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’Alessandro³⁸, 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, glutamine (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 succiny1, 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 deacylations 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, deacylation 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 dopaminergic 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 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.

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-shSIRT52311/2312. 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-RUNX1/ITN/ITR-NRS5(312), BCR-ABL(312), and MPL(315). 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). These data indicate that primary AML cells, but not normal CD34+ cells, are at least partially dependent on SIRT5.

We engineered 22 AML cell lines to express doxycycline-shSIRT52311/2312. 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

**B** Colonies (% of WT)

**C** Viable cells (% control)

**D** Cell lines

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

**F** shSIRT5-Dox

**G** shSIRT5-Dox

**H** Annexin V

**I** SKM-1 shSIRT5

**J** CMK OCI-AML3

**K** SKM-1 shSIRT5
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-inducible shSIRT52311/2312 and luciferase, and randomized at 18 hours postinjection to doxycycline-containing or regular water and followed by weekly bioluminescence imaging. At 3 weeks, groups were again randomized to continue their current regimen or switch to doxycycline-containing or regular water, respectively. Doxycycline showed no luminescence at week 3 (Fig. 2A, left), and controls were strongly positive (Fig. 2A, right). We next randomized mice to continue their regimen or switch to doxycycline-containing or regular water, respectively. In mice started on doxycycline-containing water, irrespective of randomization, we observed no luminescence until termination of the experiment (week 13; Fig. 2A, left). There was no leukemic involvement on autopsy, and BM and spleen were negative for hCD45+ (Fig. 2B, left and left middle panels). In the group started on regular water, mice switched to doxycycline-containing water 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 × 106 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 × 106 GFP+Lin+Kirt+ (LSK) 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 5-fluorouracil (5-FU)–treated Sirt5+/- and Sirt5−/− mice with BCR-ABL1p210-GFP 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

**Figure 2.** SIRT5 KD attenuates leukemia in a xenograft model. CMK cells (SIRT5-dependent) or OCI-AML3 cells (SIRT5-independent) coexpressing doxycycline-inducible shSIRT52311/2312 and luciferase were engrafted into NSG mice. A, Mice engrafted with CMK cells (N = 20) were randomized between samples containing doxycycline-containing or regular water, respectively. Doxycycline x 106 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 × 106 GFP+Lin+Kirt+ (LSK) 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 5-fluorouracil (5-FU)–treated Sirt5+/- and Sirt5−/− mice with BCR-ABL1p210-GFP 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

![Figure 2](https://bloodcancerdiscovery.aacrjournals.org/)

*Note:* Kaplan–Meier analysis of survival of CMK-engrafted mice across all experimental conditions.
A Critical Role for SIRT5 in AML Metabolism

**Figure A**
NSG mice injected with CMK<sup>shSIRT5</sup>.

**Figure B**
Human CD45 and Mouse CD45 cell surface expression.

**Figure C**
Survival rates over time with different treatment groups.

**Figure D**
OCI-AML3<sup>shSIRT5</sup> cell survival.

**Figure E**
Survival rates over time with different treatment groups.

**Figure F**
Human CD45 expression levels.

**Figure G**
Western blot for SIRT5 and α/β-tubulin.
FLT3ITDITD/Sirt5+/+ and FLT3ITDITD/Sirt5−/− genotypes. At 5 months, WBCs, granulocytes, and monocytes were reduced in FLT3ITDITD/Sirt5−/− compared with FLT3ITDITD/Sirt5+/+ mice (Fig. 3E). Altogether these data show that SIRT5 is required for leukemogenesis by several myeloid oncogenes.

**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...
A Critical Role for SIRT5 in AML Metabolism

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<sup>231/232</sup> 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–D, CMK (SIRT5-dependent) and OCI-AML3 (SIRT5-independent) cells expressing doxycycline-shSIRT5<sup>231/232</sup> 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 expressing doxycycline-shSIRT5<sup>231/232</sup> 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<sup>231/232</sup> 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-shSIRT5 and control constructs 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 tricarboxylic 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). 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 -independent cell lines with or without SIRT5 KD (Supplementary Fig. S6A). AML cells with low spare respiratory capacity are susceptible to oxidative stress (19). To test whether cell death induced by SIRT5 KD is caused by increased oxidative stress, we measured mitochondrial superoxide by flow cytometry. 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. S5A). Superoxide dismutase 2 (SOD2) detoxifies mitochondrial superoxide to oxygen and hydrogen peroxide, which is converted to oxygen and water by catalase. We compared SOD2 expression in SIRT5-dependent and SIRT5-independent AML cells but found no differences (Supplementary Fig. S6C). However, ectopic SOD2 expression blunted the superoxide increase induced by SIRT5 KD in CMK cells, reduced apoptosis, and partially restored colony formation (Fig. 5E and F). Strikingly, SOD2 KD induced growth inhibition and apoptosis only in SIRT5-dependent cell lines (Fig. 5G and H). In contrast, ectopic expression of SOD1 failed to rescue SIRT5-dependent cell lines from the effects of SIRT5 KD (Supplementary Fig. S6D). In addition, measurement of SOD2 activity revealed no change after KD of SIRT5, suggesting that SOD2 is not a direct target of SIRT5 (Supplementary Fig. S6E). We next measured the NAD+/NADH ratio and GSH level as a measure of redox buffer capacity. Both NAD+/NADH and GSH decreased upon SIRT5 KD in SIRT5-dependent but not SIRT5-independent cell lines (Fig. 5I), suggesting that SIRT5-dependent AML cell lines require SIRT5 to reduce mitochondrial superoxide and maintain redox homeostasis to prevent apoptosis.
A Critical Role for SIRT5 in AML Metabolism

