



Narrative review of targetable features of pediatric acute myeloid leukemia from molecular and metabolic perspectives

Adriana Milagros Trabal, Joya Chandra

Division of Pediatric Oncology and Patient Care, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

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Correspondence to: Adriana Milagros Trabal, MD. Division of Pediatric Oncology and Patient Care, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd. Houston, TX 77030, USA. Email: AMTrabal@mdanderson.org.

Abstract: Acute myeloid leukemia is uncommon in the pediatric population. Unlike the majority of pediatric leukemia patients, young patients with acute myeloid leukemia have not seen significant improvement in treatment outcomes. In both the adult and pediatric population, few novel treatments have developed in the past decades. For this reason, patients continue to have poor outcomes with overall survival well below pediatric patients with acute lymphoblastic leukemia. This is frequently attributed to the heterogeneity of this malignancy in terms of mutations and molecularly defined categories. Instead, broader cellular features of acute myeloid leukemia, such as altered metabolic and anti-apoptotic properties have been considered in designing new treatment strategies. Therefore, further study of cytogenetics, metabolism, and molecular properties have become increasingly important in the development and tailoring of new therapeutic options. Although some of the characteristics of adult-onset myeloid leukemia resemble those of the pediatric population, their differences should be further characterized in order to individualize treatment and improve outcome. Here we will highlight unique features of pediatric acute myeloid leukemia biology and potential ties to metabolism, as well as recent advances in therapy.

Keywords: Acute myeloid leukemia; cytogenetics; pediatric acute myeloid leukemia; treatment of acute myeloid leukemia

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Introduction

Malignancy develops in the bone marrow where hematopoietic stem cells should develop into active blood cells. There the progenitor cell differentiates into the common lymphoid progenitor and common myeloid progenitor cells. The myeloid progenitor is responsible for the development of erythrocytes, platelets, mast cells, eosinophils, basophils, macrophages, and neutrophils. This occurs as a result of several biological processes including signal transductions, transcription factors, growth factors, and DNA or RNA synthesis (1). If genetic mutations develop during this process it may lead to unregulated

cell growth and halting of differentiation. When the accumulation of myeloid blasts reaches 20% or more in the bone marrow, the diagnosis of acute myeloid leukemia (AML) can be made and treatment should begin as soon as possible. AML is responsible for around 15% of leukemia in childhood and increases in frequency to ~80% in adults (2). Although there have been significant advancements in the treatment of acute lymphocytic leukemia (ALL), the prognosis of AML remains unfavorable with overall survival at 65% decreasing more so to around 12.5% in patients over 65 years of age (3). There is dire need for optimization of therapy.

Since the development of genetic-sequencing,

cytogenetics, and molecular subgroups, they have become more essential in the management of AML (4). At diagnosis, the cytogenetic and molecular properties identified are key to influence treatment as well as prognosis. It is noted that in children, mutation frequency is low and more commonly includes fusion and copy number aberrations as compared to higher level of mutations in adults (4). This may lead to differences in efficacy of therapeutic targets and therefore their gene products and effects on metabolism should be explored closely. For this reason, we will further detail the importance of metabolism, molecular features, current treatment modalities, and how they may lead to innovative therapies.

We present the following article in accordance with the Narrative Review reporting checklist (available at <http://dx.doi.org/10.21037/pm-20-98>).

Methods

PubMed search was done for literature in English including years 2000–2020. Keywords included acute myeloid leukemia, cytogenetics, pediatric acute myeloid leukemia, treatment of acute myeloid leukemia. Search of personal library for AML was done. Relevant articles were included in this review.

Molecular features in pediatric and adult AML

The workup at diagnosis of AML, regardless of age, includes determining the cytogenetics of the leukemic cells to guide therapy and, if possible, individualize treatment tailored to the mutations found.

