The Role of AMPK/mTOR Modulators in the Therapy of Acute Myeloid Leukemia

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Abstract: Differentiation therapy of acute promyelocytic leukemia with all-trans retinoic acid represents the most successful pharmacological therapy of acute myeloid leukemia (AML). Numerous studies demonstrate that drugs that inhibit mechanistic target of rapamycin (mTOR) and activate AMP-kinase (AMPK) have beneficial effects in promoting differentiation and blocking proliferation of AML. Most of these drugs are already in use for other purposes; rapalogs as immunosuppressants, biguanides as oral antidiabetics, and 5-amino-4-imidazolecarboxamide ribonucleoside (AICAr, acadesine) as an exercise mimic. Although most of these pharmacological modulators have been widely used for decades, their mechanism of action is only partially understood. In this review, we summarize the role of AMPK and mTOR in hematological malignancies and discuss the possible role of pharmacological modulators in proliferation and differentiation of leukemia cells.

Keywords: AML, AMPK, mTOR, rapamycin, metformin, AICAR, differentiation.

1. INTRODUCTION

Acute myeloid leukemia (AML) is a heterogenous group of malignant disorders that are characterized by uncontrolled proliferation of blasts that are blocked at an early stage of differentiation. The accumulation of immature myeloblasts in the bone marrow and peripheral blood occurs at the expense of normal terminally differentiated blood cells resulting in progressive anemia, thrombocytopenia and neutropenia. AML is the most common type of acute leukemia in adults with the incidence of 3-5 cases per 100 000, a slight male predominance and a median age at diagnosis of approximately 70 years. The WHO classification that is currently in use, defines 7 main subtypes of AML according to genetic abnormalities, relationship to previous conditions and therapies, and cytochemical and morphological characteristics. The older classification, which is still in use, is the French-American-British Classification (FAB). FAB classification is based predominantly on cytomorphological features of leukemia cells, and the 8 AML subtypes (M0-M7) defined in such manner have been integrated into the WHO classification.

The standard therapy for acute myeloid leukemia (AML) has not changed significantly for the past four decades and consists of 7-10 days of cytarabine combined with 3 days of an anthracycline as a remission induction therapy. In patients <60 years of age, this therapy results in complete remission rates of 60-90%. Several courses of high dose cytarabine or allogeneic hematopoietic stem cell transplantation may be used as a consolidation therapy. In elderly patients for whom intensive therapy is not appropriate, treatment remains unsatisfactory and includes low-dose cytarabine or demethylation therapy with supportive care [reviewed in 1, 2].

Several new agents including monoclonal antibodies, drugs targeting signaling pathways, and epigenetic regulators are in early clinical trials for AML. However, the most successful pharmacological therapy for
AML is still retinoic acid-based differentiation therapy of acute promyelocytic leukemia (APL), a particular subtype of AML characterized by t(15;17) translocation. Once fatal disease with a high incidence of early hemorrhagic death, APL has now been successfully treated with all-trans retinoic acid (ATRA) and chemotherapy inducing complete remission rates of 90% and cure rates of approximately 80% [3]. The clinical outcome of the disease has been further improved by the introduction of arsenic trioxide (ATO) into the treatment of refractory or relapsed APL. Moreover, the latest results of the clinical studies instituting ATO plus ATRA as a first-line treatment suggest the possibility of leukemia treatment without any DNA-damaging chemotherapy [4].

The biochemical mechanisms responsible for differentiation of leukemia cells are not entirely understood. The high sensitivity of APL to ATRA is ascribed to the presence of the fusion protein promyelocytic leukemia (PML)/retinoic acid receptor α (RARα), which is encoded by a specific chromosomal translocation involving PML gene on chromosome 15 and RARA gene on chromosome 17. In cells containing t(15;17) translocation, the fusion protein PML/RARα acts as a co-repressor recruiting multiple repressive epigenetic modifiers to down-regulate the expression of target genes and to induce a differentiation block. It has been generally assumed that the mechanism of action of pharmacological doses of ATRA includes binding to RARα, release of co-repressors, recruitment of transcriptional activators and relief of the differentiation block [5]. However, it should be noted that differentiative properties of ATRA were first described in HL-60 cell line [6] established from peripheral blood of a patient suffering from acute myeloblastic leukemia, or the AML-M2 which actually carries no t(15;17) translocation. Therefore, paradoxically, the cells on which differentiative properties of ATRA were first described would not fulfill the current clinical criteria for ATRA-based treatment. Obviously, the mechanism of ATRA-mediated differentiation of AML could not be solely ascribed to the effects on the PML/RARα protein [7].

