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REVIEW

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# Metabolic underpinnings of leukemia pathology and treatment

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## Abstract

**Background:** Carcinogenic transformation of white blood cells during hematopoiesis leads to the development of leukemia, a cancer characterized by incompetent immune cells and a disruption of normal bone marrow function. Leukemias are diverse in type, affected population, prognosis, and treatment regimen, yet a common theme in leukemia is the dysregulated metabolism of leukemic cells and leukemic stem cells with respect to their noncancerous counterparts.

**Recent findings:** In this review, we highlight current findings that elucidate metabolic traits unique to the four major types of leukemia, which confer carcinogenic survival but can be potentially exploited for therapeutic intervention. These metabolic features can work in conjunction with or be independent of unique aspects of the bone marrow microenvironment that can also influence cell survival and proliferation, thus sustaining carcinogenesis.

**Conclusion:** Deepening our understanding of the interactions of leukemias with their niche environments in vivo will inform future treatments for leukemia, particularly for those that are refractive to tyrosine kinase inhibitors and other therapeutic mainstays.

**KEYWORDS**

metabolism, leukemia, mass spectrometry, metabolomics, stable isotope tracing, tumor microenvironment

## 1 | INTRODUCTION

Leukemias are a group of hematological malignancies caused by the rapid production of abnormal white blood cells. Such atypical white blood cells are not functional immune cells and, through space occupied, ultimately impair the ability of the bone marrow to produce sufficient numbers of red blood cells, platelets, and normal white blood cells. Several classifications exist to characterize the four major types of leukemia, each of which involves the oncogenic transformation of progenitor cells that are biased toward specific fates in the hematopoietic hierarchy. Lymphocytic leukemia refers to the transformation of marrow progenitor cells that become lymphocytes, including natural killer, T, and B cells. In myelogenous (or myeloid) leukemia, malignant transformation occurs in the marrow progenitor cells that mature into the cells of the myeloid lineage, which includes monocytes, mast cells, basophils, neutrophils, eosinophils, erythrocytes, and thrombocytes. The second classification, acute vs chronic leukemia, refers to the degree of cell maturity prior to carcinogenic transformation. Acute leukemias are malignancies of immature hematopoietic cells, and in contrast, chronic leukemias are characterized by the transformation of partially matured hematopoietic cells. In general, chronic leukemia progresses more slowly than acute cases, the latter of which, if left untreated, can result in death within weeks.

The ongoing search for effective strategies to treat hematological cancers is not unlike other cancers in that a precise and thorough understanding of their distinct genotypic and metabolic states, especially in comparison to their normal counterparts, can provide targets for selective, robust, and lasting cytoreduction of cancer cells. Investigations of hematopoiesis, particularly in the context of leukemia development, face several substantial challenges. First, although isolation of the specific progenitor populations is possible using flow cytometry, cell numbers are often low as the populations are small.

  

A second major challenge is heterogeneity among tumors of the same leukemia type resulting from unique tumor niches, genetic variability among humans, comorbidities, and as yet unidentified disease-related or disease-causing mutations. The advent of systems biology-based approaches such as omics technologies (genomics, transcriptomics, proteomics, metabolomics, among others) has transformed this research by generating complex datasets that provide a unique view into disease pathology and thus enable the development of novel therapies. Of the different tiers in the central dogma, metabolism represents that which is closest to the cellular phenotype and most responsive to environmental stimuli.[1-3] As such, metabolomics (ie, the study of small molecules <1500 Da) has emerged as the approach most amenable for detecting rapid, dynamic changes in cellular state that are biologically impactful but not manifested at the genome level due to timing or magnitude of change.

Techniques for performing wide-scale metabolomic analyses rely on nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) that provide direct readouts of complex metabolite mixtures and quantify changes occurring in response to stimuli, drug treatment, time, disease, and so on. In contrast, alternative approaches to measure single or several metabolites include autofluorescence, multiphoton, hyperspectral, and fluorescence lifetime imaging microscopy (FLIM), as well as commercial kits that spectrophotometrically quantify individual metabolites based on a surrogate reading of exogenously added enzyme activities. Extracellular flux (XF) analyzers can provide more holistic views of dynamic metabolism by determining extracellular acidification rate (ie, glycolytic flux) and oxygen consumption rate (ie, mitochondrial metabolism). These alternative approaches are utilized widely and are included in studies discussed herein. However, while valuable, these approaches are sharply limited by the breadth of information obtained in comparison to NMR and, in particular, MS experiments. This review will focus on recent investigations of metabolism in the context of leukemia that serve as major efforts to identify mechanisms of drug resistance, biological phenomena underlying relapse, and new targets for leukemia therapy.

Hematopoiesis begins at the stage of hematopoietic stem cells (HSCs), which are largely quiescent and maintain a self-renewing capacity. HSCs reside in the bone marrow, an organ marked by hypoxia and metabolic regulation by HIF-1α.[4] As a result, HSC homeostasis relies primarily on anaerobic glycolysis,[5] fatty acid metabolism,[6] mitophagy, and autophagy[7] with little contribution of oxidative phosphorylation and high antioxidant defense systems, thus protecting this limited and precious cell population against the adverse effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Once activated, HSCs progress into multipotent progenitor cells (MPPs), a population devoid of self-renewal and marked by robust proliferation (Figure 1). Through sophisticated molecular signaling that is only partially elucidated at current day, MPP subtypes (ie, MPP2, MPP3, and MPP4) are molecularly biased towards committing to specific lineages; MPP2 cells primarily form granulocyte/macrophage progenitors (GMP) and megakaryocyte-erythroid progenitors (MEP), while MPP3 cells predominantly fuel GMP production and MPP4 cells for lymphoid lineage progenitors, both via common myeloid progenitors (CMPs), or common lymphoid progenitors (CLPs, also known as MPP4).[8] Key modulators of hematopoiesis include inflammation,[9,10] the HSC microenvironment, mitochondrial dynamics,[11] reactive oxygen species,[12] and epigenetics,[13] among other factors.