Fortunately, the most common pediatric AML cytogenetics have a favorable prognosis. These include t(8;21), t(16;16), t(15;17), and 11q23/*MLL* rearrangements (5). The gene products of these mutations lead to altered replication and metabolism in malignant cells as detailed below.

T(8;21) translocation leads to the formation of *RUNX1-RUNX1T1* gene. This leads to transcriptional repressors [histone deacetylase (HDAC), SMRT, N-CoR, and mSin3] being recruited by RUNX1T1 and blocking RUNX1 target genes who are in charge of differentiation (6). It also causes alterations in replication for proliferation of leukemic cells. For example, there is over-expression of hypoxia-inducible factor 1-alpha (HIF1-alpha) which increases DNMT3a for DNA hypermethylation and upregulation of p21, an ATPase which leads to cell cycle progression. Other functions such as FOXO1 transcription factor activation

and overexpression of anti-apoptotic B-cell lymphoma 2 (Bcl-2) and Bcl-XL proteins have also been attributed to the *RUNX1-RUNX1T1* mutated cells (6). Inversion 16, on the other hand, is responsible for the *CBFB-MYH11* fusion gene which causes sequestration of CBFA2 (a transcription factor involved in the expression of hematopoietic genes) in the cytoplasm blocking differentiation of hematopoietic cells (7). Another important chromosomal alteration is t(15;17) which forms the PML/RARA fusion protein, most common in the APL subtype. Acute promyelocytic leukemia (APL) is rare in children varying from 5% to 10% of AML cases and slightly more common in 10–15% of adult AML cases in the United States (8,9). It creates an abnormal retinoid receptor which blocks retinoid-induced myeloid differentiation (10). As a result, it was found that all-trans-retinoic acid administration can bypass this blockage and improve outcome in these patients.

Some mutations conferring and adverse prognosis are similar in adults and pediatrics. FLT3, for example, is common to both. FLT3 is a transmembrane tyrosine kinase inhibitor (TKI) which, with internal tandem duplications, results in constant activation hence stimulating cell proliferation (11). Wilms' tumor 1 gene (*WT1*) is a transcriptional regulator that can both activate or repress gene transcription (12). Although not as well proven to confer an adverse prognosis on its own, it may when in combination with FLT3 mutation in pediatric patients. Although some adverse cytogenetics between the pediatric and adult population may be the same their incidence may differ. For example, in adults monosomies are known to cause an adverse prognosis, however, these, although may happen, are extremely rare in the pediatric population (5). The differences in cytogenetics between adult and pediatric AML are further detailed in *Table 1*.

AML metabolism

Leukemia is a result of increased proliferation of undifferentiated cells that leads to the accumulation of non-functional hematopoietic cells in the bone marrow. It develops as a result of genetic mutations, cytogenetic abnormalities, and/or chromosomal translocations in progenitor cells causing clonal proliferation of cells. These molecular abnormalities lead to derangements in cell metabolism in order to assist malignant cells with rapid growth. To increase proliferation of leukemic cells, they alter the regular processes of cell growth and survival. Malignant cells modify to bypass checkpoints, avoid

Table 1 Pediatric (5) and adult (13) cytogenetics

Adverse
Pediatric
–7 or del(7q)
–5 or del(5q)
t(6;11)(q27;q23)/MLL-MLLT4(AF6)
t(10;11)(p12;q23)/MLLMLLT10(AF10)
t(7;12)(q36;p13)/ETV6(TEL)-HLXB9(MNX1)
t(6;9)(p23;q34)/DEK-NUP214
t(5;11)(q35;p15.5)/NUP98-NSD1
inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/RPN1-MECOM(EVI1-MDS1-EAP)
t(9;22)(q34;q11.2)
WT1mut/FLT3-ITD
t(4;11)(q21;q23)/MLL-MLLT2(AF4)
CBF-AML t(15;17)(q22;21)
Adult
add(7q), del(7q), –7
add(5q), del(5q), –5
t(6;11)(q27;q23)
t(10;11)(p11–13;q23)
abn (3q) [excluding t(3;5), (q21–25;q31–35)]
abn(7p), –17
t(11q23)[excluding t(9;11), (p21–22;q23) and t(11;19)(q23;p13)]
inv(3)(q21q26)/t(3;3)(q21;q26)
t(9;22)
complex (≥4 unrelated abnormalities)
Favorable
Pediatric
t(8;21)(q22;q22)/RUNX1-RUNX1T1
inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB-MYH11
t(15;17)(q22;q21)/PML-RARA
NPM1-mutated
CEBPA double mutation
t(1;11)(q21;q23)/MLL-MLLT11(AF1Q)
Adult
t(8;21)(q22;q22)
inv(16)(p13q22)/t(16;16)(p13q22)
t(15;17)(q22;q21)

