



Amino Acid Metabolic Vulnerabilities in Acute and Chronic Myeloid Leukemias

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Amino acid (AA) metabolism plays an important role in many cellular processes including energy production, immune function, and purine and pyrimidine synthesis. Cancer cells therefore require increased AA uptake and undergo metabolic reprogramming to satisfy the energy demand associated with their rapid proliferation. Like many other cancers, myeloid leukemias are vulnerable to specific therapeutic strategies targeting metabolic dependencies. Herein, our review provides a comprehensive overview and TCGA data analysis of biosynthetic enzymes required for non-essential AA synthesis and their dysregulation in myeloid leukemias. Furthermore, we discuss the role of the general control nonderepressible 2 (GCN2) and-mammalian target of rapamycin (mTOR) pathways of AA sensing on metabolic vulnerability and drug resistance.

Keywords: non-essential amino acid, GCN2, general control non-derepressible 2, mTORC1, myeloid leukemias

INTRODUCTION

Myeloid leukemias are a group of disorders characterized by the presence of increased numbers of immature myeloid cells in the marrow and peripheral blood. These diseases are most common in adults with survival rates and first-line therapy options varying by age. In this review, we focus on the differences of non-essential amino acid (NEAA) metabolism in myeloid leukemias with an emphasis on the enzymes required for amino acid biosynthesis. While normal cells can synthesize NEAAs, cancers, in order to maintain their rapid growth and proliferation, can alter the expression of genes involved in amino acid biosynthesis and therefore lose the ability to synthesize specific NEAAs. This auxotrophy, or the inability of cancer cells to synthesize certain NEAA amino acids required for growth, leads to a dependency on exogenous sources of NEAAs, which can be pharmacologically targeted.

To comprehensively examine potential metabolic targets in myeloid leukemias within this review, we first introduce the disease and currently available pharmacotherapies to demonstrate the need for novel agents and to identify patients most likely to benefit from pharmacological agents targeting NEAA dependencies. We thereafter discuss the biosynthesis of NEAA, explore gene expression data analysis of potentially dysregulated pathways, and consider the supporting evidence for novel vulnerabilities in myeloid leukemias. Lastly, we detail mechanisms of cancer cell

adaptation to nutrient stress and considerations for the optimal design of strategies targeting NEAA metabolic vulnerabilities.

MYELOID LEUKEMIAS AND NEED FOR NOVEL TREATMENT OPTIONS

Novel Drug Therapies Are Needed to Improve Outcomes in Myeloid Leukemia Patients

Myeloid leukemias are classified as either chronic or acute based on their proliferation rate. Acute and chronic myeloid leukemias make up 14 and 33% of all estimated new 2020 leukemia cases in the United States, respectively (1).

Acute myeloid leukemia (AML) is the most common acute leukemia in adults. As of 2016, the 5-year overall survival (OS) of AML is approximately 24% (2), but the highest rate of AML deaths in the United States is among older patients (~90% for ages ≥ 65), underscoring the need for better therapy options. Due to the lower performance status of this demographic and inability to tolerate aggressive chemotherapy, these older patients have more limited curative pharmacotherapy options available.

Younger AML patients with good performance status can receive traditional intensive remission-induction chemotherapy with cytarabine, an anthracycline, and a FLT3 inhibitor depending on FLT3 mutation status. Alternatively, pharmacotherapy options for patients not eligible for intensive induction therapy include targeted approaches such as the hypomethylation agents decitabine or azacitidine and the BCL2 inhibitor venetoclax (3). Current approaches for post-induction therapy for low-risk patients generally include additional therapy using similar induction agents (such as high-dose cytarabine), and higher risk patients proceed to allogeneic hematopoietic cell transplantation (HCT).

The main characteristic of chronic myeloid leukemia (CML) is the translocation between chromosomes 22 and 9 (i.e. Philadelphia chromosome) that creates a *BCR-ABL* fusion oncogene with constitutively activated tyrosine kinase activity (4, 5). The cornerstone of CML treatment is the *BCR-ABL* tyrosine kinase inhibitor (6). However, there remains a need to develop novel therapies for CML patients with unfavorable prognosis, including those that are negative for the Philadelphia chromosome (7) and the 20–30% of CML patients who fail to achieve treatment milestones or develop TKI resistance. Altogether, current responses to standard AML and CML treatment regimens indicate that while many patients attain responses, there remains a need to develop therapies for those patients with poor prognosis.

Targeting AAs Dependencies in Myeloid Leukemias

Novel agents for the treatment of myeloid leukemias with unfavorable prognosis will most likely rely on certain cancer cell vulnerabilities to specifically inhibit their growth and limit treatment toxicity. Differences between the metabolic status of cancerous and normal cells are one of the primary hallmarks of

cancer (8), and changes in AA metabolism, glucose utilization, lipid consumption, and ATP generation are adaptations cancer cells can acquire for meeting their increased energy demands (9–13). These metabolic changes can lead to an “addiction” of particular fuel sources and identification of these vulnerabilities has led to the development of several agents directed toward specific molecular targets (14).

AAs are the building blocks of protein and are necessary for the sustenance of both normal and leukemic cells. There are 20 AAs classified as essential, non-essential, and conditionally essential (15). Essential AAs must be obtained exogenously from diet, whereas the body possesses the metabolic pathways to catalyze the synthesis of NEAAs. Conditionally essential AAs, like arginine and glutamine, are those that can become essential during certain physiological conditions that limit their synthesis (16). AAs can serve as precursors to many biological compounds, including those involved nucleotide synthesis (17), redox balance (18), lipogenesis (19, 20), and molecules that can fuel the TCA cycle (21). Furthermore, AAs can modulate the tumor microenvironment (22), global chromatin structures (23, 24), and epigenetic factors (25–28).

Herein, our discussion concerning myeloid leukemias and amino acid metabolism will focus on identifying potential vulnerabilities in AA biosynthesis that could be exploited for therapeutic purposes. Below, we provide a thorough discussion on NEAA biosynthesis for subsequently identifying dysregulations in potential targets that can lead to a dependency on extracellular NEAA sources for survival. Upon identification of an AA dependency, there are several targets within AA metabolic pathways that can be selectively targeted to exploit the vulnerability, including AA transporters, synthases, and transaminases that regulate biosynthesis (13, 29–31). In addition, enzymatic AA depletion therapy is an effective strategy for preferentially targeting cancer cells dependent on a specific AA for maintaining cell proliferation demands (13).