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)-shSIRT52311/2312 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 (Ann 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-shSIRT52311/2312 engineered to ectopically express SOD2 were cultured ± doxycycline for 72 hours. E, Left, percent MitoSOX+/annexin V−/DAPI− cells. Middle, percent annexin V+ 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 test. 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+/NADH ratio was measured by GLO assay in SIRT5-independent (OCI-AML3; KG1a) and SIRT5-dependent (OCI-AML2; CMK; SKM-1) cell lines expressing doxycycline-shSIRT52311/2312 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).
Figure 6. SIRT5 regulates glutamine metabolism in SIRT5-dependent AML cells. A, Overview of the predicted isotopologues for metabolites downstream to [1^13C_2,^{15}N_2] glutamine in tracing experiments. αKg, α-ketoglutarate. B, Ratio of isotope-labeled glutamate/glutamine, with comparisons by two-way ANOVA and Tukey correction for multiple comparisons. C, Oxidative stress in SIRT5-dependent and SIRT5-independent (CMK, OCI-AML2) AML cell lines, with comparisons by two-way ANOVA and Tukey correction for multiple comparisons. D, Apoptosis in SIRT5-dependent and SIRT5-independent cell lines cultured ± l-glutamine for 72 hours. E, Apoptosis in OCI-AML2 shSIRT5 cells cultured ± doxycycline ± glutamine for 48 hours. Data represent the mean ± SEM from at least three independent experiments (*, 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 produg of SIRT5 inhibitor 1 (SS11) named ethyl 3-[3-((S)-6-([S]-1-(cylobutylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-5-([3-fluorophenyl]sulfonamido)-6-oxoheptylthioureido)propanoate (NRE139; Fig. 7A; Supplementary Fig. S10). The parent inhibitor, SS11, inhibits SIRT5 with an IC_{50} of 110 nmol/L in a published
biochemical assay (58) but exhibits no activity in cell-based assays. Presumably, SS11 does not enter cells, partly due to a negatively charged carboxylate moiety. We modified SS11 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). We documented SIRT5 target (59, 60). Succinylation of SOD1 at lysine 122 was increased in the presence of NRD167 (Fig. 7B). The effect of SS11, NRE139, and NRD167 was compared against SIRT5-dependent (OCI-AML2; SKM-1) and SIRT5-independent (KG1a; Marimo) cell lines. As expected, SS11 was inactive. NRE139 and NRD167 selectively inhibited 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 IC50 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 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 of AML cell lines, while colony formation by normal CD34+ oncogenes cells was unaffected. Genetic absence of SIRT5 impairs transduction of 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−/− mice challenged with the reactive oxygen species (ROS) inducer MTPT, SOD2 expression decreases much more than in Sirt5+/+ 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 deacylation 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 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 deubiquitination, thereby increasing α-ketoglutarate concentrations (54). In contrast, SIRT5-dependent desuccinylation of GLS K164 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, [13C5]-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

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

Figure 7. (Continued) F, CD34+ cells purified from three primary AML and three CB samples were cultured in colony assays ± NRD167 for 12 to 14 days. G, Survival of NSG-SGM3 mice injected with cells from AML sample 17-268 treated in vitro with NRD167 for 72 hours. H, Schematic depicting metabolic consequences of SIRT5 KD in AML cells. ROS, reactive oxygen species.
A Critical Role for SIRT5 in AML Metabolism