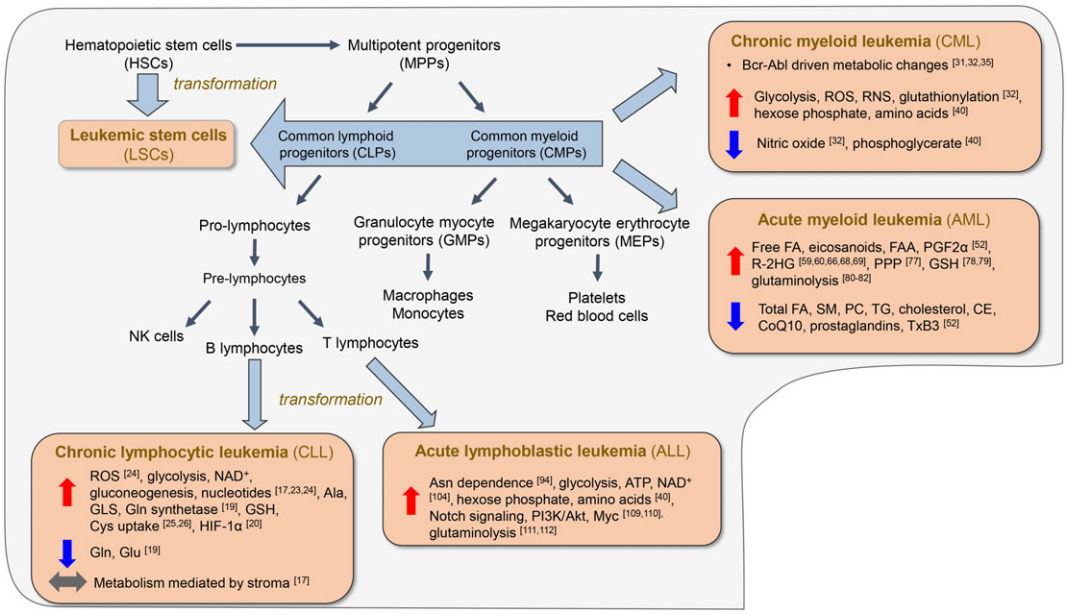


**FIGURE 1** Overview of hematopoiesis and transformation. Hematopoietic stem cells (HSCs) possess self-renewing capacity and are maintained as a small population. Generation of multipluripotent progenitor (MPP) cells devoid of self-renewal, but with active proliferation, marks the path toward differentiation to ultimately form the cellular components of blood. Carcinogenic transformation at the HSC stage generates leukemic stem cells (LSCs); transformation of mature or immature cells at points downstream generates leukemic cells. Shown here is an overview of metabolic features of the leukemia types. Abbreviations: ATP, adenosine triphosphate; CE, cholesterol ester; CoQ10, coenzyme $Q_{10}$; FA, fatty acid; FAA, fatty acid amide; GLS, glutaminase; GSH, reduced glutathione; HIF-1α, hypoxia-inducible factor 1α; NAD, nicotinamide adenine dinucleotide; PC, phosphatidylcholine; PGF2α, prostaglandin $F_{2\alpha}$; PPP, pentose phosphate pathway; R-2HG, (*R*)-2-hydroxyglutarate; RNS, reactive nitrogen species; ROS, reactive oxygen species; SM, sphingomyelin; TG, triglyceride; TxB3, thromboxane B3

Carcinogenic transformation of HSCs generates leukemic stem cells (LSCs, Figure 1), which are characterized by their impaired capacity for differentiation, enhanced proliferation, and unlimited self-renewal.[14] These cells are central components of disease progression, as well as drug resistance and relapse. Contradictory to long-term HSCs, LSCs are more dependent on oxidative respiration and have upregulated antioxidant defense systems, both adaptations which poise this cell population for rapid proliferative capacities. In addition to arising from the LSC pool,[15] leukemic cells also initiate at more mature developmental stages; we discuss next the four leukemia subtypes and how their unique metabolic features underlie current treatment approaches and provide cues for the development of therapies in the future. A prominent and common feature of leukemia cell biology is the cellular interaction with the tumor microenvironment. The stroma proves to be a key regulator of cell metabolism and redox state in a manner that controls transformation and proliferation. The various roles of the tumor microenvironment are noted within each of the leukemia subtypes, but additionally, this topic is also addressed on a larger scale in the concluding section with a particular focus on extracellular matrix protein dynamics.

## 2 | CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocyc leukemia (CLL) results from an overproduction of abnormal B cell lymphocytes by the bone marrow. CLL is a slow-progressing disease but unfortunately does not have a cure. Approximately 20 000 new cases of CLL will be diagnosed in the United States in 2018, equating to 1.2% of all newly diagnosed cancers, and 5-year survival rates were measured at 84% from 2008 to 2014 (seer.cancer.gov, Table 1). Cells associated with CLL are often quiescent and are found in bone marrow, lymph nodes, the spleen, and circulating blood. The diverse microenvironments in which CLL cells reside require them to be highly adaptive to oxygenation status and molecular signals originating in the stroma. For example, cell signaling in non-CLL cells in lymph nodes regulates CLL cell proliferation there as opposed to at other sites.[16] Additionally, the microenvironment in the bone marrow of CLL patients is characterized by increased expression at the mRNA and protein level of the BCL-2 family of proteins, crucial antiapoptotic factors that sustain CLL without affecting proliferation[17] (and references therein). Eluding apoptosis is a hallmark of CLL cells and underlies their propensity for chemoresistance, thus serving as a promising target for therapy.[18] Metabolically, CLL cells are characterized by—among other features—altered glutaminolysis, constitutive hypoxia-inducible factor 1α (HIF-1α) expression, and most critically widespread and continuous interactions with the stroma that function to regulate metabolic and redox homeostasis in these cells.

A mass spectrometry-based metabolomic study of B lymphocytes from CLL patients (n = 4) in comparison to healthy controls (n = 5) revealed alterations in glutaminolysis in B-CLL cells, specifically decreased levels of glutamine and glutamate and increased alanine, a product of glutaminolysis (Figure 2A).[19] Further evidence supporting dysregulated glutaminolysis was found in accompanying proteomic experiments which revealed increased levels of glutaminase and glutamine synthetase in CLL cells.[19] In comparison to normal lymphocytes, CLL cells have a dysregulated cell growth cycle and are primed for hypoxic response by constitutive low level expression of HIF-1α[20] even in normoxic environments such as circulating blood. Transcript-level expression of HIF-1α is variable among CLL patients[21] and has been correlated to unfavorable prognoses in a study of 88 CLL patients.[22] HIF-1α has also been implicated as a key regulator of the interactions between CLL cells and the stroma. This molecular interfacing of CLL cells with their dynamic microenvironment confers CLL plasticity, dynamically regulating their metabolism and cell cycle. Studies of the interaction between CLL cells and the stroma often utilize coculturing of patient CLL cells with stromal cell lines. For example, a 2-hour coculturing of patient CLL cells with NK Tert and other

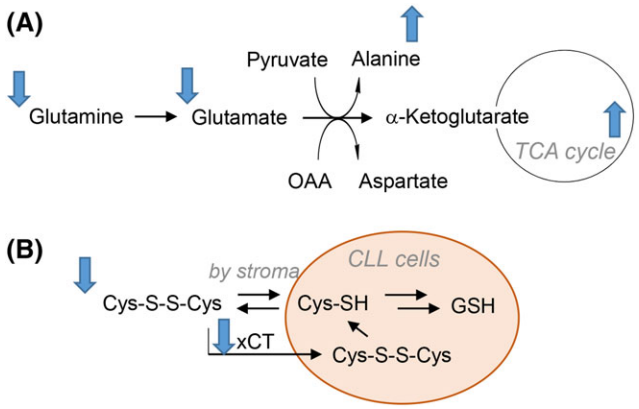


**FIGURE 2** Metabolic alterations observed in CLL cells. A, Flux through glutaminolysis is increased as evidenced by decreased Gln and Glu, elevated ala, and increased expression of glutaminase and glutamine synthetase. Coculturing with stroma increases TCA cycle activity in CLL cells. B-CLL cells have lower levels of cystine and the xCT cystine transporter; experimental evidence suggests that the stroma can regulate CLL redox homeostasis by reducing the cystine disulfide to cysteine, which enters cells and is utilized for glutathione biosynthesis

**TABLE 1** Statistics of leukemia incidence and survival in the United States

| | CLL | CML | AML | ALL | ALL—Pediatric |
|---|---|---|---|---|---|
| Est. new cases in 2018 | 20,000 | 8430 | 19,520 | 5970[a] | 3000[a] |
| Percentage of cancers diag. in 2018 | 1.2% | 0.5% | 1.1% | 0.4% | 0.2% |
| 5-year survival rate | 84% | 67.6% | 27.4% | 71% (All)[a]; 40% (Adult)[b] | 85%-90%[c] |

Statistics obtained from the National Cancer Institute's Surveillance, Epidemiology, and End Results Program (seer.cancer.gov) unless otherwise noted.