Phosphatidylinositol 3-kinase (PI3K)/Akt/ mammalian target of rapamycin (mTOR) has been generally considered one of the principal pathways responsible for transmitting proliferative and anti-apoptotic signals in AML [8]. Our previous study on ATRA-treated HL-60 and NB4 cells, the latter containing the typical t(15;17), suggested that PI3K/Akt pathway had some role in differentiative responses of leukemia cells as increases in the level of both nuclear phosphatidylinositol (3,4,5) trisphosphate (PIP3) and phosphatidylinositol (3) phosphate, as well as an increase in the activity of protein kinase B/Akt were observed, and siRNA-mediated down-modulation of Akt in HL-60 cells reduced the expression of differentiation markers in ATRA-treated cells [9]. In both the cell lines, pharmacological inhibitors of PI3K and Akt inhibited proliferation, but negatively affected differentiative capacity of the cells [10]. In contrast, use of rapamycin, which inhibits mTOR, a more distal component of the pathway, potentiated differentiation of AML cells along granulocytic pathway [10-12]. Physiologically, mTOR is negatively regulated by AMP-activated kinase (AMPK), an evolutionary conserved serine-threonine kinase that is activated whenever the energy level in the cell is low and the ratio of AMP to ATP is high. Once activated, AMPK stimulates ATP-generating pathways (glycolysis and fatty acid oxidation), and inhibits ATP-consuming pathways (gluconeogenesis and synthesis of fatty acids and cholesterol) through both direct phosphorylation of enzymes and alteration in gene expression [13]. Our recent study demonstrated that 5-amino-4-imidazolecarboxamide ribonucleoside (AICAr, acadesine), an AMPK-activator, enhances ATRA-mediated differentiation in HL-60 and NB4 cells. In monocytic U937 cells, a non-APL AML cell line, AICAr induces the expression of cell surface markers associated with mature monocytes and macrophages but the mechanisms responsible for AICAr-mediated differentiative effects are still unknown [14].

Recent studies suggested that drugs that target metabolism may have some role in the treatment of cancer [reviewed in 15]. Many of these drugs have been studied as modulators of AMPK pathway. In AML cells, several studies demonstrated involvement of PI3K/Akt/mTOR and AMPK pathways in cell cycle progression, proliferation and survival based on cytotoxicity assays. In this review, we will focus more on the possible role that both AMPK activators and mTOR inhibitors may have in the differentiation of leukemia cells. The identification of their mechanisms of action may provide some theoretical basis for further improvement of differentiation therapy of AML.

2. mTOR AS A POTENTIAL THERAPEUTIC TARGET

2.1. Regulation and Functional Role of mTOR

The mechanistic target of rapamycin (mTOR, formerly named mammalian target of rapamycin) is a serine/threonine protein kinase that was identified as a target for rapamycin, a drug discovered more than 50
years ago on the Chilean Easter Island (Polynesian name Rapa Nui) [16]. Although it was originally discovered as an antifungal agent, it was soon attributed with immunosuppressive and antitumor activity, but the exact mechanism of action was unknown until the 1990s when genetic screens for rapamycin resistance identified two genes, TOR1 and TOR2 in Saccharomyces cerevisiae [17].

mTOR is a catalytic subunit of two functionally distinct protein complexes, mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2), which are mammalian analogues of yeast complexes TORC1 and TORC2. As shown in Fig. (1), rapamycin-sensitive mTORC1 contains protein Raptor and phosphorylates p70 S6 Kinase 1 (S6K1) and eIF4E Binding Protein (4EBP-1), which are involved in regulation of protein translation [18]. On the other hand, mTORC2 is considered to be rapamycin insensitive, at least upon acute exposure. Although mTORC2 phosphorylates several PKC isoforms important for cytoskeletal remodeling and cell migration, the most important target is Akt, which is activated downstream of receptor tyrosine kinases.