oxidative stress, adjust their microenvironment, and prevent apoptosis (14).

Metabolism has been more deeply studied in adult AML, although it has yet to be well defined in pediatric AML. In adults, the advances in the study of genetics have led to the characterization of AML genomics. These have shed light on the effects of the translocations and oncogenic mutations on metabolism (15). Several cell processes have found to be altered such as glycolysis, the tricarboxylic acid (TCA) cycle, oxidative phosphorylation in the mitochondria, as well as amino acid and fatty acid metabolism (15). One of the hallmarks is the development of increased glycolysis. There is upregulation of this process and lactate production as cancer cells strive for survival in a hypoxic microenvironment (16). The TCA cycle is also modified to fit the needs of blast cells. Glutamine is transformed into glutamate which is transformed into alpha-ketoglutarate. This permits cells to maintain mitochondrial metabolism despite decreased pyruvate which is being used for glycolysis (16). There is also an increase in amino acids, such as phenylalanine and tyrosine, for degradation to maintain an active TCA cycle (17). On the other hand, there is a decrease in fatty acids levels due to upregulated lipogenesis and metabolism. The specific difference between myeloid cell metabolism in pediatric *vs.* adult patients has been understudied and requires more clarification in future studies.

The FLT3/ITD mutation, well known for conferring a poor prognosis, is present in 20–35% of adult AML patients and less commonly in around 15% of pediatric patients (18). FMS-like tyrosine kinase 3 or FLT3 is normally a transmembrane receptor tyrosine kinase in stem cells that signals cell survival and differentiation. However, its mutation leads to consistent propagation in leukemia myeloid cells (11). It upregulates hexo-kinase 2 which leads to enhancement of glycolysis and the Warburg effect (17). Bassal *et al.* also describe altered metabolism secondary to mutations in isocitrate dehydrogenase 1 and 2 (*IDH1*, *IDH2*) genes, enzymes that are part of the TCA cycle (19). These are found mutated in 5–20% of adult patients and less so in 3.5% of pediatric AML patients (20,21). Mutations in these lead to the formation of 2-hydroxyglutarate and DNA hypermethylation (15). Hence, *IDH1* and *IDH2* inhibitors such as ivosidenib and enasidenib are being incorporated in some patients with these mutations.

Oxidation-reduction (redox) also plays a part in the survival of leukemic cells. Healthy cells normally have a

balance between reactive oxygen species (ROS) and anti-oxidant enzymes for survival. ROS can promote proliferation with low levels but induce DNA damage and limit proliferation with increasing levels (22). Although leukemic cells tend to have an elevated ROS, they use the DNA damage as a mechanism to alter genes to continue to grow or acquire drug resistance. FLT3-ITD, for instance, has been shown to have increased ROS in AML cell lines (22). Relapsed AML patients have also been shown to have an increase in xanthine oxidase which contributes to increased ROS as well. On the other hand, they can also alter anti-oxidant capabilities. They may alter the activity and production of antioxidant proteins to upregulate the formation of ROS or increase antioxidant activity. Nrf2, for example, is a transcription factor which causes transcriptional activation of antioxidants. *In vitro* it has been shown that AML cell lines will upregulate Nrf2 expression when under stress (22). Catalase enzymes, also confer resistance to ROS damage and have been found to be upregulated in AML (22). Similarly, Bcl-2 is an anti-apoptotic protein that can induce antioxidant proteins inhibiting ROS and promoting cell survival. Yusuf *et al.* recently published how myeloid leukemia cells, in contrast from healthy cells, expressed dependency on the enzyme aldehyde dehydrogenase 3a2 (23). This enzyme is responsible for oxidizing long-chain aliphatic aldehydes preventing cellular damage and therefore preventing cell death. The inhibition of this enzyme in their study led to oxidative cell death and could present a potential therapeutic target (23).