BIOSYNTHESIS OF NEAA

Glutamate Plays a Central Role in the Biosynthesis of Several NEAA

The concentration of glutamate in the body is tightly regulated to balance its important role in various biological processes (32). The biosynthesis of several NEAAs is interlinked with each other with glutamate playing a central role in the synthesis of several NEAAs as a precursor of alanine, proline, glutamine, aspartate, and serine (**Figure 1**). There are several biochemical pathways leading to the synthesis of glutamate, with a majority of this NEAA derived from the hydrolysis of glutamine by the amidohydrolase enzyme, glutaminase (GLS). Glutamate can also be derived from branch-chain amino acids (BCAAs) and proline (**Figure 1**). BCAA aminotransferase (BCAT) catalyzes the conversion of BCAAs and α -ketoglutarate into glutamate and branched chain α -keto acids, whereas oxidation of proline occurs *via* two enzymes: proline dehydrogenase (PRODH) and P5C dehydrogenase (P5CDH). In addition, glutamate is

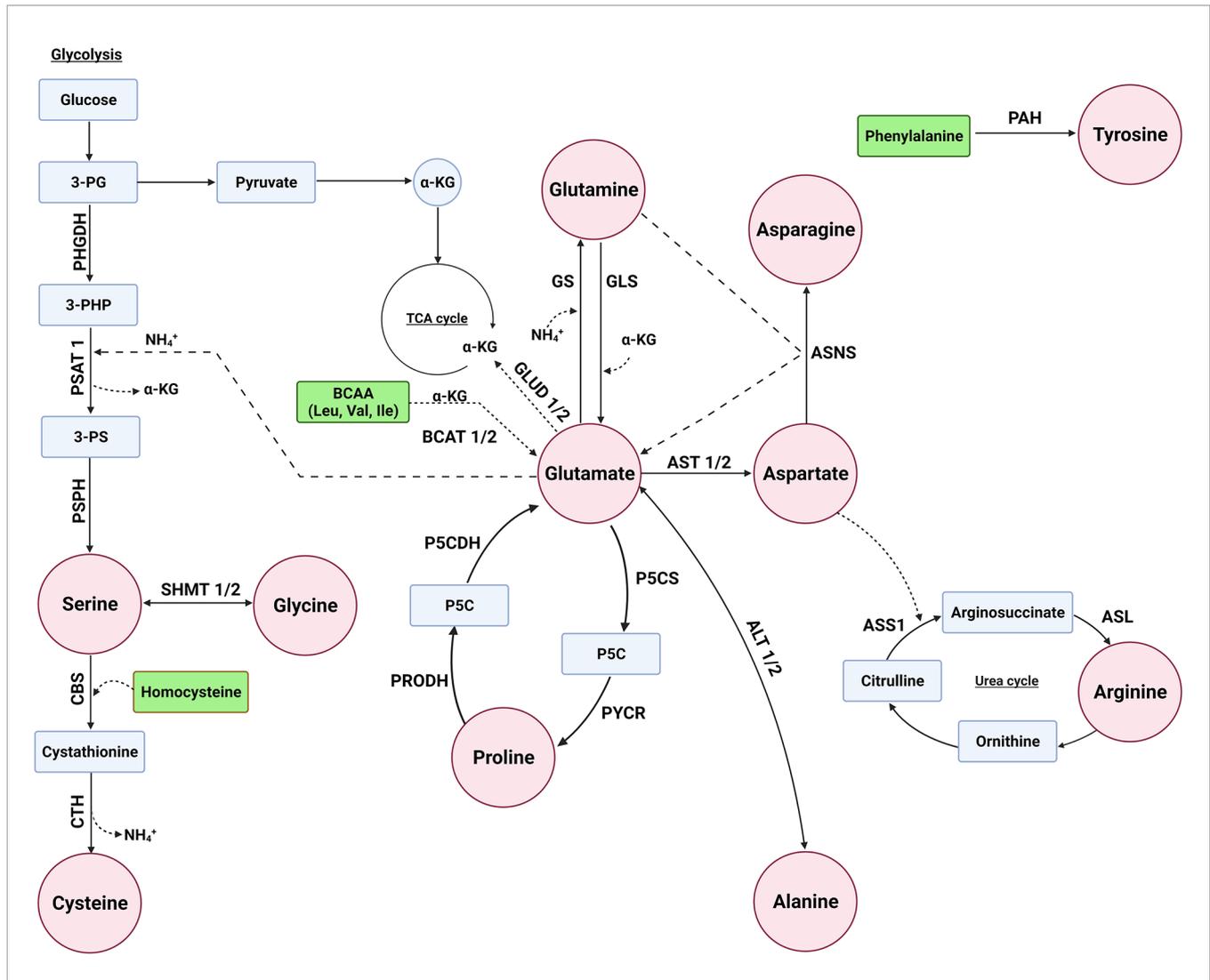


FIGURE 1 | Several enzymes are involved in the biosynthesis of non-essential amino acids. Glutamate can serve as a precursor for the biosynthesis of alanine, aspartate, glutamine, proline, and serine. The enzymes glutaminase (GLS/GLS2), proline dehydrogenase 1 (PRODH), P5C dehydrogenase (P5CDH or ALDH4A1), and branched chain amino acid transaminase (BCAT1/2) are involved in glutamate biosynthesis, whereas glutamate dehydrogenase (GLUD1/2) is involved in its metabolism. The enzymes alanine aminotransferase (ALT1/2 or GPT/2), aspartate aminotransferase (AST1/2 or GOT1/2), and glutamine synthetase (GS or GLUL) convert glutamate to alanine, aspartate, and glutamine, respectively. Pyrroline-5-carboxylate synthase (P5CS/ALDH18A1) and pyrroline-5-carboxylate reductase 1 (PYCR1) are used for proline biosynthesis from glutamate, whereas serine biosynthesis involves the transamination of 3-phosphohydroxypyruvate to 3-phosphoserine (3-PS) by phosphoserine aminotransferase (PSAT1) with glutamate as the amino donor and phosphoserine phosphatase (PSPH) to convert 3-PS to serine. Aspartate is a precursor of asparagine and arginine. The enzyme asparagine synthetase (ASNS), argininosuccinate synthase 1 (ASS1), and argininosuccinate lyase (ASL) are required for converting aspartate to asparagine and arginine, respectively. Serine is a precursor of glycine and cysteine, where serine is converted to glycine by serine hydroxymethyltransferase (SHMT1/2). Cysteine is synthesized through a process called the reverse transsulfuration pathway involving cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH). Tyrosine biosynthesis not linked to glutamate, but rather the enzyme phenylalanine hydroxylases (PAH) converts phenylalanine to tyrosine.

metabolized by glutamate dehydrogenase (GLUD1/2) back to α -ketoglutarate (33).

Alanine, Aspartate, Glutamine, Proline, and Serine Can Be Synthesized From Glutamate

As previously mentioned, glutamate serves as a substrate for several enzymes involved in the synthesis of several NEAAs. For alanine, the enzyme alanine aminotransferase (ALT/GPT)

catalyzes the transamination of pyruvate, with glutamate as the amino donor, to synthesize alanine (34). Similar to ALT, aspartate aminotransferase (AST/GOT) transfers an amine group from glutamate to oxaloacetate of the TCA cycle producing aspartate and α -ketoglutarate (35).

Glutamine is the most abundant amino acid in blood and is indispensable for the survival and growth of cancers. While humans acquire glutamine mostly through diet, glutamine synthetase (GS or GLUL, **Figure 1**) can synthesize glutamine

through the condensation of glutamate and ammonia (36). For proline biosynthesis (**Figure 1**), pyrroline-5-carboxylate synthase (P5CS) produces P5C from glutamate, which is converted to proline by pyrroline-5-carboxylate reductase (PYCR) (37).