Consistent with a potential role of SIRT5 in promoting entrance of glutamate-derived carbon atoms into the TCA cycle, we find that SIRT5 KD reduces the steady-state levels and $^{13}$C$_5$N$_2$-glutamine-derived labeled isotoptologues of TCA cycle metabolites in multiple SIRT5-dependent cell lines. This may explain the reduction of OXPHOS and spare oxidative reserve capacity in response to SIRT5 KD, a consistent finding in Seahorse experiments on SIRT5-dependent cell lines. This feature appears to be at least in part constrained by decreased flux through $\alpha$-ketoglutarate dehydrogenase (which binds to and feeds complex I; ref. 62) downstream of glutaminolysis. Alternatively, by regulating glutathione synthesis via glutaminolysis, SIRT5 may participate in glutathionylation 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 $\rho_0$ derivatives from SIRT5-dependent 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 SDH/β 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 cell lines aligns well with our inability to generate $\rho_0$ 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 ($N_{\text{new}} = 48$), relapsed/refractory AML ($N_{\text{relapse}} = 5$), 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 S3). The shRNA constructs were designed in a pRSI16 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 $1.56 \times 10^6$ TU/mL.

Infection of Primary AML Cells

MNCs were cultured at up to $5 \times 10^6$ 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 $10^7$/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 HEPS buffer (Invitrogen; 10 mmol/L). 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% CO$_2$ and 37°C. The medium was...
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) FACSAria 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 × 10⁵ 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 FACSAria 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 Qiangen Buffer P1 supplemented with 100 μg/mL RNase A (Qiagen) at a concentration of 10⁵ 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. Additive 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 IC₅₀ 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 SIRT5-targeting shRNAs with the highest depletion during the screening (shSIRT5α11 and shSIRT5α12) were inserted into a doxycycline-inducible vector (pRSRT16-U6 Tet-shCMV-TetR-2A-TagRFP-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 SIRT5 was assessed by measuring mRNA transcripts using SYBR Green– and SIRT5-specific primers (Supplementary Table S6) in a CFX96 Real-Time System (Bio-Rad). For cell lines, the KD of SIRT5 was assayed by immunoblotting.

CRISPR
SIRT5 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′-CATCGATGAGC TGCACCGCA-3′, gRNA-B: 5′-GTCCGACCTTCAGAGGAC-3′, and gRNA-C: 5′-AAGCACATGTCATCATCCTC-3′) targeting SIRT5 were selected from a published database of predicted high-specificity protospacer adjacent motif (PAM) target sites for human SIRT5. In addition, a nontargeting oligo control was cloned (5′-TGACAT CAATTATACATCAT-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 the extracted DNA plasmids (Qiagen) were verified by Sanger sequencing. The four plasmids created in this fashion were as follows: Lentiviral plasmids transduced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with pPA2X (15 μg) and pSVH-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Lentivirus Production.** Lentiviral plasmids transduced as a stoichiometric mixture (21 μg) in HEK293T/17 cells using Lipofectamine 2000 and Plus Reagent (Invitrogen) together with pPA2X (15 μg) and pSVH-g (10 μg) to generate lentiviral particles. The virus was concentrated with PEG and stored at −80°C.

**Generation of SIRT5 Knockout Cell Line.** 5 × 10⁴ cells were plated in a 6-well plate in standard media in the presence of polybrene (8 μg/mL) and infected at high MOI. Four days after infection, the cells were sorted for ZsGreen1 expression and expanded in culture for 1 week. Subsequently, the cells were single-cell sorted in 96-well plates and cultured in RPMI media for 2 to 3 weeks. When single-cell colonies were formed and covered more than 50% of the well area, the cells were transferred to 12-well plates for expansion. Again, the cells were verified for ZsGreen1 expression, and only pure population clones were further analyzed. The clones obtained in this manner were processed for RNA/DNA extraction and for protein expression analysis. SIRT5 expression was analyzed by qPCR and immunoblot. For all clones negative for SIRT5 expression, fragments of about 800-bp size around each gRNA locus were PCR amplified from genomic DNA, gel purified, and analyzed by Sanger sequencing to confirm genomic DNA editing.

**Cell Culture**

**AML Cell Lines.** AML cell lines (Supplementary Table S4) were grown in RPMI1640 medium (Invitrogen) containing 10% FBS, 2 mmol/L L-glutamine, and 100 U/mL penicillin/streptomycin. In the case of OCI-AML5, TF-1, UT-7, M-07e cells, GM-CSF (10 ng/mL) was added to the culture medium. Cell lines were authenticated using the GenePrint 24 Kit (Promega) and DSMZ Online STR Analysis database at the DNA Sequencing Core at the University of Utah (Salt Lake City, UT). All cell lines were confirmed for Mycoplasma by using the MycoAlert Mycoplasma Detection Kit (Lonza).