mTORC1 is at the intersection of many signaling pathways, but one of the best described pathways is phosphatidylinositol 3-kinase class I/Akt/mTOR (PI3KC1/ Akt/mTOR) downstream of insulin receptor. As shown in Fig. (1), activation of PI3K by growth factors leads to generation of PIP3, which recruits inactive PKB/Akt to the plasma membrane. Once activated, Akt phosphorylates tuberous sclerosis complex 2 (TSC2) and activate mTORC1 through Ras homologue enriched in brain (Rheb). In addition, Akt directly activates mTORC1 through phosphorylation of proline-rich AKT substrate 40 (PRAS40). Activated mTOR allows for normal cell growth by stimulating protein, lipid and nucleotide synthesis in case of nutrient abundance and growth factor stimulation. mTOR is a master regulator of translational pathways controlling biosynthesis of proteins crucial for cell survival, like Bcl-2, Mcl-1 and Survivin. The functional role of mTOR in growth, survival, aging, metabolism and cancer have recently been reviewed in depth by several authors [18-21].

The PI3KC1/Akt/mTOR signaling pathway is constitutively activated in many sporadic human cancers, but also plays a role in familiar cancer syndromes. In most cases, the activation is due to the amplification/mutation of catalytic or regulatory subunits of PI3K, receptor tyrosine kinases (RTK), Akt, TSC1/2, mTOR, or deletion/inactivation of tumor suppressors, including phosphatase and tensin homolog (PTEN) and
liver kinase B1 (LKB1). Loss-of-function mutation of TSC1/2 is associated with multiorgan hamartomas and multiple benign tumors in tuberous sclerosis and lymphangioleiomyomatosis; the loss of PTEN, the main negative regulator of PI3K/Akt that removes the 3’-phosphate from PIP₃, is mutated in 70% of patients with Cowden syndrome; and LKB1 is mutated in Peutz-Jeghers syndrome characterized by intestinal polyps [22]. Using publicly available tumor genome sequencing data Grabiner et al. [23] generated a catalogue of 33 MTOR mutations in various cancer types that confer pathway hyperactivation and these mutated cells were highly sensitive to rapamycin.

The PI3KCl/Akt/mTOR signaling pathway has been found to be constitutively activated in 50-80% of human primary AML cells [24-28], mostly due to activating mutations in tyrosine kinases upstream of PI3K/Akt axis. Although several studies have demonstrated beneficial effects of drugs targeting either PI3K and/or Akt in preclinical models of AML, results of clinical trials are modest or disappointing [reviewed in 29-31]. Two inhibitors selectively targeting Akt, MK-2206 and UCN-01, had promising effects in preclinical studies, but failed to exert their action on AML patients in clinical trials [32,33]. A recent phase I trial of budsiparlisib, an oral PI3K inhibitor, demonstrated acceptable tolerability and preliminary activity in a subset of patients with advanced leukemia [34].

The activity of mTOR is high in majority of AML samples, even in the absence of an elevated PI3K and Akt signaling. One of the possible Akt-independent mechanisms of mTOR-activation involves an upstream activation of the oncogenic Lyn kinase that has been shown to be augmented in AML cells [35]. Amplified levels of Rheb and Raptor, or down-regulated TSC2 may lead to increased activity of mTORC1. Other possible mechanisms of constitutive mTOR activation involve the activity of ERK, WNT or HIF-1 [29-31].

The role of mTOR in promoting leukemia have been documented in several murine models. In a model of leukemogenesis evoked by PTEN deficiency, deletion of Raptor, an activator of mTORC1, significantly prolonged survival of mice [36]. In a mouse model of MLL-AF9-driven AML, conditional deletion of Raptor significantly suppressed leukemia progression, prevented leukemia initiation and prolonged animal survival [37], and the similar results were obtained when S6K1, downstream target of mTORC1 [38], or Rheb1, an upstream activator of mTORC1 [39], were deleted. Taken together, these results suggested that mTORC1 promoted leukemogenesis and that pharmacological inhibition of mTORC1 might be a promising strategy in the treatment of leukemia.