[a]American Society for Clinical Oncology (cancer.net).

[b]Goldstone et al. Blood. 2008, 111, 1827.

[c]Pui et al. Leukemia. 2014, 28, 2336.

stromal cell lines increased oxygen consumption rate and maximum respiratory capacity and reduced glucose uptake relative to CLL cells alone.[17] Several mitochondrial features in CLL cells were unaffected by coculturing, including levels of reactive oxygen species (ROS), outer mitochondrial membrane potential, mtDNA copy number, and expression levels of proteins involved in oxidative phosphorylation.[17] In addition, glycolytic flux, as judged by extracellular acidification rate (ECAR), was unaltered by coculturing with NK Tert cells for 2 or 24 hours.[17]

Mass spectrometry-based metabolomics revealed that coculturing increased $NAD^+$ along with levels of metabolites involved in the tricarboxylic acid (TCA) cycle, gluconeogenesis, and de novo synthesis of nucleotides. Furthermore, levels of nucleotide triphosphates in CLL cells were elevated at later time points such as at 24 and 48 hours of coculturing.[17] Taken together, these results help to elucidate the manners by which the stroma regulates the energy demands of CLL cells and reveals that such effects are evident as quickly as 2 hours following initial interaction of these cell types. Longer term coculturing has been performed using patient CLL cells cocultured with stromal cell line HS-5 for 6 days and revealed instead increased glucose uptake and expression of GLUT3 along with elevated glycolytic activity, as judged by increases in the expression of glycolytic enzymes, ATP levels, and ECAR relative to CLL cells alone.[23]

Redox homeostasis and metabolism are crucial in CLL cells, which possess higher basal levels of reactive oxygen species (ROS) than normal lymphocytes,[24] thus rendering these cells more susceptible to oxidative stressors. Recent findings revealed that the synthesis of glutathione (GSH), a key tripeptide antioxidant, in CLL cells is regulated by cells in the bone marrow stroma. Coculturing did not affect protein-level expression of a rate-limiting enzyme of GSH synthesis, and additional experiments revealed that CLL cells have reduced cystine content and lower expression of the xCT cystine transporter compared to normal lymphocytes (Figure 2B).[25] It was found in this study that the stroma reduces cystine to cysteine which is then taken up by CLL cells for GSH synthesis, revealing a mechanism by which the stroma regulates the redox environment of CLL cells.[25] The reliance of CLL cells and other cancer cells on extracellular import of cysteine/cystine to sustain GSH synthesis has been exploited as a treatment option for these cancers. An engineered form of human cysteinase with two activating point mutations was recently shown to deplete the extracellular cysteine/cystine pool and kill CLL cells from patients and a mouse model.[26] Cysteinase treatment reduced tumor size in breast and prostate cancer xenografts and prolonged (doubled) survival in a mouse model of CLL.[26] Lastly, in patient cells and mouse splenocytes, a treatment of 0.1 μM cysteinase significantly reduced intracellular GSH and increased superoxide levels, disrupting intracellular redox homeostasis in a manner sufficient to induce cell death.[26]

## 3 | CHRONIC MYELOID LEUKEMIA

Chronic myeloid leukemia (CML), also known as chronic myelogenous leukemia, is a cancer caused by transformed and growth-unrestricted myeloid cells (Figure 1). In the United States this year, an estimated 8430 new cases of CML will be diagnosed, corresponding to 0.5% of newly diagnosed cancers (seer.cancer.gov). CML is rare in children and has a 5-year survival rate of 67.6% in the United States (seer.cancer.gov, Table 1). CML is marked by a translocation between chromosomes 9 and 22, termed the Philadelphia chromosome, which results in the genetic fusion of the BCR and ABL genes, leading to the expression of cytosolic Bcr-Abl kinase. Bcr-Abl is a constitutively active tyrosine kinase whose presence leads to sustained activation of signaling involving the MAPK,[27] JAK/STAT,[28] MYC, RAS,[29] and PI3K[30] axes (reviewed in Cilloni and Saglio[31]). In addition, Bcr-Abl increases the rate of cell proliferation and interferes with cell cycle signaling and quality control mechanisms, particularly those involved in DNA repair, allowing for rapid growth and propagation of mutations.[32]

Treatments for CML often utilize tyrosine kinase inhibitors (TKIs) to target the Abl kinase ATP-binding domain, and although they are largely successful at inducing a remission state, TKIs are not curative for CML. Commonly used TKI therapies for CML include imatinib mesylate (marketed as Gleevec) and subsequent generation drugs nilotinib and dasatinib. The second-generation drugs provide faster responses and are more potent but do not affect the overall survival rate for CML patients compared to first-generation imatinib.[33] Third-generation drug ponatinib provides a treatment option for CML patients with the T315I mutation of Bcr-Abl,[34] but very likely, there are other mutations and other molecular mechanisms not yet identified that contribute to inefficacy in TKI compounds. Resistance to TKI therapy is a significant challenge in treatment of the disease; thus, it is crucial to understand the full picture of Bcr-Abl's far-reaching effects in order to better target future treatments.

Bcr-Abl increases glucose transport and renders CML cells more glycolytic than their normal counterparts,[35] promoting the Warburg effect that is commonly seen in many cancers. Imatinib has been shown to decrease flux through glycolysis through both reduction in glucose uptake and inactivation of glycolytic enzymes.[36,37] In one study, a continuous exposure of K562, an erythroleukemia CML cell line, to imatinib lead to inhibition of glycolysis and induction of autophagy by mechanisms involving activation of AMPK and suppression of S6K1.[38]

In addition to investigations of glycolysis, several studies have aimed to develop a more comprehensive cellular context for how CML cells differ metabolically from their healthy counterparts and to which degree the various TKI treatments recapitulate the phenotype of noncancerous cells. In one such study, the plasma of newly diagnosed CML patients before and after TKI treatment was compared to the plasma of age-matched healthy control patients using gas chromatography-mass spectrometry-based metabolomics.[39] A comparison of newly diagnosed patient (prior to TKI exposure, n = 26) plasma to the plasma of healthy controls (n = 26) revealed nine metabolites significantly altered: lactate, isoleucine, glycine, glucose, galactose, and myo-inositol elevated in CML; glycerol, myristate, and sorbitol decreased.[39] TKI therapy recipients were classified using clinical criteria as being resistant (n = 26) or sensitive (n = 26) to therapy.[39] In the plasma of these patients, myristate and glycerol emerged as potential biomarkers for TKI responsiveness, both elevated in the sensitive CML group.