Glutamate is also used for the biosynthesis of serine (**Figure 1**), where the glycolysis intermediate 3-phosphoglycerate (3-PG) is oxidized by phosphoglycerate dehydrogenase (PHGDH) to generate 3-phosphohydroxypyruvate (3-PHP). Phosphoserine aminotransferase (PSAT) catalyzes the transamination of 3-phosphohydroxypyruvate to 3-phosphoserine (3-PS) with glutamate as the amino donor, and phosphoserine phosphatase (PSPH) hydrolyzes 3-phosphoserine to serine (38).

Aspartate Is a Precursor for the Biosynthesis of Asparagine and Arginine

Similar to glutamate, other NEAAs, such as aspartate, serve as precursors for the biosynthesis of other AAs. Asparagine is synthesized from aspartate and glutamine *via* asparagine synthetase (ASNS, **Figure 1**), which transfers an amine group of glutamine to aspartate (39). Similarly, aspartate can be used for the synthesis of arginine through a multi-enzyme pathway in which the enzyme argininosuccinate synthase 1 (ASS1) initiates the process by catalyzing the formation of argininosuccinate from citrulline, which is an intermediate of urea synthesis, and aspartate (**Figure 1**). Subsequent cleavage of argininosuccinate by argininosuccinate lyase (ASL) produces arginine and fumarate (40).

Serine Is a Precursor of Both Cysteine and Glycine

Serine can serve as a precursor for several AAs, including cysteine and glycine (**Figure 1**). While cancers can dysregulate cysteine biosynthesis (41–43), typically cysteine is derived from extracellular sources (44–46). The metabolic pathway that leads to the generation of several sulfur metabolites, including cysteine, is called the transsulfuration pathway, where mammalian cells can only obtain cysteine through the reverse transsulfuration pathway (47–49). This pathway for generating cysteine in mammals involves the condensation of homocysteine (derived from methionine) with serine to generate cystathionine through the enzyme cystathionine β -synthase (CBS). Cystathionine γ -lyase (CTH) then converts cystathionine to cysteine (50).

Serine can also be converted to glycine by serine hydroxymethyltransferase (SHMT), which transfers the β -carbon of serine to tetrahydrofolate to produce glycine and 5,10-methylene tetrahydrofolate (51). In humans, there are two SHMT genes: cytoplasmic SHMT1 and mitochondrial SHMT2 (52).

Tyrosine Is Synthesized by Phenylalanine Hydroxylases (PAH) From Phenylalanine

While the biosynthesis of other NEAA is linked to each other, mammals synthesize tyrosine from the essential amino acid phenylalanine. The enzyme phenylalanine hydroxylases (PAH) hydroxylates the aromatic side-chain of phenylalanine to generate tyrosine (53).

DYSREGULATION OF ENZYMES INVOLVED IN NEAA BIOSYNTHESIS IN MYELOID MALIGNANCIES

Gene Expression Analysis of TCGA AML Samples

The Expression of Enzymes Involved in the Biosynthesis of Glutamate Is Dysregulated in AML

A gene expression analysis of enzymes involved in NEAA biosynthesis was performed using the Gene Expression Profiling Interactive Analysis (GEPIA) web server (54, 55). GEPIA uses RNA-Seq datasets based on the UCSC Xena project₂ that has a standard processing pipeline allowing users to compare gene and transcript expression from The Cancer Genome Atlas (TCGA) tumor samples to corresponding Genotype-Tissue Expression (GTEx) normal samples. GEPIA includes over 8,000 normal samples from TCGA and the GTEx projects, albeit from unrelated donors (56, 57). For AML, GEPIA was used to screen for dysregulated enzymes by comparing transcript expression between AML (n = 173) and corresponding normal GTEx samples (n = 70).

Using GEPIA and TCGA AML samples, a gene expression analysis of enzymes involved in the synthesis of glutamate was performed. Consistent with the central role glutamate plays in the biosynthesis of several NEAA, we found that the expression of the glutaminase isoforms *GLS* and *GLS2* in AML is upregulated relative to normal controls (P < 0.01, **Figures 2A, B**). Similar to *GLS*, *PRODH* was significantly upregulated in AML samples (P < 0.01, **Figure 2C**), whereas expression levels of *P5CDH* (i.e., *ALDH4A1*, data not shown) showed a similar but non-statistically significant trend. Interestingly, the two isoforms of *BCAT* (cytosolic *BCAT1* and mitochondrial *BCAT2*) were downregulated in AML TCGA samples (P < 0.01, **Figures 2D, E**), indicating a potential strong dependence on glutamine and proline for sources of glutamate. For glutamate dehydrogenase, which is involved in glutamate metabolism, no association was identified for *GLUD1*, whereas the *GLUD2* genes was significantly downregulated (P < 0.01, **Figure 2F**), consistent with a glutamate dependency in AML.

The Expression of Enzymes Involved in the Biosynthesis of NEAA Requiring Glutamate as a Precursor Is Decreased in AML

Given that several enzymes of glutamate biosynthesis were dysregulated in AML, it was possible that the corresponding effect on glutamate levels in AML had an influence on other enzymes involved in the biosynthesis of NEAAs requiring glutamate as a precursor. An analysis of the 10 enzymes involved in the biosynthesis of alanine, aspartate, glutamine, proline, and serine identified significant downregulations in *AST* (*GOT1* and *GOT2*), *GLUL*, *PYCR1*, *PSAT1*, and *PHGDH* (P < 0.01, **Figures 3A–F**), but no statistical dysregulations in *ALT* (*GPT* and *GPT2*), *P5CS* (*ALDH18A1*), or *PSPH* (data not shown). Overall, there was a downregulation in 6 of 10 enzymes involved in the biosynthesis of these NEAAs, with alanine being the only NEAA with no association. Therefore, while there seems