As previously mentioned, AML has a high relapse rate. It is thought to be secondary to leukemic stem cells (LSC) in the bone marrow which are more resistant to chemotherapy and lead to re-generation of blast cells after the patient had achieved remission. Therefore, therapeutic interventions should include targets that are specific to the LSC and not present on healthy stem cells. Raffel *et al.* aimed to further study *in vitro* metabolic features and proteins limited to LSC population as potential targets. They found proteins specific to LSC from adult AML blood samples whose presence led to altered metabolism (24). MBOAT7, a phospholipid-remodeling enzyme, was upregulated in LSC as well as cysteine rich protein 2 (CRIP2) which was the most highly expressed. Upregulation of CRIP2 is responsible for increasing oxidative phosphorylation and affecting glycolysis (24). Its knockdown has been shown to lead to increased glycolysis and repressed oxidative phosphorylation (25). CD36 and sesittrin-1 expression was

also higher in LSC which are responsible for enhancing oxidative phosphorylation and metabolic homeostasis respectively (24). As such, alterations in the leukemic cell metabolism and oxidative phosphorylation are significant as these changes may lead to potential targets for novel therapies.

AML front-line therapy has been based on cytotoxic chemotherapy with a portion of patients requiring stem cell transplant (SCT). However, the new discoveries of gene mutations and metabolism alterations have led to emerging treatment modalities.

AML front-line therapy

As in any malignancy, the goal of treatment in AML is curative with decreased toxicity or long-term secondary effects. Front-line treatment of AML has not been altered significantly in the recent years. In adults, it includes the combination of anthracycline and a nucleoside analogue as per the National Comprehensive Cancer Network (NCCN) guidelines. On the other hand, treatment of pediatric AML in the United States is largely dictated by the standard of care in Children's Oncology Group (COG) protocols. Nonetheless, therapy also consists of a combination of an anthracycline (Daunorubicin, Idarubicin or Mitoxantrone) and a nucleoside analogue (Cytarabine). Both guidelines typically include 7 days of Cytarabine and 3 days of an anthracycline. One to two courses of induction are administered followed by 2–5 courses of consolidation. After induction, complete remission is achieved slightly more often in pediatrics than in adults, with children achieving complete remission in 80–90% of times and adults 50–75% in *de-novo* AML (26,27). This is possibly due to differences in mutations or age-specific factors that affect response. Unlike in ALL, maintenance therapy in AML has not been proven to be efficacious. Consolidation chemotherapy normally includes the combination of drugs used in induction but with Cytarabine used in higher doses. After complete remission is achieved, there have been debates about the use of allogenic SCT as another form of consolidation. Those with favorable risk factors usually do not undergo transplant while its use in higher risk patients is debated (5). After first relapse, the use of SCT is favored. In adults, however, use of cytotoxic chemotherapy front-line is limited by its likelihood of tolerance in elderly patients.

As previously mentioned, anthracyclines are one of the components of front-line therapy. The one most

commonly used is Daunorubicin. Daunorubicin has both anti-mitotic and cytotoxic properties. It is metabolized by the liver to become daunorubicinol, its active metabolite. It works by intercalating between DNA base pairs inhibiting topoisomerase II and leading to DNA strand breaks thus interfering with replication (28).