In a wider metabolomic approach, leukocytes and plasma of CML patients were obtained at the time of diagnosis, following hydroxyurea

(HU) treatment (a common first cytoreductive step), and following subsequent TKI treatment using imatinib, nilotinib, or dasatinib.[40] Principal component analysis (PCA) revealed a clustering of newly diagnosed patients with the samples obtained following HU treatment. This group was separated from a clustering of patient samples following TKI to healthy control samples, suggesting a metabolic rewiring induced by TKIs that render the cells more phenotypically similar to non-CML leukocytes. The PCA trend existed in the patient plasma as well but was more evident in the leukocytes. A prominent theme of the findings in this study is that measurable metabolic disruptions that occur in the newly diagnosed patients in comparison to healthy controls are normalized, though not necessarily fully, by TKI treatment.[40] For example, such conclusions were observed for hexose phosphate content in cells (increased in CML), hexose content in plasma (low in CML), decreased late glycolysis intermediate phosphoglycerate (decreased in CML), and leukocyte and plasma amino acid levels (increased and decreased, respectively, in CML).[40] In a comparison of the three TKIs used, samples from dasatinib-treated patients were found to cluster apart from the other TKIs, perhaps reflective of the known higher incidence this drug has for off-target effects.

Resistance to imatinib and other TKIs, which occurs in a minority of patients, is believed to be a consequence of other mutations in the Abl domain that affect drug binding. As a result, alternative therapies for CML continue to be explored. One such effort, reported earlier this year, utilizes ivermectin, an antiparasitic compound. Ivermectin induces autophagy in breast cancer tumors by interfering with the mTOR/Akt pathway,[41,42] and has been shown to induce cell death in AML cells via chloride-dependent membrane hyperpolarization and increased ROS.[43] This study compared CML cell line K562 to primary CML cells and normal bone marrow cells, the latter two both CD-34+ (ie, expressing a cell surface marker indicating stemness) and found that ivermectin induced caspase-dependent apoptosis in CML but not normal hematopoietc cells via mitochondrial dysfunction and oxidative stress.[44] Molecular signaling propagated by kinases in the AKT/mTOR pathway was reduced following ivermectin treatment, and increases in levels of superoxide and total intracellular ROS were both observed. Additionally, ivermectin was found to synergize with TKIs nilotinib and dasatinib to increase the amount of CML apoptosis.[44] TKIs efficiently target differentiated cells, allowing leukemic stem cells to persist, thus further motivating the development of innovative treatment approaches for CML. A recent study utilized MS-based metabolomics to profile CD-34+ cells versus CD-34− (differentiated) cells from four CML patients.[45] CD-34+ cells were marked by increased fatty acid oxidation (FAO) as illustrated by decreased levels of free fatty acids, increased acylcarnitine content, and increased utilization of [U-$^{13}$C] palmitate into the TCA cycle and amino acids (Figure 3).[45] In addition, decreased lactate levels in CD-34+ cells suggested a greater fraction of pyruvate carbons being utilized in the TCA cycle. Indeed, [U-$^{13}$C] glucose tracing revealed that although no changes were observed in pyruvate dehydrogenase (responsible for pyruvate decarboxylation and the formation of acetyl-CoA), the activity of anaplerotic enzyme pyruvate carboxylase (conversion of pyruvate to oxaloacetate) was increased in CD-34+ CML cells.[45] Further experiments utilized CD-34+ cells from control (non-CML) patients to confirm via palmitate and glucose tracing that

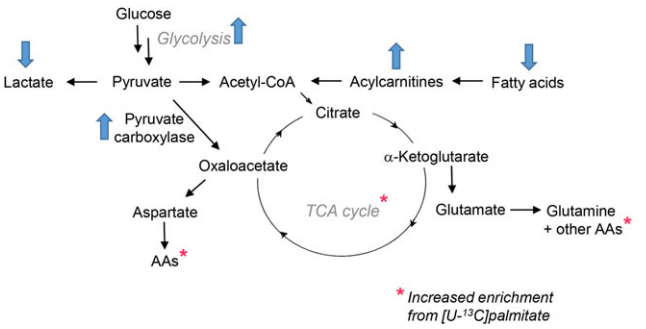


**FIGURE 3** Metabolic alterations observed in CD-34+ CML cells: Increased fatty acid oxidation leads to elevated levels of acylcarnitines and increased enrichment of TCA cycle metabolites and amino acids from stable isotope labeled palmitate. TCA flux is also increased via the enhanced activity of anaplerotic enzyme pyruvate carboxylase

the observed increases in anaplerosis and oxidative metabolism in CD-34+ CML cells are in fact a unique phenotype and are not reproducible in control CD-34+ cells absent of CML.[45] Such thorough studies of the fine details involved in how CML reprograms cell metabolism, both in stem and differentiated cells, are crucial to identify new avenues for selective and efficacious treatments, particularly for patients that are insensitive to TKI therapy.

## 4 | ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia (AML) is a hematological cancer that develops specifically in the myeloid lineage of the hematopoietic cellular hierarchy and results in the accumulation of immature myeloblasts that have a blockade in downstream cellular differentiation. An estimated nearly 20 000 patients were diagnosed in 2017 (seer.cancer.gov, Table 1). While great strides have been made during the last half century to treat AML thus improving survival rates, it remains an aggressive malignancy with poor prognoses of less than 30% 5-year relative survival rates for all patients (seer.cancer.gov). While outcomes are much better for patients younger than 60 years with cure rates near 35% to 40%, prognoses for patients over the age of 60 are particularly poor in which only 5% to 15% of patients are in remission.[46] These poor prognostic rates arise due to lack of response, or high rate of relapse, to the initial therapy.[47]

AML is heterogeneous and classified principally by cytological and genetic characteristics. While patient blood smear assessments and characterization of cell-specific markers by flow cytometry have been historically used to diagnose AML, the advent and continuous innovation of gene and transcript sequencing technologies has expanded classification of molecular genetic subgroups.[48] An analysis of 200 AML patient genomes enabled the functional categorization of several mutations thus detailing how multivariate data can be organized and potentially translated into new therapeutic strategies.[49] These categories include impaired cell signaling, chromosomal dynamics, epigenetic homeostasis, and deregulation of both the transcription and splicing processes. Given the dense connectivity of biological networks, it is not surprising that the deregulated processes identified on the genetic tier also have significant metabolic components.