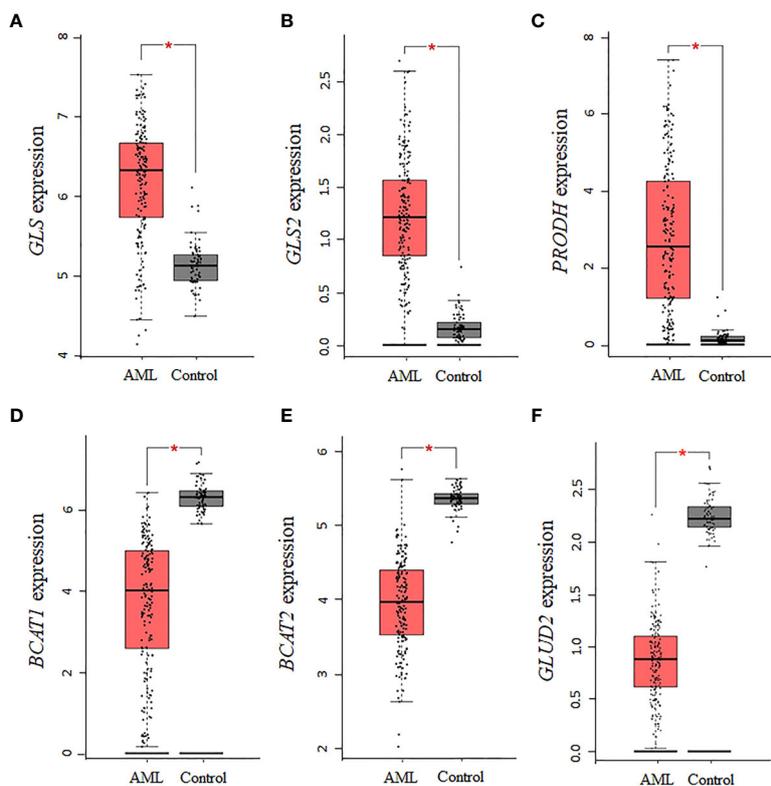


FIGURE 2 | Several enzymes involved in the biosynthesis of glutamate are dysregulated in AML. The expression levels of genes involved in the biosynthesis and metabolism of glutamate were assessed in AML TCGA samples using GEPIA. The data indicate that the expression of (A) *GLS*, (B) *GLS2*, and (C) *PRODH* is upregulated, whereas the expression of (D) *BCAT1*, (E) *BCAT2*, and (F) *GLUD2* is downregulated. Log₂ (TPM + 1) was used for log-scale and P values ≤ 0.01 are denoted with an asterisk (*) and indicate significance ($n_{\text{AML}} = 173$; $n_{\text{control}} = 70$).

to be a dependence on glutamine and proline for sources of glutamate, many of the pathways involved in the biosynthesis of NEAA requiring glutamate as precursor were repressed.

The Expression of Enzymes Involved in the Biosynthesis of NEAA Requiring Aspartate or Serine as Precursors Is Decreased in AML

Aspartate is a precursor for the biosynthesis of asparagine and arginine, whereas serine is a precursor of cysteine and glycine. Of the seven collective enzymes involved in the biosynthesis of asparagine, arginine, cysteine, and glycine, we found that *ASNS*, *ASS1*, *ASL*, *CTH*, and *SHMT2* were all statistically downregulated in AML samples (Figures 4A–E), whereas *CBS* and *SHMT1* showed non-statistically significant downregulations relative to controls (data not shown). Altogether, our analysis using the AML samples available through TCGA shows that there is a consistent decrease in the expression of enzymes involved in the biosynthesis of NEAAs that are interconnected through glutamate. In contrast, for tyrosine, whose biosynthesis is not linked to glutamate, there was no statistical difference in the expression of *PAH* between AML and control samples.

In summary, using GEPIA and TCGA AML samples we identified statistically significant dysregulations in enzymes

involved in the biosynthesis of glutamate, aspartate, glutamine, proline, serine, asparagine, arginine, cysteine, and glycine. Therefore, future efforts should focus on validating these associations across various AML subtypes to best identify specific patient groups with potential NEAA metabolism vulnerabilities, which was not possible using GEPIA.

Gene Expression Analysis of NEAA Biosynthesis in CML

The Expression of Enzymes Involved in Regulating Glutamate, Aspartate, Alanine, Asparagine, and Cysteine Levels Is Upregulated in CML

CML samples were not available through the TCGA database; rather, we used the largest publicly available data set disaggregated by CML phase with gene expression for all 26 genes involved in the biosynthesis of NEAA (Gene Expression Omnibus, GEO accession no. GSE47927)₃. Unlike AML, we found few statistical associations for genes encoding enzymes involved in NEAA biosynthesis (Figure 5). Of the 26 genes encoding enzymes involved in the biosynthesis of NEAAs used for our AML analysis, only five statistically significant dysregulated genes were identified in the combined CML cohort. We identified a significant downregulation in *PRODH* expression in combined and chronic phase CML samples

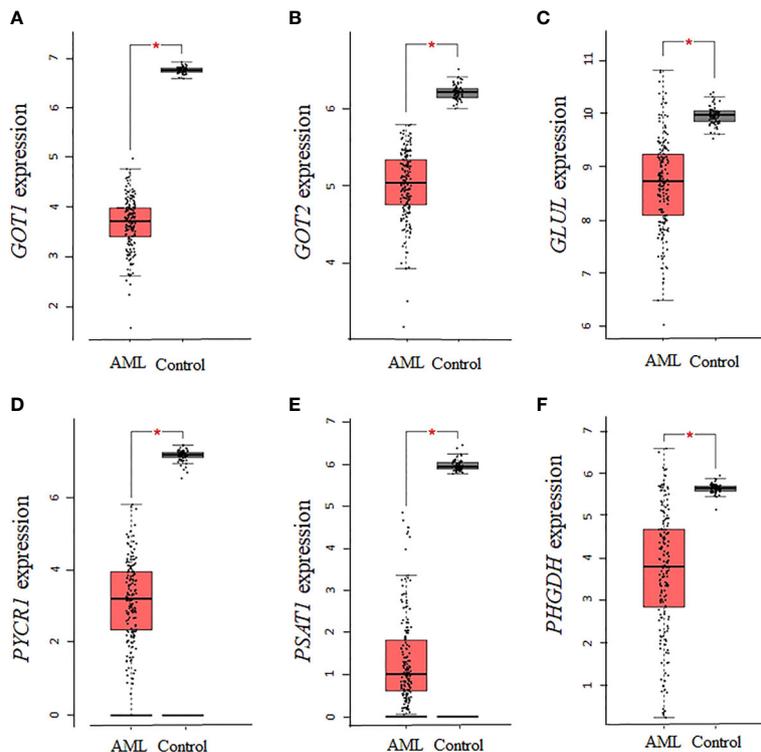


FIGURE 3 | The expression of genes involved in the biosynthesis of alanine, aspartate, glutamine, proline, and serine is decreased in AML. The expression of genes involved in the biosynthesis of alanine, aspartate, glutamine, proline, and serine was analyzed using TCGA AML samples. **(A)**, *GOT1*, **(B)** *GOT2*, **(C)** *GLUL*, **(D)** *PYCR1*, **(E)** *PSAT1*, and **(F)** *PHGDH* gene expression was significantly downregulated in AML. Log₂ (TPM + 1) was used for log-scale and P values ≤ 0.01 are denoted with an asterisk (*) and indicate significance (n_{AML} = 173; n_{control} = 70).

(**Figure 5A**, P_{Comb} = 4.6 × 10⁻², P_{Chronic} = 9.4 × 10⁻³), and significant upregulations in *AST1* (**Figure 5B**, P_{Comb} = 0.02, P_{Chronic} = 0.03), *GPT/ALT1* (**Figure 5C**, P_{Comb} = 0.03; P_{Chronic} = 0.04), *ASNS* (**Figure 5D**, P_{Comb} = 0.04; P_{Chronic} = 0.02), and *CTH*

(**Figure 5E**, P_{Comb} = 3 × 10⁻⁴; P_{Chronic} < 1 × 10⁻⁴) in the combined and chronic phase CML sample analysis. Of the genes that were identified as significant in the combined CML analysis, only *AST1* upregulation was significant in blast phase