**Short-term Liquid Culture of Primary AML and CB CD34+ Cells.** FACS-sorted RFP+ CD34+ cells were plated at 5 × 10⁴–2 × 10⁵ cells/well in 96-well plates in RPMI supplemented with 10% FBS and cytokines [for AML: CC100, GM-CSF (10 ng/mL), and G-CSF (10 ng/mL); for CB: CC100 only, ± doxycycline (100 ng/mL)], for 3 to 5 days.

**Cell Proliferation Assays**

Cells were seeded in triplicate in 96-well plates (5–9 × 10³ cells/well) ± doxycycline (100 ng/mL). Viable cells were measured using CellTiter 96 AQueous One Solution MTS Reagent (Promega) on an Epoch microplate spectrophotometer (BioTek Instruments).

**Colony-Forming Assays**

**AML Cell Lines.** Cells were seeded in duplicate at 0.1–0.2 × 10⁴ cells/dish in MethoCult H4230 (StemCell Technologies) ± 100 ng/mL doxycycline or in the presence and absence of graded concentrations of NRD167. Colonies were counted 7 to 10 days after planting.

**Primary AML and CB CD34+ Cells.** FACS-sorted RFP+ (AML patient cells; 2–4 × 10⁴ cells/dish; CB: 1 × 10⁴ cells/dish) were plated in duplicate in MethoCult H4230 with CC100, GM-CSF (10 ng/mL), and G-CSF (10 ng/mL; AML cells) or CC100 only (CB), ± doxycycline (100 ng/mL). For inhibitor studies, nontransduced cells were plated with graded concentrations of NRD167. Colonies were scored after 10 to 15 days. AML colony-forming assays were considered informative if an average of at least 15 colonies/dish was present in the control (no doxycycline).

**Mouse BM Cell**

**Effects of SIRT5 Knockout on Leukemogenicity.** Mouse BM cells transduced with leukemia-associated oncogenes (1 × 10⁴ cells/dish) were plated in duplicate in methylcellulose medium without cytokine (Methocult M3231, StemCell Technologies) to detect cytokine-independent CFU-GM colonies.

**Effects of SIRT5 Knockout on Physiologic BM Function.** Mouse BM cells (2 × 10⁴ cells/dish) from wild-type or Sirt5−/− mice were plated in duplicate in complete methylcellulose medium (Methocult M3434, StemCell Technologies). Colonies were scored under an inverted microscope at day 7.

**Real-Time Quantitative RT-PCR**

RNA was purified with the RNeasy Kit (Qiagen), and cDNA was synthesized with iScript RT Supermix by Bio-Rad. Sirtuin family members 1 to 7 were analyzed with TaqMan Fast Advanced Master Mix (Applied Biosystems) and probe assays from Life Technologies (Supplementary Table S6). Routine monitoring of SIRT5 following induction of shRNA was performed with SsoFast EvaGreen Supermix by Bio-Rad.

**Immunoblot Analysis**

For immunoblotting, cells were lysed in RIPA lysis buffer containing phosphatase and protease inhibitors (Cell Signaling Technology) with freshly added phenylmethylsulfonyl fluoride (PMSF; 2 mmol/L). Protein concentrations were measured using the Bradford method. Protein lysates were boiled in Laemmli sample buffer for 5 to 10 minutes and separated on Tris-glycine/SDS-PAGE gels (Bio-Rad), followed by transfer to nitrocellulose membranes. For antibodies used in immunoblotting experiments, see Supplementary Table S7. Images were collected with an Odyssey Fluorescent Imaging System (LI-COR) or a Bio-Rad ChemiDoc and quantified with ImageJ.

**Plasmids**

Plasmids were obtained from various sources. For complete information, see Supplementary Table S8.

**Mitochondrial Protein Fractionation**

Cells (1–5 × 10⁴) were pelleted, washed, and resuspended in 4 mL sucrose buffer [250 mmol/L sucrose, 1 mmol/L EDTA, 10 mmol/L Tris-HCl (pH 7.4), filtered at 0.2 μm] with PMSF (2 mmol/L) and kept on ice for 15 minutes before the cells expanded as visualized under a microscope. The cell suspension was pulled through an 18-gauge needle and expelled onto the wall of a 50-mL tube through a 26-gauge needle. This process was repeated 15 to 20 times, and break-up of cells was ascertained by inspection under a light microscope. The suspension was then centrifuged at 1,200 × g for 5 minutes at 4°C, and 200 μL of the supernatant was set aside for analysis of total protein. The remaining supernatant was centrifuged at 12,000 × g for 10 minutes at 4°C. Of this supernatant, 200 μL was set aside for analysis of cytosolic protein. The pellets (mitochondria) were washed one to two times with 1 mL sucrose buffer, and then lysed in 2× Laemmli sample buffer, incubated at 100°C for 6 minutes, and stored at −80°C.