Cytarabine has also long been used as part of the front-line therapy in AML. It is a nucleoside analogue that works by entering into the leukemic cell where it is metabolized into its active metabolite, arabinosylcytosine (ARA-C) triphosphate, which competes with deoxycytidine triphosphate to be incorporated into DNA or RNA and therefore, hinders its synthesis and attacks replicating cells (29). Despite the long-term use of this chemotherapeutic agent, AML continues with a high relapse rate. Farge *et al.* theorizes that one of the mechanisms of resistance to Cytarabine is an altered oxidative phosphorylation status in LSC (30). Their patient-derived xenograft mice models were treated with Cytarabine and resistant cells were examined and noted with higher ROS levels, increased mitochondrial mass, and higher mitochondrial oxygen consumption, consistent with a high oxidative phosphorylation status. With induction of a low oxidative phosphorylation status, those cells would become chemosensitive (30). Similarly, Buelow *et al.* showed that if OCTN1 (SLC22A4) expression, a transporter in AML blasts, had increased methylation it was associated with a poor response to Cytarabine due to decreased uptake among cell lines. *In vitro* use of hypomethylating agents increased its expression and therefore increased sensitivity to Cytarabine (31). This denotes the importance of cellular metabolism in drug resistance. These are only one of the ways leukemic cells alter to overcome resistance.

AML innovative therapy

Despite front-line therapy, AML patients have a high relapse rate in both adults and children. This has led to the need for the development of more and more novel therapeutic agents. As a result of genomic sequencing, molecular targeting has become a new treatment modality in AML. Several strategies have been implicated in new genetic therapy, such as targeting fusion oncogenes and their functional mutations, promoting cell death, attacking leukemic cell metabolism, and development of antibodies against clusters of differentiation in malignant cells.

Tyrosine kinase inhibitors

Fusion oncoproteins are notoriously responsible for AML resistance to treatment. Targeting fusion proteins may, therefore, lead to improvement in outcome. Tyrosine kinases are responsible for phosphorylation of tyrosine residues. This phosphorylation may lead to signal transductions responsible for cell differentiation, proliferation or death. Mutation or overexpression of tyrosine kinases receptors lead to constant activation resulting in leukemic cells (32). For instance, the FLT3 receptor is often mutated by an internal tandem duplication or point mutation at the tyrosine kinase domain causing sustained activation (33). Understanding of the role of tyrosine kinases led to the development of TKIs. The most recognized of which has been Imatinib, whose target is the tyrosine kinase protein, result of the *BCR-ABL1* fusion gene (34). This is used in the management of chronic myeloid leukemia and some types of ALL (33). In AML the use of FLT3 inhibitors has become more and more common. Midostaurin was the first FLT3 TKI to be FDA approved in adults with AML, after having shown improvement in overall and event-free survival. In pediatrics, a phase II study evaluating its use in *de novo* AML patients with FLT3 mutation is still ongoing (35). Sorafenib, another first generation FLT3 inhibitor, showed improvement in 3-year event-free survival and complete remission after induction I and II in COG protocol AAML1031 (36). Similarly, Quizartinib, a second generation FLT3 inhibitor, was developed and is therefore more selective. Cooper *et al.* recently published a phase I trial using Quizartinib in relapsed AML patients in the pediatric population. It had favorable toxicity and of the 7 patients with FLT3-ITD mutation most achieved complete remission with or without recovery of counts or had stable disease (37). A phase I/II study evaluating its safety and efficacy in the pediatric population is ongoing (NCT03793478). Gilteritinib, another second generation FLT3 inhibitor, has shown efficacy in the adult population. Recently, a phase I/II study investigating its use in relapsed or refractory pediatric patients with FLT3-ITD mutation has started recruiting patients (NCT04240002). Others, that vary in selectivity, include Sunitinib, Lestaurtinib, and Tandutinib (4).