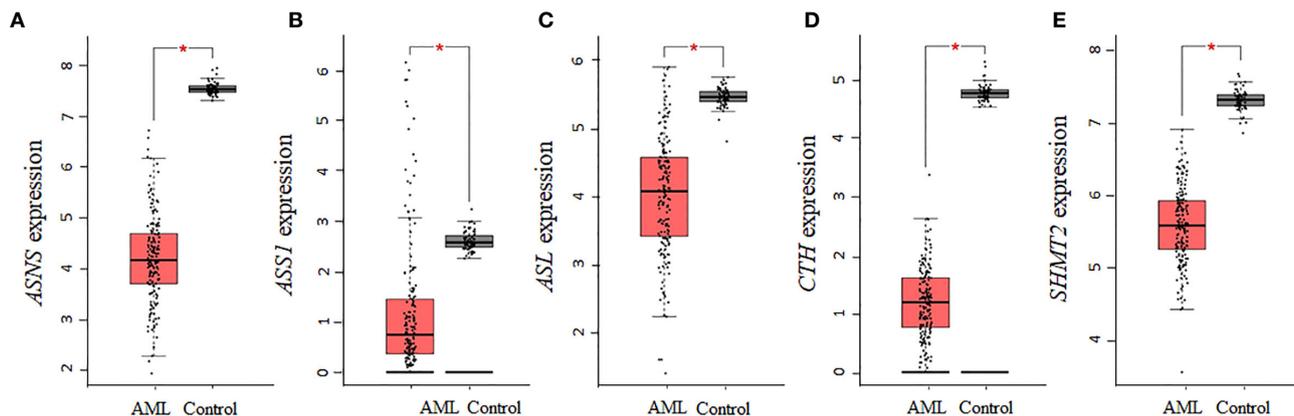
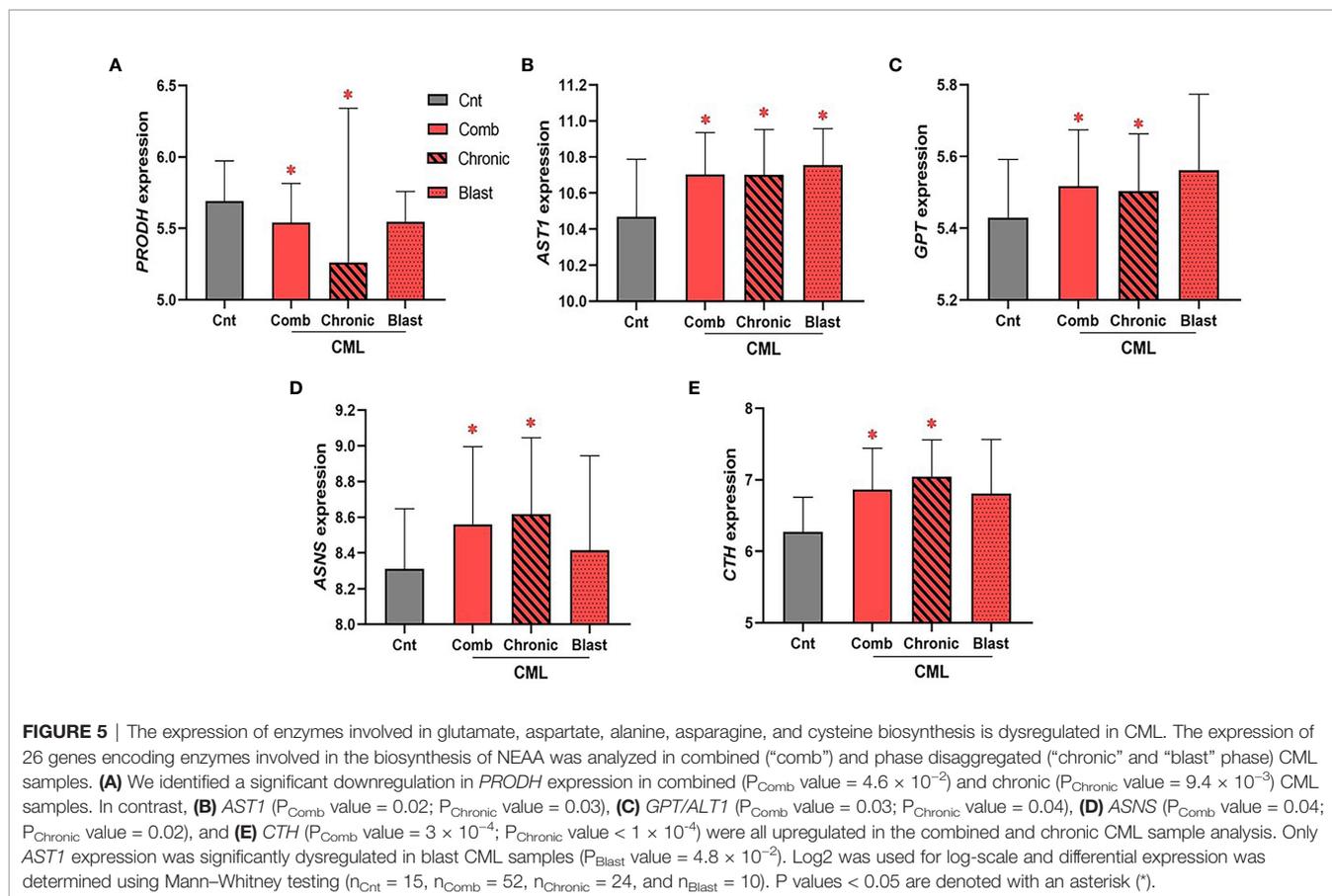


FIGURE 4 | The gene expression of enzymes involved in the biosynthesis of asparagine, arginine, cysteine, and glycine is decreased in AML. AML TCGA analysis of genes involved in the biosynthesis of asparagine and arginine identified a statistical downregulation in **(A)** *ASNS*, **(B)** *ASS1*, and **(C)** *ASL*. Analysis of genes involved in cysteine and glycine biosynthesis identified a statistical downregulation in **(D)** *CTH* and **(E)** *SHMT2*. Log₂ (TPM + 1) was used for log-scale and P values ≤ 0.01 are denoted with an asterisk (*) and indicate significance (n_{AML} = 173; n_{control} = 70).



CML samples (**Figure 5B**, $P_{\text{Blast}} = 4.8 \times 10^{-2}$). While no consistent similarities between AML and CML were identified, dysregulation of genes involved in sustaining glutamate (i.e., *PRODH*), aspartate (i.e., *AST1*), alanine (i.e., *GPT*), asparagine (i.e., *ASNS*), and cysteine (i.e., *CTH*) levels suggest potential vulnerabilities involving these amino acids.

EVIDENCE SUPPORTING NEAA VULNERABILITIES IN AML AND CML

Various factors can lead to genetic alterations in cancers that result in the downregulation of an enzyme involved in the biosynthesis of a NEAA, thus rendering the cancer cells dependent on extracellular sources of the NEAA for proliferation and growth. Based on the expression changes observed in AML and CML samples described in the previous section, significant NEAA biosynthesis pathways identified will be reviewed to identify high-priority NEAA vulnerabilities in AML and CML.