**Superoxide Dismutase Activity Assay**

Superoxide dismutase activity was measured in OCI-AML2 cells 36 hours after the addition of doxycycline with the Superoxide Dismutase (SOD) Colorimetric Activity Kit (Invitrogen) using...
1.5 x 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 FLIT3-ITD mice were purchased from The Jackson Laboratory (stock number 011112) and were homozygous for the FLIT3-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 FLIT3-ITD mice to generate final experimental mice: FLIT3-ITD/Sirt5+/− and FLIT3-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 I2gretm1Wjl/SzJ (NSG) mice (8 weeks old, male, Jackson) were irradiated (200 rad, RadSource 2000, RadSource Technologies), and then injected intravenously with 5 x 10^7 cells/mouse. BM cells were then maintained on that regimen for 3 weeks. Half of each group mice were randomized between doxycycline and regular water and treated with vehicle or NRD167 (100 μmol/L) for 72 hours (2.3 μmol/L) for 72 hours (2.3 μmol/L) for 72 hours (2.3 μmol/L) for 72 hours. After treatment, cells were harvested and washed once with cold PBS, and then injected via tail vein into 10-week-old sublethally irradiated 2 Gy) male NOD.Cg-Prkdcscid I2gretm1Wjl Tg(CMV-IκBΔN:MYC)1Eng (Invitrogen) supplemented with 15% FBS, 15% WEHI, murine IL3 (7 ng/mL), murine IL6 (12 ng/mL), and murine SCF (56 ng/mL; PeproTech, for all mouse cytokines). Half of BM cells were then transduced with pMSCV-GFP retrovirus for expression of leukemia-associated oncogenes by two rounds of spinoculation with 10 μg/mL polybrene and HEPES (10 mmol/L) at 2,500 rpm for 90 minutes at 32°C. Oncogenes tested were BCR-ABL1, FLIT3-ITD, MLL-AF9, RUNX1-RUNX1T1/CRB1, and MPLW511L. Transduced cells were used in colony assays or transplantation models.

BM Transplantation
BCR-ABL1+ mice, lineage-negative, Sca-1+Kit+ cells were sorted from transduced C57BL/6 Sirt5+/− or Sirt5−/− BM 48 hours after first infection. Five thousand GFP-Lin Sca-1+Kit+ cells plus 3 x 10^5 untransduced BM cells from Sirt5+/+ or Sirt5−/− donors, respectively, were injected into lethally irradiated Sirt5+/− recipient mice (two doses of 450 rad, 4 hours apart). For maintenance of identical genetic background, all recipients were 6 to 11 weeks old and gender-matched Sirt5+/− littersmates from the same colony. Mice were monitored by daily inspection, weekly body weight measurement, weekly complete blood counts, and weekly FACS for GFP. Moribund animals (see xenograft studies) were sacrificed and subjected to detailed autopsy, FACS analysis, and histology as appropriate.

MLL-AF9. Following transduction, 3 x 10^7 cells/mouse were injected into lethally irradiated Sirt5+/− recipient mice (two doses of 450 rad, 4 hours apart). For maintenance of identical genetic background, all recipients were 6 to 11 weeks old and gender-matched Sirt5+/− littersmates from the same colony. Mice were monitored as described above. For secondary transplants, 5,000 GFP-Lin Kit− cells sorted from the BM of primary recipients were injected into secondary Sirt5+/− recipients, with 3 x 10^5 untransduced BM cells from Sirt5+/+ or Sirt5−/− donors, respectively, for support. Mice were followed as described above for BCR-ABL1+ mice.

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 nmol/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 – ND1) 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, 2.5 mmol/L CaCl2, 2 mmol/L MgCl2, and 0.1% bovine serum albumin (BSA) at room temperature for 15 minutes in the dark.
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 LSRFortessa (BD Biosciences) flow cytometer and analyzed with FlowJo analysis software (Treestar; ref. 62).

**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 Pheno
typing Core Facility at the University of Utah (Salt Lake City, UT) according to the manufacturer’s instructions. Briefly, Seahorse XFP96 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 × 10⁶ 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 (5 mmol/L; for Mito Stress test) on the glutamine (for glycolytic stress test) or pyruvate (1 mmol/L), glucose (5 mmol/L; for Mito Stress test) on the day of assay and adjusted to 7.4 with 1 N NaOH at 37°C. Respiration was measured under basal conditions, with sequential addition of 10 mmol/L glucose, 1 μmol/L oligomycin (ATP synthase inhibitor), 1 μmol/L carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP, mitochondrial oxidative phosphorylation uncoupler), and 0.5 μmol/L rotenone and antimycin A (electron transport chain complex I and III inhibitors). Glycolysis was measured under basal conditions, with sequential addition of 10 mmol/L glucose, 1 μmol/L oligomycin, and 50 mmol/L 2-deoxy-glucose (hexokinase inhibitor).

