N9O4SF2Caled 703.3); HRMS m/z 703.2745 ([M+H]+, C23H40N9O4SF2Caled 703.2742). FpH = 3-fluorophen-1-yl.

**Authors’ Disclosures**

C.C. Mason reports other support from Intermountain Healthcare and Primary Children’s Hospital Foundation, and grants from NIH during the conduct of the study. A.B. Patel reports research to deuterated solvent as internal standard (ΔH DMSO-d6, 2.50 ppm; ΔC DMSO-d6, 39.52 ppm). Assignments of NMR spectra are based on two-dimensional correlation spectroscopy (COSY, HMQC, and HMBC spectra).
A Critical Role for SIRT5 in AML Metabolism

A. S.チン

Ses. They also thank the Metabolic Phenotyping Core Facility at the Institute. The authors thank Benjamin C. Brown (University of Colorado, and R01HL146442 by the National Heart, Lung and Blood Institute. N. Rajabi was supported by funds from the Boettcher Webb-Waring Investigator Award, and the Division of Hematology/Oncology. A.B. Patel was supported by fellowships from the Leukemia & Lymphoma Society (LLS). C.C. Mason was funded by the Pediatric Cancer Program that is supported by the NCI through Award Number 1P30CA042014-24. The V Foundation (T2017-008), Lady Tata Memorial Trust, Leukemia & Lymphoma Society, American Society of Hematology, and NIH (1S10RR026802-01) during the conduct of the study. No disclosures were reported by the other authors.

Acknowledgments

This work was supported by NCI (NIH) grant R21CA20593601 (to M.W. Deininger), R01CA254534 (to M.W. Deininger), The V Foundation for Cancer Research Translational Award T2017-008 (to M.W. Deininger and T. O’Hare), and a Catalyst award from the University of Utah College of Pharmacy. The Cancer Center Support (NCI P30-CA042014) awarded to the Huntsman Cancer Institute at the University of Utah provided pilot project funds and supported shared resources critical to this project. D. Yan is supported by the International Award from the Lady Tata Memorial Trust, United Kingdom. D. Yan and J.S. Khorashad were supported by the Special Fellow Awards from the Leukemia & Lymphoma Society (LLS). C.C. Mason was funded by the Pediatric Cancer Program that is supported by the Intermountain Healthcare and Primary Children’s Hospital Foundations, University of Utah, Department of Pediatrics, and the Division of Hematology/Oncology. A.B. Patel was supported by a Research Training Award for Fellows from American Society of Hematology. N. Rajabi was supported by the Novo Nordisk Foundation (grant number NNF17OC0029464). C.L. Jones acknowledges funds from LLN and American Cancer Society. A. D’Alessandro was supported by funds from the Boettcher Webb-Waring Investigator Award, RM1GM131968 by the National Institute of General Medical Sciences, and R01HL146442 by the National Heart, Lung and Blood Institute. The authors thank Benjamin C. Brown (University of Colorado, Aurora, CO) for technical assistance with metabolomics analyses. They also thank the Metabolic Phenotyping Core Facility at the University of Utah for the assistance with the experiments. This work was supported by the University of Utah Flow Cytometry Facility in addition to the NCI through Award Number 1P30CA042014-24 and the National Center for Research Resources of the NIH under Award Number 1S10RR026802-01. Research reported in this publication utilized the High-Throughput Genomics and Bioinformatic Analysis Shared Resource at the University of Utah Huntsman Cancer Institute, supported by the NCI of the NIH under Award Number P30CA042014. Claire Davis, HCl Communications, assisted with illustrations. The authors thank Dr. Dennis Winge (Division of Hematologic and Hematologic Malignancies, University of Utah, Salt Lake City, UT), Dr. Jared Rutter (Department of Biochemistry, University of Utah, Salt Lake City, UT, and Howard Hughes Medical Institute, Chevy Chase, MD), Dr. David Lombard (Department of Pathology, University of Michigan, Ann Arbor, MI), and Dr. Hening Lin (Department of Chemistry, Cornell University, New York, NY, and Howard Hughes Medical Institute, Chevy Chase, MD) for thoughtful discussion and feedback.

Received September 18, 2020; revised January 19, 2021; accepted March 5, 2021; published first April 10, 2021.

REFERENCES

SIRT5 Is a Druggable Metabolic Vulnerability in Acute Myeloid Leukemia

Dongqing Yan, Anca Franzini, Anthony D. Pomicter, et al.


Updated version
Access the most recent version of this article at:
doi: 10.1158/2643-3230.BCD-20-0168

Supplementary Material
Access the most recent supplemental material at:
http://bloodcancerdiscov.aacrjournals.org/content/suppl/2021/03/09/2643-3230.BCD-20-0168.DC1

Cited articles
This article cites 74 articles, 25 of which you can access for free at:
http://bloodcancerdiscov.aacrjournals.org/content/2/3/266.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://bloodcancerdiscov.aacrjournals.org/content/2/3/266.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://bloodcancerdiscov.aacrjournals.org/content/2/3/266.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.