BCL-2 inhibitors

Other emerging therapies promote apoptosis, given malignant cells' ability to avoid programmed cell death. The

Bcl-2 (B-cell CLL/Lymphoma 2) family is an essential part of this process. Pro-apoptotic proteins in this family such as BAX (Bcl-2 associated X protein) and BAK (Bcl-2 antagonist killer 1) cause permeabilization of the mitochondrial membrane through creation of the proteolipid pore, which in turn leads to the release of caspase-activating proteins and apoptosis (38). On the other hand, anti-apoptotic proteins such as Bcl-2, Bcl-XL, Bcl-W, and myeloid cell leukemia 1 (MCL-1), usually located within the outer membrane of the mitochondria, restrain the pro-apoptotic proteins BAX and BAK. Overexpression of these anti-apoptotic proteins lead to malignancy and may lead to resistance to chemotherapy. Interaction between the pro-apoptotic molecule and BH3 promotes apoptosis. Therefore, targeted medications that mimic BH3 can lead to apoptosis of leukemic cells (4). Recent medications with this mechanism of action include ABT-199 or Venetoclax. ABT-737, another BH3 mimetic, targets Bcl-2, Bcl-XL, and BCL-W. There has also been published data that suggests that Bcl-2 inhibition by this drug might also lead to alteration in cellular metabolism by inducing glutathione depletion and increasing ROS which promotes apoptosis (39). There is little to no scientific evidence on the difference between Bcl-2 expression in childhood AML or its effect on their cellular metabolism. Nonetheless, the efficacy of Bcl-2 targeted medications in adults has led to innovative trials investigating their use in the pediatric population. Current trials available are evaluating the safety and efficacy of Bcl-2 inhibitors in combination chemotherapy in the pediatric population (Table 2).

Targeting cell surface molecules

Therapeutic agents have been developed against surface molecules. CD33 is a myeloid differentiation surface antigen that is frequently expressed on AML blasts. It has endocytic properties by which it internalizes antigens when it is antibody-bound (40). AML cells with adverse cytogenetics such as those expressing FLT3-ITD⁺, NPM1⁺, and CEBPA⁺ have been linked to higher CD33 expression, making it a significant target (40). Antibodies are hence created against these cell surface receptors or antigens on leukemia cells. A well-known antibody is Gemtuzumab, a humanized murine IgG4 antibody who targets CD33. It binds CD33 on surface cells and is internalized, delivering the drug inside the cell. *In vitro*, it leads to cell-cycle arrest and mitochondrial-induced apoptosis (41). It was initially approved in adults where it showed improvement in 2-year event-free

survival, overall survival, and relapse-free survival (41). Later, COG AAML0531 protocol also demonstrated improvement in 3-year event-free survival, relapse risk, and disease-free survival in pediatric patients (42).

Liposomal formulations

More recently, in 2017, we saw the approval of CPX-351 in adults. CPX-351 is an encapsulated liposomal formulation of Cytarabine and Daunorubicin at a 5:1 ratio (43). A phase II trial published by Lancet *et al.* showed improvement in event-free survival and overall survival in patients with secondary AML (myelodysplasia-related or therapy-related) in elderly patients 60 to 75 years old (44). Since then, multiple trials have emerged utilizing CPX-351 in combination chemotherapy in adults. In children, however, its safety and efficacy are still being investigated. A phase I study published by Absalon *et al.* in patients 1 to 21 years old showed it was well tolerated albeit with a small sample size (45). Three clinical trials in pediatric patients with AML are still ongoing (NCT04293562, NCT03568994, and NCT03826992).