**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 2311-2312 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 μg/mL 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-methoxylamine 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 μm Phenomenex ZB-5 MS column with a 5 m 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.

**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 Sequant zic-pHILIC 2.1 × 100 mm column (Millipore Sigma) with Phenomenex Krud-katcher (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/min. Data were collected using MassHunter 7.0 software (Agilent). Molecular features were identified using MassHunter Profiler 8.0 and their peak area was recorded using MassHunter Quant 7.0. 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 METLIN library. Altered metabolites were identified, and statistical analysis was performed using Mass Profiler Professional (Agilent).

**Metabolic Flux**

SKM-1 cells expressing doxycycline-shSIRT5 2311-2312 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 t-glutamine, 21870–076, Gibco) supplemented with [1³C₅] t-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,–³C₄ 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 frozencell 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 replicate 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 and Materials for 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, 230–400). TLC plates were visualized under UV light and by in situ detection of potassium permanganate (10 g/L), potassium carbonate (67 g/L), and sodium hydroxide (1.83 g/L) in water; (ii) a solution of ninhydrin (3 g/L) in 3% acetic acid in water (v/v); or (iii) a solution of molybdate-phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) in 3% conc. sulfuric acid in water (v/v); or (iii) a solution of molybdate-phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) in 3% conc. sulfuric acid in water (v/v) followed by heating with a heat gun. Vacuum liquid chromatography was performed with silica gel 60 (particle size 15–40 μm). After column chromatography, appropriate fractions were pooled and dried at high vacuum (<2 mbar) for at least 12 hours to give obtained products in high purity (>95%) unless otherwise stated. Evaporation of solvents was carried out under reduced pressure at a temperature below 40°C. UPLC-MS analyses were performed on a Phenomenex Kinetex column (1.7 μm, 150 × 4.6 mm) using an Agilent 1260 LC system equipped with a diode array UV detector and an evaporative light scattering detector (ELSD). Gradient A with eluent I (0.1% HCOOH in H2O) and eluent II (0.1% HCOOH in MeCN) rising linearly from 0% to 95% of II during t = 0–0.5 minutes and then isocratically at 95% during t = 45–50 minutes was applied at a flow rate of 0.6 mL/minute. Preparative reverse-phase high-performance liquid chromatography (HPLC) purification was performed on a C18 Phenomenex Luna column (3 μm μm, 100 Å, 50 × 4.6 mm) using an Agilent 1100 series system equipped with a diode array UV detector. Gradient C using eluent III and eluent IV, rising linearly from 0% to 95% of IV during t = 5–45 minutes and then isocratically at 95% during t = 45–50 minutes was applied at a flow rate of 0.6 mL/minute. High-resolution mass spectrometry (HRMS) measurements were recorded either on a maxis G3 quadrapole time-of-flight (TOF) mass spectrometer (Bruker Daltonics) equipped with an electrospray ionization (ESI) source or on an Agilent 1290 UHPLC equipped with a diode array detector and coupled to Agilent 6550 QTOF mass spectrometer operated in positive electrospray or on a Bruker Solarix WR by either matrix assisted laser desorption/ionization, or ESI. Nuclear magnetic resonance (NMR) spectra were recorded either on a Bruker Avance III HD equipped with a cryogenically cooled probe (1H NMR and 13C NMR recorded at 600 and 151 MHz respectively) or on a Bruker Avance III (1H NMR, 13C NMR, and 19F NMR recorded at 400, 101, and 377 MHz, respectively). All spectra were recorded at 298 K unless otherwise stated. Chemical shifts are reported in ppm relative to deuterated solvent as internal standard (dH DMSO-d6, 2.50 ppm; DC DMSO-d6, 39.52 ppm). Assignments of NMR spectra are based on two-dimensional correlation spectroscopy (COSY, HSQC, and HMBC spectra).

**NRE139**

The Boc group in S1 (Supplementary Fig. S10) was removed by pTFA-SiH (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 coevaporation with CH2Cl2/toluene (1:1, 2 × 100 mL), CH3Cl/acetone/CH3OH (1:10:0.6, 2 × 100 mL) and CH3Cl/CH3OH (1:0.06, 100 mL), affording an off-white solid, tentatively assigned as the resulting TFA salt (UPLC-MS tR 1.29 minutes, m/z 544.2; [M+H]+, C27H35N5O4SF; Calcd 544.2), which was used without further purification. A solution of HCl/3-alanine tbutyl ester (50 mg, 0.32 mmol) and pTFA-Ne (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-derivative protected of compound S1 (98 mg, −0.15 mmol) were dissolved in anhydrous DMF (4.0 mL). Then, pTFA-Ne 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) (17 mg, 37% from S1) as a colorless fluffy material after lyophilization.