Metabolomic approaches have been successfully applied to elucidate metabolic programs of AML cells both basally and in response to drug treatments, as well as provide diagnostic and prognostic biomarkers (Figure 1 for summary). Wang and colleagues applied NMR to characterize features in 183 patients with AML compared to 232 age-matched and gender-matched healthy controls to identify a number of distinct metabolic features of disease.[50] This study detailed distinct metabolic pathways pertinent to energy metabolism including glycolysis, TCA cycle, amino acid (AA), and fatty acid (FA) metabolism. The steady-state levels of these compounds were also able to distinguish between various disease burdens, highlighting the value of metabolomics as an analytical readout. Additional work expanded on these findings with the use of GC-MS and provided preliminary evidence that lipid levels can also potentially be used to distinguish between leukemia subtypes.[51,52] FAs are released from membrane lipids by lipases, then conjugated first to CoA by acyl-CoA ligases, and then to carnitine by carnitine palmitoyltransferase 1 (CPT1) at the outer membrane of the mitochondria. Upon transport into the mitochondria, they undergo FAO and generate acetyl-CoA to fuel the TCA cycle. Interestingly, the FA transporter CD36 has been suggested as a prognostic marker in AML.[53] A unique population of CD36+ leukemic stem cells (LSCs) identified in murine gonadal adipose tissue was reliant on FAO and was similar to both CD36+ CML and AML cells in humans.[54] In addition, coculture of AML blasts and bone marrow adipocytes increased lipolysis-FA transport axis that was abrogated with inhibition of CPT1A.[55] These metabolic adaptations protected LSCs from chemotherapy[54] and are also exploited in additional models of AML drug resistance.[56] Furthermore, AML cell populations that are not enriched for LSCs but are drug resistant, such as those resistant to cytarabine, also upregulate CD36, fatty acid oxidation, and overall mitochondrial activity.[57] Taken together, these studies highlight a central role for mitochondria in AML development and drug resistance, further supported by the finding that inhibiting mitochondrial translation can serve as a therapeutic strategy in treating AML.[58]

Molecular subtype can also directly contribute to metabolic programs in AML. One such category of mutations seen in roughly 20% of AML occurs in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2, which represent the cytosolic and mitochondrial isoforms, respectively). These NADP(+)-dependent enzyme isoforms catalyze the oxidative decarboxylation of isocitrate to produce α-ketoglutarate (αKG) and $CO_2$ and play integral roles in the TCA cycle as well as the maintenance of cytosolic redox homeostasis, among other functions. Mutations in these genes result in the dysregulation of cell maintenance and proliferation, and ultimately contribute to tumorigenesis in large part due to a gain of function that enables IDH to convert αKG into (*R*)-2-hydroxyglutarate (R-2HG),[59,60] the enantiomer of (*S*)-2HG produced by lactate dehydrogenase or malate dehydrogenase under hypoxic or low pH conditions (Figure 4A).[61-63] Classified as a central oncometabolite, R-2HG is a competitive inhibitor of αKG-dependent dioxygenases such as ten-eleven translocation 2 protein (TET2), which converts 5-methylcytosine to 5-hydroxymethylcytosine in the process of DNA methylation (reviewed in Parker and Metallo[64] and Sciacovelli and Frezza[65]). Accumulation of R-2HG can thus destabilize the epigenetic and metabolic landscape of hematopoietic

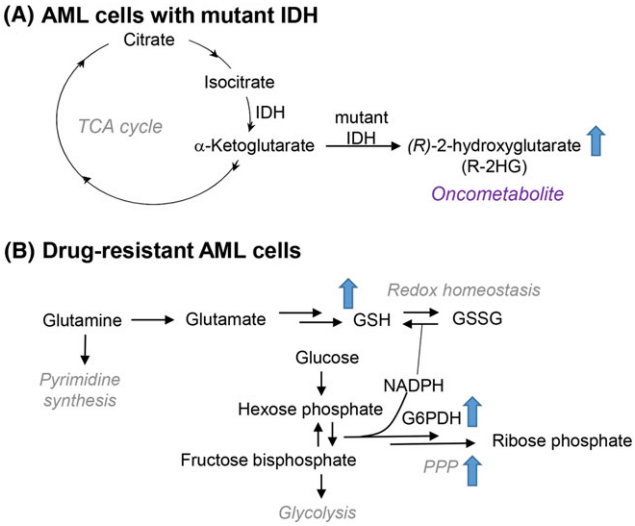


**FIGURE 4** Metabolic features of AML cells. A, Mutant isocitrate dehydrogenase (IDH) generates oncometabolite (R)-2-hydroxyglutarate from a-ketoglutarate. B, Hallmarks of drug-resistant AML cells include a reliance on glutamine to fuel glutathione (GSH) biosynthesis and pyrimidine synthesis. Additionally, drug-resistant cells have elevated pentose phosphate pathway (PPP) activity particularly of rate-limiting enzyme glucose 6-phosphate dehydrogenase (G6PDH)

precursor cells, poising them for leukemic transformation.[66-68] Given the active role IDH mutations play in leukemic development and progression, a number of small molecule inhibitors of IDH have been developed and are currently being tested or have already received FDA approval (more extensively reviewed in Ragon and DiNardo[69]). In addition, 2HG can alone be used as a prognostic marker in AML patients positive for IDH1/2 mutations,[50] or in combination with the abundance of pyruvate, lactate, αKG, and glycerol-3-phosphate to predict survival outcomes.[70] The implementation of high-throughput metabolomic technologies in the future will enable the rapid screening of patient samples[71,72] and expand upon the utility of metabolomics in clinical biochemistry.[73]

Another common set of mutations that predict for poor outcome occur in signaling genes including activating mutations caused by internal tandem duplications (ITD) in FMS-like tyrosine kinase 3 (FLT3).[74] This mutation has become a sought-after target in the treatment of AML due to the successful development and implementation of TK inhibitors. While inhibition of FLT3 has proven effective in treating AML,[75,76] short-lived remission rates have prompted subsequent research into possible cellular adaptive mechanisms and have pointed to significant metabolic contributions. Studies into the downstream effects of FLT3 inhibition have determined that these cells are acutely susceptible to oxidative stress, particularly due to accumulation of mitochondrial reactive oxygen species that induce apoptosis.[77] As revealed by synthetic lethal shRNA screening in combination with FLT3 inhibition, drug-resistant cells can upregulate the activity of ataxia telangiectasia mutated (ATM) and its downstream target glucose 6-phosphate dehydrogenase (G6PD) to activate the pentose phosphate pathway, thus enabling the cells to maintain reduced glutathione at levels sufficient to cope with oxidative stress.[77] Indeed, AML cells depend strongly upon glutathione metabolism[78,79] and

glutaminolysis to generate glutamate for glutathione synthesis (Figure 4B).[80,81] Glutamine utilization may also be required for pyrimidine synthesis, which represents an additional target for AML.[82] Taken together, these studies have defined a viable therapeutic strategy to eliminate drug-resistant cells by targeting FLT3 signaling in combination with glutamine metabolism and redox balance to treat AML (Gregory bioRxiv 2018).[83]