Chimeric-antigen receptor T-cells

In the age of immunotherapy and given the success of chimeric antigen receptor T cells (CAR-T) in ALL, CAR-T cells are being explored in the treatment of AML. The targets under investigation also include CD33 on myeloid cells as well as CD123, CD38, CD19, C-type lectin-like molecule 1 (CLL-1), and CD7. However, there are more limitations in the CAR-T cell therapy in AML, given that there is not a surface antigen unique to myeloid malignant cells and therefore targeting a surface antigen that is also present in non-malignant cells may cause significant myelosuppression (46). Additionally, the AML microenvironment interferes with the efficacy of CAR-T cells by several mechanisms including prevention of T-cell activation, increasing regulatory T cells to limit cytolytic T cell expansion, and inhibiting natural killer cell activity (47). As a result of these multiple negative interactions between the microenvironment and the CAR-T cells, their efficacy in AML has been limited. There are ongoing trials in both the adult and pediatric population.

Immune checkpoint inhibitors

Immune checkpoint inhibitor development has also been

Table 2 Current trials with Venetoclax in the pediatric population

Trials	Identifier	Age range
Study of Venetoclax in Combination with Chemotherapy in Pediatric Patients with Refractory or Relapsed Acute Myeloid Leukemia or Acute Leukemia of Ambiguous Lineage	NCT03194932	2 to 20 years old
A Study Evaluating the Safety and Efficacy of Venetoclax in Combination with Azacitidine Versus Standard of Care After Allogeneic Stem Cell Transplantation (SCT) in Participants with Acute Myeloid Leukemia (AML) (VIALE-T)	NCT04161885	12 to 20 years old
A Study of Effectiveness of Venetoclax in Combination with Azacitidine or Decitabine in an Outpatient Setting in Patients with Acute Myeloid Leukemia (AML) Ineligible for Intensive Chemotherapy	NCT03941964	12 years old and older
Venetoclax and Sequential Busulfan, Cladribine, and Fludarabine Phosphate Before Donor Stem Cell Transplant in Treating Patients with Acute Myeloid Leukemia (AML) or Myelodysplastic Syndrome	NCT02250937	2 to 70 years old
A Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Preliminary Activity of Idasanutlin in Combination with Either Chemotherapy or Venetoclax in the Treatment of Pediatric and Young Adult Participants with Relapsed/Refractory Acute Leukemias or Solid Tumors	NCT04029688	Up to 30 years old
Personalized Targeted Preparative Regimen Before T-depleted Allogeneic HSCT in Children with Chemoresistant Acute Leukemias (*not yet recruiting)	NCT04000698	Up to 27 years old
A Study of the Safety and Pharmacokinetics of Venetoclax in Pediatric and Young Adult Patients with Relapsed or Refractory Malignancies	NCT03236857	Up to 25 years old
An Extension Study of Venetoclax for Subjects who have Completed a Prior Venetoclax Trial (*recruitment by invitation)	NCT03844048	Child
Venetoclax combined with Vyxeos (CPX-351) For Participants with Relapsed or Refractory Acute Leukemia	NCT03826992	1 to 39 years old

increasing. Their significance stems from the knowledge that malignant cells have to escape immune cells in order to proliferate. In normal cells, T-cell activation depends on a balance between stimulatory and inhibitory signals. Inhibitory receptors like CTL-4 and PD-1 hinder T-cell activation and hence immune response leading to blast proliferation (48). As a result, antibodies against these receptors avoids blockage of the immune response. The first antibody developed against CTL-4 was Ipilimumab, initially used against melanoma in adults. However, the use of immune checkpoint inhibitors, including Ipilimumab, has extended to AML and other hematological malignancies. One of these, Nivolumab, is an anti-PD-1 receptor antibody that was also developed initially for melanoma in adults. However, PD-L1 (PD-1 receptor ligand) is also expressed in myeloid cells making it a potential target for AML. Its use, along with the use of other anti-PD-1 receptor antibodies such as Pembrolizumab, continue to be studied in AML in both the adult and pediatric population (48).