1H NMR (600 MHz, DMSO-d6) & 10.80 (d, J = 2.4 Hz, 1H, NH Dashboard), 8.09 (d, J = 8.6 Hz, 1H, NH Dashboard), 8.05 (d, J = 7.9 Hz, 1H, H2 Dashboard), 7.51–7.47 (m, 2H, H4 Dashboard, H6 Dashboard), 7.43 (s, 1H, NH Dashboard), 7.39–7.33 (m, 3H, H2 FPh, H5 FPh, NH(Ch3)CO2Et), 7.31 (dd, J = 8.1, 0.9 Hz, 1H, H2 Dashboard), 7.05 (dd, J = 8.1, 7.0, 1.1 Hz, 1H, H6 Dashboard), 7.01 (d, J = 2.3 Hz, 1H, H2 Dashboard), 6.96 (dd, J = 8.0, 6.9, 1.0 Hz, 1H, H6 Dashboard), 4.26–4.21 (m, 1H, H2 Dashboard), 4.13–4.02 (m, 3H, CH3CH2, H1 Cyclobutyl), 3.76 (td, J = 8.6, 5.5 Hz, 1H, H Dashboard), 3.59 (br s, 2H, CH2CH2CO2Et), 3.20 (br s, 2H, CH2CH2CO2Et), 3.19–3.17 (m, 2H, H2 Cyclobutyl), 2.02–1.93 (m, 3H, H1 Cyclobutyl), 1.84–1.74 (m, 1H, H2 Cyclobutyl), 1.68–1.60 (m, 1H, H4 Cyclobutyl), 1.49 (m, 2H, H3 Cyclobutyl), 1.49–1.27 (m, 4H, H4 FPh, H5 FPh), 1.23–1.13 (m, 4H, H5 FPh, H6 FPh, H2 Cyclobutyl), 1.05 (ddt, J = 17.0, 12.9, 4.0 Hz, 1H, H6 FPh, 1C NMR (151 MHz, DMSO) Δ 171.5 (CO2Et), 170.1 (CO Lys), 169.6 (CO Trp), 161.5 (d, J = 17.4 Hz, C3 α Indole), 127.4 (C1 Indole), 119.3 (d, J = 17.4 Hz, C2 α Indole), 116.5 (d, J = 298.0 Hz, CF3 Si TFA residual TFA), 113.6 (d, J = 142.1 Hz, C1 Trp), 112.5 (d, J = 142.8 Hz, C2 Trp), 111.4 (C7 Indole), 109.6 (C3 Indole), 59.9 (CH2 CH3), 56.2 (C2 Cyclobutyl), 53.2 (C2 Cyclobutyl), 43.9 (C1 Cyclobutyl), 43.3 (C3 Lys), 38.7 (CH3 CH2CO2Et), 33.6 (CH2 CO2 Et), 32.6 (C3 Lys), 30.0 (C6 Indole), 29.9 (C6 Cyclobutyl), 28.1 (C2 Cyclobutyl), 27.7 (C2 Cyclobutyl), 22.5 (C4 Lys), 14.6 (C3 Cyclobutyl), 14.1 (CH2 CH3), 13.9 (H2 Cyclobutyl). The peak for C = S was not visible in 13C NMR, presumably due to fast quadrupolar relaxation via the nearby 15N-nuclei. UPLC-MS tR 1.93 minutes, m/z 703.4 ([M+H]+, C37H49N5O6S2F; Calcd 703.3); HRMS m/z 703.2745 ([M+H]+, C37H49N5O6S2F; Calcd 703.2742). FpH = 3-fluoropenham-1-yl.

**Authors’ Disclosures**

C.C. Mason reports other support from Intermountain Healthcare Foundation and Primary Children’s Hospital Foundation, and grants from NIH during the conduct of the study. A.B. Patel reports research...
A Critical Role for SIRT5 in AML Metabolism

S. Reza.

They also thank the Metabolic Phenotyping Core Facility at the

rado, Aurora, CO) for technical assistance with metabolomics analy-

Institute. The authors thank Benjamin C. Brown (University of Colo-

Sciences, and R01HL146442 by the National Heart, Lung and Blood

funding from Genentech and American Society of Hematology. J.E. Cox

reports grants from NIH/National Institute of Diabetes and Digestive

and Kidney Diseases (NIDDK) during the conduct of the study. C.A. Olsen

reports grants from Novo Nordisk Foundation during the conduct of the

study. M.W. Deininger reports grants from NIH/NCI (R21CA20593601,

R01CA254354, and P30-CA42014-24), The V Foundation (T2017-008),

Lady Tata Memorial Trust, Leukemia & Lymphoma Society, American

Society of Hematology, and NIH (1S10RR268802-01) during the conduct

of the study. No disclosures were reported by the other authors.