## 5 | ACUTE LYMPHOBLASTIC LEUKEMIA

Acute lymphoblastic leukemia (ALL) results from the transformation of hematopoietic B cell or T cell progenitors. ALL is the most common pediatric cancer, accounting for 80% of childhood leukemias.[84] Adult-onset ALL is most prevalent in white males age >70 years (seer.cancer.gov, Table 1). For pediatric B-ALL, the remission rate is 95% with 5-year survival in the range of 85% to 90%.[85] For adults, remission is achieved in 85% of patients but 5-year survival is only ~40%[86] and decreases to 7% if a relapse occurs.[87] Independent of age, relapsed ALL is associated with poor survival rates. Known genetic alterations, including mutations, fusions, deletions, and other modifications, are associated with 50% of adult and ~70% of pediatric ALL cases.[88] ALL is a heterogeneous disease marked by reprogrammed metabolism and atypical molecular signaling and, like CML, can be induced by the presence of the Philadelphia chromosome and expression of Bcr-Abl, particularly in adult patients. We will focus here on the most recent investigations of ALL cell metabolism as fuller pictures of ALL subtypes are recently and individually reviewed (B-ALL[89,90]; T-ALL[91-93]).

All cases of pediatric ALL, along with many adolescent, young adult, and adult cases, are treated with asparaginase (ASNase) therapy as a frontline approach to cytoreduction. It has been known for a half century that asparagine (Asn), normally a nonessential amino acid, is essential to ALL cells due to deficient activity of asparagine synthetase.[94] A sharp reduction of the cellular Asn pool using ASNase, which hydrolyzes the Asn amide to generate aspartate and ammonia, sufficiently retards mRNA translation to ultimately induce apoptosis in ALL cells.[95] Unfortunately, although ALL cells are particularly reliant on Asn, cross-reactivity of ASNase with other cell types results in significant side effects to an extent that has limited the use of ASNase in adult ALL cases. To rectify the off-target effects, other sources and preparations of ASNase are under investigation. For example, a recent study compared retrospectively the standard of care *Escherichia coli* enzyme with a polyethylene glycol conjugated (PEGylated) version in 122 ALL patients over age 14.[96] Major findings included a prolonged half-life of the PEGylated form, a 2-month increase in relapse-free survival time (~11 vs ~9 months with the non-PEG-conjugated enzyme) in patients under age 35, but no change in outcomes measured by metrics such as remission rate and survival time.[96] The occurrence and the intensity of side effects measured (such as liver and kidney damage) were also comparable between the two ASNase forms.[96] Resistance to ASNase therapy can occur through several mechanisms; from a metabolic standpoint, glutamine/glutamate metabolism has attracted attention as these can also serve as precursors for Asn (see Marini et al[95] for a recent review on asparaginase therapy in ALL).

Within the context of longer term treatment strategies, nearly all pediatric and adult cases of ALL are treated with methotrexate (MTX). MTX is a potent inhibitor of dihydrofolate reductase, an indispensable folate pathway enzyme required to generate folate cofactors for the synthesis of nucleotides and *S*-adenosylmethionine. Thus, MTX confers cytotoxicity by interfering with nucleic acid synthesis along with other molecular mechanisms. MTX is utilized in a variety of cancer types; however, adverse effects are common, and can include acute kidney injury,[97] cognitive/memory impairment,[98] leukoencephalopathy,[99] and myelopathy. As MTX is the subject of at least hundreds of clinical trials, we will highlight here only several of the most recent developments regarding its role in the treatment of ALL with a particular focus on multiple metabolic manipulations used in conjunction.

First, a CRISPR-Cas9 screen of the HEL (erythroblast-like) cell line identified formimidoyltransferase cyclodeaminase (FTCD) as being linked to MTX sensitivity.[100] FTCD is a crucial enzyme in the histidine degradation pathway, which utilizes tetrahydrofolate. Depletion of genes in this pathway decreased MTX sensitivity—in particular, CRISPR-Cas9-induced knockdown of FTCD in CML cells reduced both flux through the histidine catabolic pathway and MTX sensitivity.[100] Accordingly, His supplementation was hypothesized to increase MTX sensitivity by further disrupting the folate pathway. As anticipated, tumors grown in mice treated with HEL cells were decreased in size upon combination treatment of MTX + His vs either agent alone; importantly, no adverse effects on other organs were observed up to 15 days.[100] Histidine supplementation thus provides an intriguing potential route for facile dietary enhancement of MTX for leukemias.

A separate study investigated the effects of MTX when used in conjunction with mTOR inhibitors for the treatment of B-ALL. Elevated mTOR activity is associated with poor prognoses in B-ALL cases, but the effects of mTOR inhibitors when used alongside de novo DNA synthesis inhibitors like MTX or 6-mercaptopurine (6-MP) were not clear. Here, $Ph^+$ B-ALL cell lines BV173 and SUP-B15 were treated for 48 hours with MTX (30 nM) or 6-MP (10 μM) in the presence of mTORC inhibitors MLN0128 (100 nM) or rapamycin (10 nM).[101] In all cases, mTORC inhibitors protected the cells from killing via MTX or 6-MP.[101] Interestingly, TKI inhibitor dasatinib also protected cells from the effects of MTX via decreased mTOR signaling.[101] From a mechanistic viewpoint, inhibition of mTORC1, known to slow cell proliferation, is believed to render cells less sensitive to MTX and other agents that interfere with DNA/RNA synthesis and quality control.[101]

Returning to the unique features of ALL metabolism, another approach to ALL treatment utilizes glucocorticoids such as dexamethasone and prednisolone to inhibit cell proliferation by impeding glycolysis, upon which ALL cells are highly reliant to produce ATP and NAD+. In general, glucocorticoids interfere with glycolysis by mechanisms such as blocking glucose uptake and reducing the expression of glycolytic enzymes like pyruvate kinase; however, these drugs are not sufficient to induce remission when used alone. Cancer cells exposed to glucocorticoids rewire away from glycolysis toward mitochondrial metabolism and processes like autophagy in order to meet energetic demands for growth and proliferation and thus become more sensitive to drugs that target these aspects of cell metabolism.

One such recent study profiled CCRF-CEM clones exposed to dexamethasone alone or in combination with etoposide, an inhibitor of topoisomerase 2[102] that disrupts mitochondrial homeostasis,[103] and other anticancer drugs. Three phenotypic subtypes emerged: (1) cells that were resistant to dexamethasone independent of etoposide treatment, (2) cells where an additive effect was observed (termed CEM-ADD), (3) cells responsive to dexamethasone but with no observed synergy in the presence of etoposide (termed CEM-NON).[104] Reduced glycolysis was measured in both CEM-ADD and CEM-NON, as judged by decreased lactate levels, decreased mRNA expression of glycolytic genes hexokinase 2 (HK2) and lactate dehydrogenase A (LDHA), and decreased protein expression of HK2.[104] CEM-ADD cells exposed to dexamethasone for 48 hours followed by treatment for 72 hours with oligomycin, an inhibitor of ATP synthase, died in greater numbers than untreated cells and cells exposed to either agent alone.