Oxidative phosphorylation inhibitors

As discussed previously, leukemic cells alter the cellular metabolism to promote proliferation. One of the ways it does that is by altering mitochondrial function. Mitochondria are the energy-house of the cell and are responsible for the production of ATP, NADH/NADPH, and ROS. Leukemic blasts upregulate the use of aerobic glycolysis and also modify oxidative phosphorylation. However, LSC, responsible for chemotherapy resistance and relapse, have been found to be more dependent on oxidative phosphorylation than glycolysis (49). This made oxidative phosphorylation a therapeutic target and led to development of IACS-010759. It is an inhibitor of complex I in the mitochondria of the electron transport chain. This make cells, like LSC, that are more reliant on oxidative phosphorylation susceptible to this drug (50). There is an active phase I clinical trial in relapsed/refractory AML patients in the adult population (NCT02882321). Atovaquone and Metformin are also being studied as

oxidative phosphorylation inhibitors in AML given pre-clinical data has shown efficacy.

Glutaminase inhibitors

Innovative therapies continue to arise against metabolic derangements in myeloid leukemic cells. Many cancer cells are dependent on glutamine. Glutamine is used for distinct purposes within the cell. It serves in signal transduction pathways, redox homeostasis, replenishes the TCA cycle, and produces glutathione (51,52). The enzyme glutaminase converts glutamine to glutamate. Therefore, glutaminase inhibition provides a therapeutic opportunity which has been studied in solid tumors and, more recently, in hematologic malignancies. *In vitro*, Matre *et al.* showed apoptosis induction of cells, cell growth suppression, and downstream decrease of glutaminase metabolites in several AML cell lines by CB-839 (Telaglenastat), a glutaminase inhibitor (52,53). More recently, in adults, a phase I study completed (NCT02071927) showed it was well tolerated (54). Other trials, such as NCT03047993, studying its use in AML with multilineage dysplasia and myelodysplastic syndromes are still ongoing.

Discussion

AML is uncommon in the pediatric population with patients under 20 years of age only accounting for 4.5% of new cases of AML in 2013–2017 (55). AML is a complex disease with cytogenetics being an important part of diagnosis and treatment. The study of cytogenetics provides information on the changes in the structural component of chromosomes. These lead to alterations in metabolism of leukemic cells including changes in cell growth, apoptosis, oxidative stress, and the bypassing of usual checkpoints. However, molecular features in pediatric AML differ from those in adults in number of mutations as well as incidence. Therefore, metabolic alterations could differ from those in adults. This should be studied more in depth as these differences may confer unique targets for innovative therapies in the pediatric field. However, despite significant advancements in treatment of pediatric ALL, management of AML has remained challenging due to its unique properties.

Treatment in the past decades has been limited to a combination of an anthracycline and a nucleoside analogue, which is common to all patients. Although it has a good initial response rate, relapse rates remain high. Therefore,

recent scientific studies have been focusing on targeted therapies. However, studies that focus on specific metabolic changes unique to pediatric AML have been lacking. For this reason, the authors encountered several limitations in this review. Differences between adult and pediatric features, including metabolism, were difficult to detect as there was seldom literature on this topic. Similarly, efficacy of innovative therapy was more focused on adult studies given that most clinical trials in the pediatric population are still ongoing. This is not unusual as pediatric trials usually start once their efficacy and safety have been studied more thoroughly in the adult population. Therefore, it emphasizes the importance of continued studies focused on pediatric AML cytogenetics, translocations, and metabolism that could lead to improvement in outcome in this population.

Conclusions

AML is a hematological malignancy with adverse prognosis. Leukemic cells alter replication properties and metabolism to escape apoptosis and promote proliferation. Cytogenetic characterization further highlights properties that lead to increased or decreased risk of resistance and relapse. Front-line chemotherapy has remained the same over several decades and prognosis remains relatively unchanged. As a result, of the high resistance and relapse rate, new emerging therapies are targeting specific molecular and metabolic components of the leukemic cells. Given the variations in cytogenetics between the adult and pediatric population, further characterization of metabolic differences should also be studied for more specific therapeutic targets in future research.

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