There Is Strong Clinical Evidence Supporting Vulnerabilities to Aspartate-Derived NEAAs in Leukemias

The best evidence supporting amino acid vulnerabilities for the treatment of leukemias is based on the efficacy of the

chemotherapeutic L-asparaginase (58–63), which is a bacteria-derived enzyme that hydrolyzes asparagine into aspartate and ammonia (64, 65). The enzyme also possesses off-target residual glutaminase activity (66, 67) and several studies have indicated that both its asparaginase and glutaminase activity are required for antileukemic efficacy depending on the expression of ASNS by the cancer (68–72). Generally, asparaginase may be beneficial for malignancies expressing low or no ASNS activity (68, 70, 73, 74), and several studies have investigated the use of asparaginase in non-hematologic malignancies, such as pancreatic, ovarian, and breast cancers (75–79). Furthermore, there is substantial clinical evidence that including asparaginase during the treatment of AML results in better treatment outcomes (80–87). While few studies in CML patients are available to assess the clinical efficacy of asparaginase (88–91), preclinical studies indicate that asparaginase may also be beneficial for CML treatment (92, 93).

Similar to asparaginase, many human cancers, such as melanoma, lymphoma, glioma, and prostate cancer, have low or no detectable expression of ASS1 (94–97). Therefore, several approaches have been investigated for depleting arginine in various cancer types and have led to the development of two pharmacological agents: arginine deiminase derived from bacteria (98–100) and human arginase 1 (101–110). Both are currently undergoing clinical trials (NCT03449901, NCT04587830, NCT02709512 and NCT03455140) and

encouraging clinical efficacy has been reported (107, 110–116), including for AML (112, 117).

In our analysis, enzymes involved in the biosynthesis of aspartate (i.e., AST/GOT) were downregulated in AML (Figures 3A, B), where aspartate serves as a precursor of asparagine and arginine (Figure 1). Interestingly, most human cells express low levels of the mitochondrial aspartate/glutamate SLC25A12 transporter, thereby depending on AST-dependent aspartate synthesis (118–120). Furthermore, levels of aspartate has been linked to cancer cell proliferation (118–122) and have been shown to provide a competitive growth advantage to cancer cells under hypoxia by contributing to the formation of oxaloacetate and enabling NADH recycling for glycolysis (119, 120, 123, 124). Aminoxyacetic acid (125, 126), hydrazinosuccinic acid (126–129), and iGOT1-01 (130) have been identified as AST inhibitors. While limited information is available regarding the potential clinical efficacy of targeting the aspartate biosynthesis pathway in cancer, *in vitro* studies using these inhibitors have demonstrated that they can decrease the proliferation of MDA-MB-231 breast adenocarcinoma cells (125), PaTu-8902 pancreatic cancer cells (125), PaTu-8902 pancreatic cancer cells (130), and DLD1 colon cancer cells (130).

Restriction of Glutamate or Glutamine Metabolism Via Inhibitors of Glutaminase Are Effective Against Myelodysplastic Syndrome

As previously mentioned, glutaminase (GLS) controls the formation of glutamate (Figure 1) and is used for various biosynthetic purposes by cells, in addition to NEAA biosynthesis, including to generate TCA cycle intermediates, glutathione (GSH), NADPH, nucleotides, and fatty acids (131). Therefore, successful strategies targeting the role of glutamate in cancers have focused on restricting glutamine metabolism *via* glutaminase inhibition (132). Studies investigating the non-competitive allosteric GLS1 inhibitor CB-839 have demonstrated that blocking glutamine metabolism has anticancer activity against triple-negative breast cancer, lung adenocarcinoma, chondrosarcoma, lymphomas, esophageal squamous cell carcinoma, and hepatocellular carcinoma (132–138). Currently, there are 12 ongoing clinical trials evaluating the CB-839 in patients with colorectal cancer (NCT02861300 and NCT03263429), myelodysplastic syndrome (NCT03047993), advanced stage non-small cell lung cancer (NCT04250545 and NCT03831932), diffuse astrocytoma (NCT03528642), ovarian cancer (NCT03944902), refractory multiple myeloma (NCT03798678), advanced or metastatic solid tumors (NCT03965845), metastatic renal cell carcinoma (NCT03428217), malignancies with NF1, KEAP1/NRF2, or STK11/LKB1 mutations (NCT03872427), and non-squamous non-small-cell lung cancer (NCT04265534). Additionally, recent interim results from a phase Ib clinical study of CB-839 in combination with azacitidine in patients with advanced myelodysplastic syndrome demonstrate the possible potential of targeting glutamate sources in leukemias, where the regimen was safely tolerated and 70% of MDS patients achieved a

complete response to therapy (139). In addition to CB-839, compound 968 (C968) is another small molecule inhibitor of GLS (140) that has been demonstrated to have anticancer activity in ovarian, brain, pancreatic, and breast cancer (140–142).

Restriction of Other NEAAs Derived From Glutamate Decrease Myeloid Leukemia Proliferation

The biosynthesis of serine and its derivatives were also identified as possible targets in AML and CML (Figures 3E, F and Figure 5E), and consistent with the gene expression analysis, substantial evidence supports that serine, cysteine, and glycine play important roles in cancers, including myeloid malignancies. One-carbon metabolism is required for the synthesis of proteins, lipids, and nucleic acids, with serine being the main source of one-carbon units for methylation reactions that occur through the generation of S-adenosylmethionine (SAM) (143). Glycine is also a major source of methyl groups for one-carbon pools and is required for the biosynthesis of GSH and purines (51), whereas cysteine contributes to redox control and ATP production as a carbon source for biomass and energy production (144). Furthermore, restriction of serine (145–148), glycine (145, 148, 149), or cysteine (150) can decrease cancer cell proliferation. Strategies suppressing PHGDH, SHMT, or cysteine levels *via* the enzyme cyst(e)inase have demonstrated encouraging results. For AML, there is evidence that serine restriction or PHGDH inhibition can attenuate cell proliferation and that *PHGDH* expression is prognostic of AML overall survival (151). In addition, cysteine has been demonstrated to be critical for the survival of leukemic stem cells (LSCs) in AML patients, whereas glycine deprivation has been demonstrated to suppress AML cell proliferation (152). For CML, similar to our analysis, several studies have shown that levels of glutamate, serine, which is a precursor of cysteine, and alanine are increased by CML (153–155). Consistent with these NEAAs playing an important role in CML, restriction of serine (156, 157) and its derivatives glycine (156), or cysteine (158, 159) decreases CML proliferation, supporting that targeting these NEAA pathways may be a potential target for CML.

In addition to serine, glycine, and cysteine, several studies support that inhibiting proline biosynthesis in melanoma and breast cancers can impede cell growth (160–162). Furthermore, PYCR1 inhibitors with anticancer activity have been identified to explore the role of proline biosynthesis in AML (163, 164). Nevertheless, limited information is available regarding the effect of inhibiting either enzymes involved in proline biosynthesis or directly restricting proline in AML. Rather AML studies available have indicated that proline uptake is elevated in LSCs isolated from *de novo* AML patients (165), and that the proline metabolism pathway is significantly impacted by differences in the oncogenic receptor tyrosine kinase FLT3 status of pediatric AML samples (166) or in AML cells overexpressing the proto-oncogene *EVII* (167).