In a separate study, the effects of antibiotic tigecycline were investigated for cytotoxicity against ALL cells. Using pediatric ALL cell lines,[3] primary ALL CD34+ progenitors and lymphocytes, normal bone marrow CD34+ cells, and peripheral blood mononuclear cells (PBMCs), a synergistic effect of tigecycline with doxorubin and (separately) vincristine was identified.[105] In the CCRF-CEM cell line, primary ALL 34+ progenitors, and primary ALL lymphocytes, mitochondrial respiration was adversely impacted by 2 hours following treatment with tigecycline alone. Glycolysis increased up to 6 hours, perhaps as compensation, but sharply decreased thereafter (final time point at 24 hours). All levels of tigecycline tested (5-20 μM) for the three cell populations resulted in decreased ATP levels (luminescence assay), increased ROS (DCF assay/flow cytometry), increased content of oxidized DNA (8-hydroxyguanine by ELISA), and increased protein oxidation (protein carbonyl content by ELISA).[105] Interestingly, no difference was observed in mitochondrial membrane potential at baseline when comparing the primary ALL CD34+ progenitors, ALL lymphocytes, normal bone marrow CD34+ progenitors, and PBMCs; however, in these experiments, the primary ALL cells were found to have greater mitochondrial mass, reserve respiratory capacity, and ATP. Finally, a xenograft mouse model (CCRF-CEM cells) found that the combinations of tigecycline with vincristine and (separately) with doxorubicin were most effective in limiting tumor size within the range of the 14 days tested.

Muschen and colleagues have recently reported new insights into the regulation of glucose metabolism in normal and malignant B cells, particularly in contrast to malignant myeloid cells. In one such study, the transcription factors PAX5 and IKZF1, which regulate genes involved in glucose uptake and metabolism, were identified as limiting ATP production via glycolysis and metabolite flow into the TCA cycle in manners that protect against transformation.[106] Lesions in the PAX5 and IKZF1 genes and/or altered expression are found in the majority of adult and pediatric B-ALL cases, leading to a loss of gatekeeper functionality that results in enhanced glucose metabolism and ATP generation (Figure 5). To illustrate, the introduction of Bcr-Abl in mice with wild-type PAX5 did not alter glucose uptake or utilization (as judged by ATP generation), but in mice haploinsufficient for PAX5, Bcr-Abl increased glucose uptake 50-fold and ATP levels 25-fold.[106] The response to glucocorticoids, which are

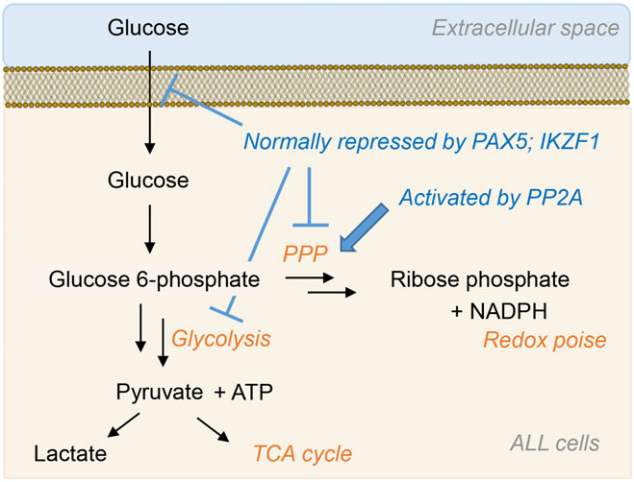


**FIGURE 5** Glucose metabolism in ALL cells. Gatekeeper transcription factors PAX5 and IKZF1 limit ATP production through glycolysis to prevent transformation. Together, genetic lesions and Bcr-Abl rewire metabolism to allow for rapid glycolytic flux. PP2A is required by ALL cells and activates the PPP to sustain redox homeostasis alongside rapid proliferation

effective for B-lymphoid ALL but not in myeloid leukemias, was linked to glucose metabolism via the NR3C1 protein, which is increased 6-fold to 20-fold in B-lymphoid cells vs myeloid leukemia cells.[106] NR3C1 is positively regulated by IKZF1 and PAX5, and several experiments revealed that modulation of the levels of these transcription factors correlated with the response to glucocorticoid prednisolone.[106]

In a subsequent report, serine/threonine phosphatase 2A (PP2A) was revealed to be essential to B cell tumors with a primary function of shifting glucose metabolism from glycolysis to the PPP to generate reducing equivalents (ie, NADPH) that counteract oxidative stress (Figure 5).[107] Normal B cells possess low PPP activity due to the repressed expression of PPP enzymes by PAX5 and IKZF1 (Figure 5).[107] Loss of PP2A in B cell leukemic cells increased glucose utilization and glycolysis products ATP and lactate; such changes were not observed in myeloid leukemic cells, and mitochondrial metabolism was unchanged in both cell types.[107] Deletion of PP2A in B-lymphoid cells decreased flux through the PPP, which also was not observed in myeloid cells. The PP2A deletion was found to increase phosphorylation of 6-phosphofructokinase/fructose 2,6-bisphosphatase 2 (Pfkfb2) at Ser483, enhancing its kinase activity (at the expense of its phosphatase activity), thereby promoting glycolysis over the PPP and illustrating a critical metabolic switch regulated by PP2A.[107] The effects of this deletion were reversed by overexpression of fructose 2,6-bisphosphate phosphatase 2 TIGAR in B-ALL cells lacking PP2A, as judged by the restoration of NADPH/NADP ratios. Overall, these and accompanying findings identify a unique reliance of malignant B cells on the PPP and thus define a vulnerable metabolic pathway for therapeutic intervention.[107]

Activation of protooncogene MYC is well documented for various cancers and, through transcriptional regulation, increases metabolic activity through glycolysis and glutaminolysis (reviewed in Hsieh et al[108]). Notch signaling upregulates MYC, and consequently the PI3K/Akt pathway,[109] and approximately half of T-ALL patients

possess activating mutations in NOTCH1.[110] Recently, in the context of ALL, resistance to γ-secretase inhibitors (GSIs), used to impede Notch signaling, was found to be associated with the loss of tumor suppressor PTEN.[111] Treatment of $PTEN^{+/+}$ ALL mice with GSI DBZ revealed using MS-based metabolomics increases of glucose and its downstream metabolites, both in glycolysis and the PPP, in comparison to untreated $PTEN^{+/+}$ ALL mice, and DBZ treated and untreated $PTEN^{-/-}$ ALL mice.[111] Leukemic mice lacking PTEN had higher lactate levels and a restored glucose metabolic phenotype (in comparison to $PTEN^{+/+}$ untreated) independent of DBZ exposure. Growth and proliferation of DND1, a $PTEN^{+/+}$ cell line with mutated NOTCH1, was hampered by DBZ and phenotypically rescued by treatment with either methyl pyruvate or dimethyl αKG, membrane permeable sources of TCA cycle carbons.[111] Finally, a comparison of stable isotope tracing using [$^{13}C_6$]-glucose or [$^{13}C_5$]-glutamine in NOTCH1-induced primary ALL cells revealed that glutaminolysis is the major source of carbons for mitochondrial metabolism; thus, this pathway is a promising target for metabolic manipulation in the treatment of Notch-implicated T-ALL cases.[111,112] However, this reliance on glutamine-dependent anaplerosis has been observed to decrease in cells that have acquired drug resistance.[56] These metabolic changes occur concomitantly with increased glycolysis and altered FAO, further evidencing the role of metabolism in responses to environmental stimuli. Interestingly, CCRF-CEM cells are able to acquire resistance to danorubicin in part due to increased expression of *DNAJC15*, which encodes for the HSP40 family member methylation-controlled J protein (MCJ). This protein has been found to modulate mitochondrial respiration via the disruption of mitochondrial supercomplexes,[113,114] thereby decreasing mitochondrial utilization and explaining a shift towards glycolysis in drug resistance.