Disclaimer

The content is solely the responsibility of the authors and does not

necessarily represent the official views of the NIH.

Authors’ Contributions

D. Yan: Conceptualization, data curation, formal analysis, supervi-

sion, investigation, writing–original draft, writing–review and editing.

A. Franzini: Supervision, investigation, writing–review and editing.

A.D. Pomicter: Data curation, formal analysis, supervision, investiga-

tion, writing–original draft, writing–review and editing. B.J. Halversen:

Investigation. O. Antelope: Investigation. C.C. Mason: Formal anal-

ysis, investigation, writing–review and editing. J.M. Ahmann: Investi-


C.L. Jones: Investigation. M.S. Zabriskie: Investigation. H. Than:


A.B. Patel: Investigation. P.M. Clair: Investigation. W.L. Heaton:

Investigation. S.C. Owen: Supervision, methodology. J.L. Andersen:

Investigation. C.M. Egbert: Investigation. J.A. Reitz: Data curation,

investigation. A. D’Alessandro: Data curation, supervision, investiga-


Investigation. S.M. Iyer: Investigation. J.S. Khorashad: Conceptualization,

supervision, investigation. N. Rajabi: Resources, investigation. C.A. Olsen:

Resources, supervision, investigation. T. O’Hare: Conceptualization,

supervision, investigation, writing–original draft, project administration,

writing–review and editing. M.W. Deininger: Conceptualization, resources,

supervision, funding acquisition, writing–original draft, project administra-

tion, writing–review and editing.

Acknowledgments

This work was supported by NCI (NIH) grant R21CA20593601 (to

M.W. Deininger), R01CA254354 (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 Huntsman Cancer Institute. 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 sup-

ported 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 Novo Nordisk Foundation

(grant number NNF17OC0029464). C.L. Jones acknowledges funds from

LLS 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 and Medical Sciences, and R01HL146442 by the National Heart, Lung and Blood

Institute. The authors thank Benjamin C. Brown (University of Colo-

rado, Aurora, CO) for technical assistance with metabolomics analy-

sies. 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 addi-

tion to the NCI through Award Number SP30CA042014–24 and the

National Center for Research Resources of the NIH under Award

Number 1S10RR268802–01. 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

P30CA42014. Claire Davis, HCI Communications, assisted with

illustrations. The authors thank Dr. Dennis Winge (Division of

Hematologic and Hematicologic 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, Chey 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, Chey 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

1. Burnett A, Wetzler M, Lowenberg B. Therapeutic advances in acute


al. Treatment of acute myelocytic leukemia: a study by cancer and leukemia


al. Cytoxane arabinoside with daunorubicin or adriamycin for therapy of


4. Lo-Coco F, Avvisati G, Vignetti M, Thiede C, Orlando SM, Jacobelli S,

et al. Retinoic acid and arsenic trioxide for acute promyelocytic leuke-


5. Yang X, Sexauer A, Levis M. Bone marrow stroma-mediated resistance

to FLT3 inhibitors in FLT3-ITD AML is mediated by persistent activa-


7. Stein EM, DiNardo CD, Pollyea DA, Farhi AT, Robor GJ, Altman JK,

et al. Enasidenib in mutant IDH2 relapsed or refractory acute mye-


8. Amantoglo MD, Quak L, Shih A, Stein EM, Roshal M, David MD,

et al. Enasidenib induces acute myeloid leukemia cell differentiation


9. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S,

Bloomfield CD, et al. Midostaurin plus chemotherapy for acute mye-


10. Stone RM, Larson RA, Dohner H. Midostaurin in FLT3-mutated acute


11. DiNardo CD, Pratz K, Pullarkat V, Jonas BA, Arellano M, Becker PS,

et al. Venetoclax combined with decitabine or azacitidine in treatment-


12. DiNardo CD, Stein EM, de Botton S, Robor GJ, Altman JK, Mims AS,

et al. Durable remissions with venetoclax and decitabine in patients with


et al. Targeting mitochondrial structure sensitizes acute myeloid leukemia


Downloaded from https://bloodcancerdiscovery.aacrjournals.org by guest on February 3, 2022. Copyright 2021 American Association for Cancer Research.


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.