Alanine is secreted by skeletal muscles and provides the carbon source for hepatic gluconeogenesis (168, 169), yet limited information is available about the role of alanine metabolism in myeloid leukemias. Nevertheless, evidence

supports that alanine provides a source of α -ketoglutarate for pancreatic and breast cancers that can be used to fuel the TCA cycle (170, 171) or remodel the extracellular matrix for metastasis (172). Additionally, KRAS mutations have been demonstrated to induce the expression of ALT2/GPT2 and drive α -ketoglutarate production and cell growth (173). Few studies have investigated pharmacological strategies for targeting alanine metabolism, yet l-cycloserine has been identified as an inhibitor of ALT/GPT that can attenuate the *in vitro* and *in vivo* growth of LLC1 Lewis lung carcinoma cells (173). Taken together, there is substantial evidence that the NEAA biosynthesis pathways dysregulated in AML and CML are promising targets for myeloid leukemias.

GCN2 AND MTOR PATHWAYS CAN MODULATE RESISTANCE TO STRATEGIES TARGETING NEAA VULNERABILITIES

Strategies targeting NEAA biosynthesis vulnerabilities can be a promising approach for myeloid malignancies. However, cancer cell adaptation to nutrient stress can lead to drug resistance or treatment failure (174). This adaptation can be due to upregulation of amino acid transporters and/or cell changes leading to increased availability of NEAA pools (8, 174, 175). Therefore, understanding the mechanism by which myeloid leukemias adapt and sense NEAA restriction is important for optimizing therapeutic approaches targeting NEAA metabolic vulnerabilities. Two essential kinases that are involved in the adaptation to nutrient stress are mammalian target of rapamycin (mTOR) and general control nonderepressible-2 kinase (GCN2). Collectively, these pathways can decrease the cellular demand for amino acids while concurrently increasing their synthesis to overcome the nutrient stress and lead to resistance (Figure 6).

Activation of the Amino Acid Response Pathway (AAR) Can Lead to Nutrient Stress Resistance

The GCN2 kinase activates the amino acid response (AAR) pathway by sensing amino acid insufficiency *via* direct binding to uncharged tRNAs that accumulate during nutrient stress (176). Upon activation *via* autophosphorylation, GCN2 phosphorylates its only known substrate, the α subunit of the translation eukaryotic initiation factor (eIF2) (177). Phosphorylation of eIF2 α has two essential consequences that can affect cancer cell responses to amino acid deprivation (Figure 6). First, it attenuates the general protein translation of most mRNAs by blocking the activity of the eIF2B guanylate exchange factor, thereby limiting the availability of ternary complex required for initiating translation (178–180). Second, it activates the AAR pathway by concomitantly increasing the translation of a subset of mRNAs with upstream ORFs that regulate translation of the downstream main ORF (179, 181–183). The AAR pathway leads to the upregulation of amino acid

transporters, aminoacyl-tRNA synthetases, enzymes involved in the biosynthesis of amino acids, and proteins involved in autophagy (29, 184–186).

In contrast to GCN2, which directly senses amino acid depletion, mTORC1 indirectly senses amino acid sufficiency through mechanisms that were recently elucidated (187–190). mTORC1 is a serine/threonine protein kinase that acts as an essential regulator of cell growth and metabolism in response to nutrient changes. SLC38A9, Sestrin1/2, and CASTOR1 have been identified as amino acid sensors upstream of mTORC1 that sense the availability of arginine and leucine (187–191). SLC38A9 stimulates mTORC1 activity through the regulation of Rag proteins, whereas Sestrin1/2 and CASTOR1 dissociate from GATOR2, which is a positive regulator of mTORC1, in the presence of leucine or arginine. Activated mTORC1 phosphorylates 4EBP1 and leads to the dissociation of 4EBP1 from EIF4E, enabling the formation of the translation initiation complex. In contrast, amino acid deficiency inactivates mTORC1 and suppresses 4EBP1 phosphorylation and protein synthesis (192–195). In addition to translation, mTORC1 is a negative regulator of autophagy, which is an intracellular process that allows orderly degradation and recycling of cellular components, including amino acids. Autophagy is regulated by mTORC1 through its phosphorylation of UNC-51-like kinase 1 (ULK) (196–200). Therefore, mTORC1 signaling plays a role in AA deprivation adaptation by limiting protein translation and increasing amino acid pools through autophagy (Figure 6).

Interestingly, there is substantial evidence supporting cross-talk between the GCN2/eIF2 α pathway and mTORC1 signaling. Activating transcription factor 4 (ATF4) is induced by phosphorylation of eIF2 α and several of its targets are mTORC1 inhibitors, including REDD1, GADD34, and Sestrin2 (201–203). Furthermore, autophagy regulation can be directly affected by ATF4 *via* the upregulation of many autophagy genes, including ATG16L, ATG12, ATG3, BECN1, LC3B, and p62 (185). Several studies have also demonstrated that inhibiting the GCN2/eIF2 α pathway during amino acid deprivation prevents mTORC1 inactivation (204–206), whereas mTORC1 inhibition by rapamycin has been demonstrated to result in activation of the GCN2/eIF2 α pathway (207).

Because mTORC1 and the GCN2/eIF2 α pathway provide mechanisms of resistance to amino acid deprivation strategies, it is conceivable that blocking these adaptation pathways would further sensitize cancer cells to the nutrient stress. Consistent with that hypothesis, the development of a GCN2 inhibitor has been shown to enhance the antileukemic efficacy of L-asparaginase (208). Other studies have demonstrated similar adaptations and GCN2-dependent sensitizations (174, 209–215). While constitutive activation of mTORC1 can lead to resistance against targeted therapies (216), in the context of amino acid vulnerabilities, blocking the generation of amino acids from autophagic protein degradation can sensitize cancer cells to amino acid deprivation approaches (217). Consistent with this concept, blocking autophagy during asparagine (93, 218–222), arginine (106, 108, 223–229), and glutamine (230) restriction leads to enhanced efficacy.