In another metabolic intervention approach to targeting ALL cells, thiopurine compounds, such as 6-mercaptopurine (also termed purinethol) and 6-thioguanine (or tioguanine), are utilized in the treatment of ALL for their ability to stall DNA replication and induce cell death. Such thiopurines have sufficient structural similarities to hypoxanthine and guanine, respectively, to be converted into nucleotides and incorporated into DNA and RNA; however, they impede protein machinery involved in processes like repair, replication, and translation, ultimately causing cytotoxicity. A recent study investigated ALL resistance to thiopurines by clonal selection of ALL cells resistant to each of the two thiopurines described and found through exome sequencing that the insensitivity of such cells to the thioguanine nucleotides occurs in the presence of mutated hypoxanthine-guanine phosphoribosyltransferase (HGPRT), the enzyme responsible for converting hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate.[115]

In total, ALL tumor cells are characterized by a high metabolic rate that implies a reliance on amino acids (specifically asparagine) and a robust supply and utilization of glucose through glycolysis and the PPP. Recent efforts have uncovered the metabolic rewiring that underlies transformation in these cells along with features of ALL cells that distinguish their biochemistry from their myeloid counterparts, providing new opportunities for treating this disease in a selective and effective manner. A challenging aspect of ALL treatment, not unlike for other cancers, is the potential and likely presence of as-yet unknown genetic mutations associated with the disease etiology and/or impacting treatment response.

## 6 | CONCLUSION—TUMOR MICROENVIRONMENT

The role of the microenvironment in harboring and promoting the growth and development of leukemic cells is becoming an increasingly appreciated aspect of hematological cancer research. The characteristics of the bone marrow depend upon a complex network of cells of hematopoietic and mesenchymal lineages, cellular signaling through chemokine receptors and adhesion molecules, vasculature, and the dense protein meshwork of the extracellular matrix (ECM) (see Tabe and Konopleva[116] for a detailed review). This microenvironment provides cues for cellular homing, quiescence, self-renewal, differentiation, and proliferation, all of which become dysregulated in LSCs and leukemia cells. One aspect of the niche with substantial metabolic implications is oxygen tension, as hypoxia is a critical regulator of HIF-1α activity and subsequent metabolic programs. Indeed, hypoxia can contribute to chemoresistance in AML cells[117,118] and potentially serve as a prognostic marker.[117] In addition, lower oxygen tension can influence interactions between transformed and stromal cells by upregulating the expression of important adhesion molecules[119,120] to attract cancer cells to hypoxic environments. As age is an important risk factor for leukemic ongogenesis (seer.cancer.gov), it is important to note the age-associated increase in bone marrow adipocytes, which can influence oncogenesis through chemokine signaling, as well providing fatty acid substrates for FAO,[121,122] a key metabolic adaptation observed in some leukemic cells and discussed earlier in this review. Additional niche metabolites can also contribute to HSC and cancer cell behavior, with studies looking at the levels of arginine[123] and asparagine[124] on leukemic cell response. Valine has also been recently revealed as an important effector of the bone marrow microenvironment,[125] although more work will be required to elucidate implications for oncogenesis.

As the microenvironment promotes oncogenesis, transformed cells also alter the surrounding microenvironment into a pro-oncogenic niche and contribute to adaptive oncogenesis.[126] The adaptations of LSCs enable these cells to outcompete HSCs and take control of the bone marrow niche. AML cells can transform the niche into a proleukemic environment by promoting osteogenic differentiation of mesenchymal stem cells[127] or by secreting exosomes.[128] Leukemic blasts can also reprogram surrounding adipocytes to upregulate lipase activity thereby providing substrates for FAO.[55] Adipocytes are also a source of proinflammatory cytokines,[129] some of which can alter the balance between normal and pathologic hematopoietic cell behavior.[130-132]

These effectors may also influence the surrounding ECM, which can harbor chemosignaling and mechanosignaling cues, the latter of which are based upon ECM stiffness and architecture.[133,134] Indeed, three-dimensional mechanosignaling can influence leukemic cell response to therapy.[135] ECM components tenascin C[136] and laminins[137] contribute to hematopoietic cycling and homing to the bone marrow. ECM remodeling through the activities of matrix

metalloproteinases has been shown to play a role in the pathogenesis of multiple myeloma.[138] The resulting architecture, as well as the individual components present in the bone marrow niche, affects tissue stiffness and mechanosignaling through axes such as YAP/TAZ.[139] This transduction pathway is also tied to nutrient signaling[140] and nucleotide metabolism.[141] Interestingly, while YAP/TAZ is mediated by mevalonate metabolism, the cholesterol-lowering drug lovastatin can inhibit LSCs in coculture with MSCs,[142,143] highlighting a possible connection between the ECM and LSC metabolism.

In summary, the investigations reviewed here aim not only to elucidate the fine tuning of leukemic cell metabolism particularly in contrast to each type's healthy counterpart cells but also to expand on the larger context of how the leukemia subtypes differ. Such a detailed understanding will improve treatment approaches and provide new technical approaches to investigate metabolism at the levels of genes, proteins, and metabolites. Although the ECM in particular has been challenging to study from a systems level due to inherent difficulties in quantifying insoluble proteins by liquid chromatography and mass spectrometry, innovations in sample preparation and handling[144,145] will help to reveal unique holistic changes to the bone marrow microenvironment in the future. While current therapeutic development has centered upon direct leukemic cell targeting, expanding this focus to include ECM dynamics in combination with metabolic reprogramming during oncogenesis holds promise for the development of new classes of leukemia therapies.

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## CONFLICT OF INTEREST

Although unrelated to the contents of the manuscript, the authors disclose that AD and TN are members of Omix Technologies, Inc.

## AUTHORS' CONTRIBUTION

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, T.N. and J.A.R. F.M.L.; *Writing - Original Draft*, T.N. and J.A.R. F.M.L.; *Writing - Review & Editing*, T.N., A.D., and J.A.R. F.M.L.; *Visualization*, T.N., A.D., and J.A.R. F.M.L.; *Supervision*, J.A.R. F.M.L.; *Funding Acquisition*, T.N. and A.D. F.M.L.

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