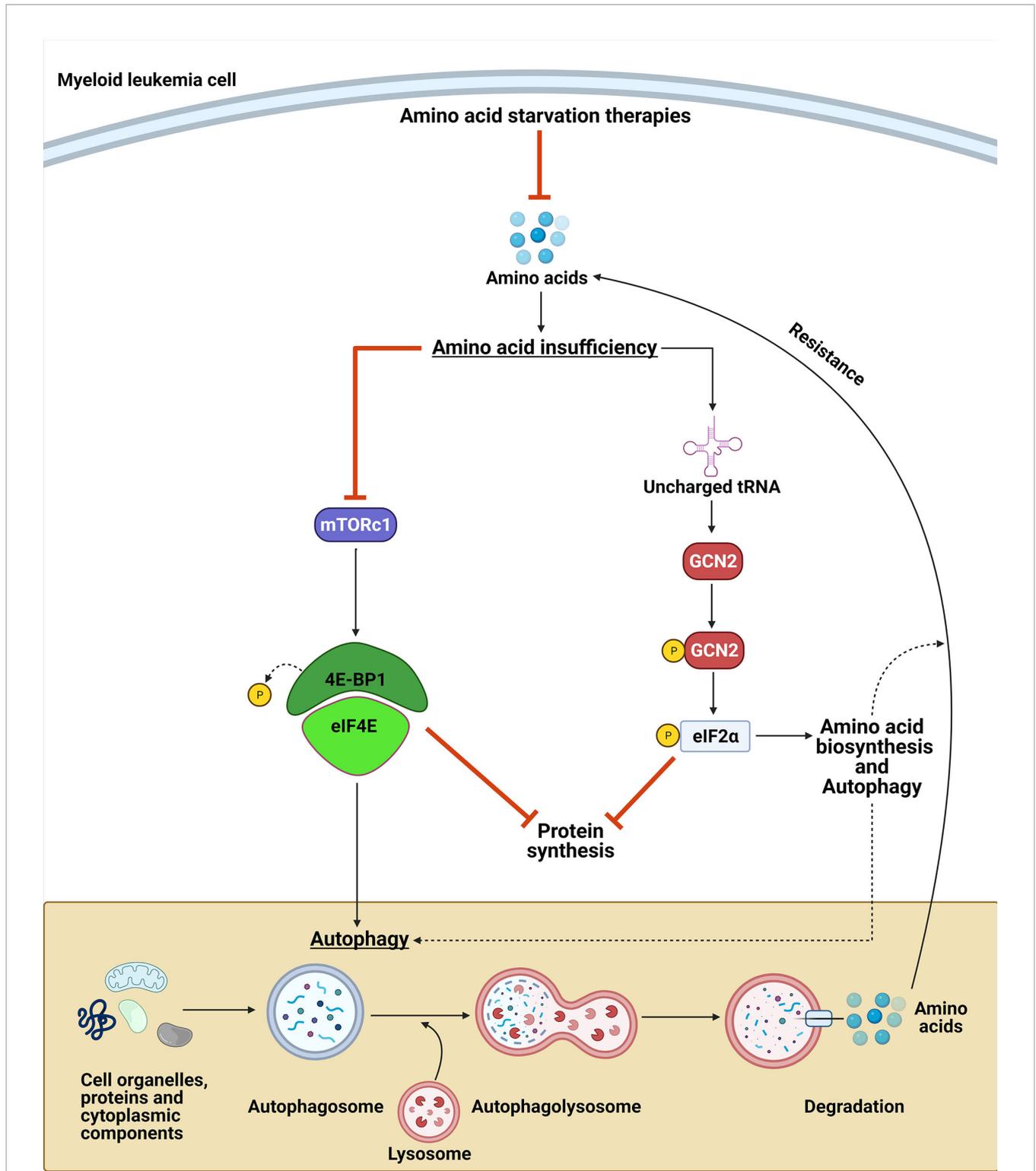


FIGURE 6 | GCN2 and mTORC1 contribute to amino acid deprivation resistance. Upon amino acid deprivation, the accumulation of uncharged tRNA leads to the activation of the GCN2 kinase, which phosphorylates eIF2 α . GCN2 activation can lead to a resistance to the amino acid restriction by inhibiting protein synthesis and by upregulating the translation of amino acid transporters, enzymes involved in amino acid biosynthesis, and proteins involved in autophagy. In contrast, amino acid suppression leads to inactivation of mTORC1, which induces autophagy and leads to a decrease in protein synthesis. Collectively, these mechanisms provide amino acid sources to maintain myeloid leukemia cell proliferation upon NEAA deprivation.

CONCLUSIONS AND FUTURE PERSPECTIVES

Cancers including myeloid leukemias use various energy sources to maintain their abnormal proliferation rate and therefore can become auxotrophic for particular NEAAs. Identifying these vulnerabilities in myeloid leukemias can therefore lead to new treatment options and improve survival rates. To date, L-asparaginase is the most successful clinical example of exploiting cancer nutrient dependencies. While outside of the scope of this review, there are several fuel sources aside from NEAA not considered here that contribute to metabolic vulnerabilities, including glucose, essential amino acids, fatty acids, lactate, and acetate (8–11). Furthermore, additional factors, including the expression of amino acid transporters (231–236) and the effect of stromal cells on the microenvironment, can contribute to cancer metabolism and proliferation (8). It is also clear that optimal treatment strategies will consider mechanisms of cell adaptation to nutrient stress. Our AML analysis identified that the biosynthesis of glutamate, aspartate, glutamine, proline, serine, asparagine, arginine, cysteine, and glycine are altered in TCGA samples, indicating that targeting multiple NEAAs may have a greater effect than a single agent approach. It is feasible that a combination approach to NEAA metabolic vulnerabilities may require lower drug exposures versus a single agent approach, and therefore can lead to better antileukemic efficacy while decreasing the risk of toxicities. Successful novel regimens with reduced risks of toxicities can lead to the development of new first-line therapy

options for myeloid leukemia patients with limited treatment options.

FOOTNOTES

1. SEER*Explorer: An interactive website for SEER cancer statistics. Surveillance Research Program, National Cancer Institute. Available from <https://seer.cancer.gov/explorer/>. [Cited 2021 April 1]
2. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE47927>

AUTHOR CONTRIBUTIONS

CF and AB performed all data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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GLOSSARY

AA	Amino acid
AAR	Amino acid response
ALT1/GPT	Cytosolic alanine aminotransaminase 1/Glutamic pyruvic transaminase
ALT2/GPT2	Mitochondrial alanine transaminase 2/Glutamic pyruvic transaminase 2
ASL	Argininosuccinate lyase
ASNS	Asparagine synthetase
ASS1	Argininosuccinate synthase 1
AST1/GOT1	Aspartate Aminotransferase/glutamic-oxaloacetic transaminase 1
AST2/GOT2	Aspartate Aminotransferase/glutamic-oxaloacetic transaminase 2
ATF4	Activating transcription factor 4
BCAA	Branch-chain amino acid
BCAT1/2	Branched chain amino acid transaminase ½
CBS	Cystathionine beta-synthase
CML	Chronic myeloid leukemia
CTH	Cystathionine gamma-lyase
eIF2 α	Eukaryotic translation initiation factor 2 alpha
GCN2	General control nonderepressible-2
GLS/2	Glutaminase/2
GLUD1/2	Glutamate dehydrogenase ½
GS/GLUL	Glutamine synthetase
GSH	Glutathione
HCT	Hematopoietic cell transplantations
HSC	Hematopoietic stem cell
LSC	Leukemic stem cell
mTOR	Mammalian target of rapamycin
NCCN	National Comprehensive Cancer Network
NEAA	Non-essential amino acid
OxPhos	Oxidative phosphorylation
3-PG	3-Phosphoglycerate
3-PHP	3-phosphohydroxypyruvate
3-PS	3-Phosphoserine
P5CDH/ ALDH4A1	Pyrroline-5-carboxylate dehydrogenase/Aldehyde dehydrogenase 4 family member A1
P5CS/ ALDH18A1	Pyrroline-5-carboxylate synthase/Aldehyde dehydrogenase 18 family member A1
PAH	Phenylalanine hydroxylase
PHGDH	Phosphoglycerate dehydrogenase
PRODH	Proline dehydrogenase 1
PSAT1	Phosphoserine aminotransferase 1
PSPH	Phosphoserine phosphatase
PYCR1	Pyrroline-5-carboxylate reductase 1
SAM	S-Adenosylmethionine
SHMT1/2	Serine hydroxymethyltransferase ½
ULK	UNC-51-like kinase.