2.2. Rapamycin and Analogs

Rapamycin or sirolimus (Rapamune) is approved as an immunosuppressant drug for the prophylaxis of organ rejection in patients receiving renal transplants and for treating lymphangioleiomyomatosis. Besides oral formulation, it is also used for coating of drug-eluting coronary stents to prevent recurrence of stenosis. Limitations such as poor solubility and pharmacokinetics resulted in the development of its analogs, temsirolimus and everolimus that are approved by U.S. Food and Drug Administration for the treatment of advanced renal cell carcinoma. Everolimus has been also approved for several other tumors and for the treatment of advanced-stage, hormone receptor-positive, HER2-negative breast cancer in combination with aromatase inhibitor [40].

Rapamycin binds FK506-binding protein (FKBP12) and its complex acts as an inhibitor of mTORC1, resulting in inhibition of protein translation. Immunosuppressive properties of rapamycin were first attributed to its antiproliferative activity and promotion of cell cycle, but experiments on T cells showed modest antiproliferative effects of rapamycin and pointed to the role of rapamycin in differentiation and anergy of T cells, as well as a complex set of roles in other immune cell populations. Recently, rapamycin was found to extend animal lifespan and reduce aging-dependent phenotypes, including neurodegenerative disorders. Still, rapamycin has been proven in human subjects to have serious adverse effects like nephrotoxicity, immunosuppression, insulin resistance, hyperlipidemia and hypercholesterolemia [reviewed in 20, 41-43].

Early after the discovery of immunosuppressive properties, rapamycin was proven to exert antineoplastic activity. In the first reports, rapamycin was shown to reduce tumor growth in several transplantable tumor models [44] and to inhibit proliferation of hepatoma cell line through decreased activity of p70 S6K [45]. In hematologic malignancies, the use of rapamycin was first considered for prevention of transplantation-related lymphoma, due to its known effects on lymphocyte proliferation [46]. Furthermore, antileukemic activity of rapamycin has been demonstrated against B-acute lymphoblastic leukemia (ALL) [47], childhood ALL [48], and BCR-ABL positive chronic myeloid leukemia (CML) in combination with imatinib [49]. Although preclinical studies have shown cytostatic effects of mTOR inhibitors on primary AML cells [28,
50], results of the clinical trials using rapamycin derivatives as a single agent have shown very limited response in AML patients treated with sirolimus [28], deforolimus [51] or everolimus [52]. Even with the addition of cytotoxic chemotherapy, sirolimus did not enhance antileukemic effects of chemotherapy [53]. Temsiroliimus [54] and everolimus [55] demonstrated acceptable toxicity, but modest clinical effects. Table I summarizes results of completed clinical trials using mTORC1 inhibitors for AML and gives a listing of trials currently underway with mTORC1 and PI3K/mTOR inhibitors.

There are several recent reviews regarding the use of mTOR inhibitors in various hematological malignancies, including AML, which provide possible explanations for limited potential of rapalogs in treating cancer [29,30,56]. Besides the lack of inhibition of all mTORC1 substrates or induction of autophagy, an important role is ascribed to the lack of negative feedback exerted by mTORC1 on the proximal PI3K/Akt signaling pathway. As an increase in PI3K/Akt activity has been demonstrated in several rapalogs-treated cells, dual inhibitors of PI3K/mTOR (e.g. PI-103, BEZ235) and/or dual mTORC1/2 inhibitors (OSI-027, PP242) have been used to increase antiproliferative effects. However, although blocking two components of PI3K/Akt/mTOR is more effective in inhibiting proliferation, it may compromise differentiation that was also shown to depend on the increase in the activity of PI3K/Akt. In ATRA-treated cells, an increase in the level of PI3K [57], or phosphorylated Akt [9] has been detected in nuclei, and specific down-regulation of Akt [9] or p85a-subunit of PI3K [57] reduced CD11b expression in ATRA-stimulated leukemia cells. In murine AML cells, constitutive activation of Akt promotes myeloid differentiation and these effects were mediated by FOXO [58].

Rapamycin was shown to induce differentiation of variety of cells [59-61]. In leukemia cell lines, an early study reported that rapamycin alone can induce differentiation, but these data were obtained at doses that were up to two orders of magnitude greater than the range of therapeutically achievable doses [62]. Several studies later on confirmed that rapamycin alone at doses of 20-50 nM had no effects on the expression of differentiation markers in vitro [10-12,63,64]. However, rapamycin enhanced differentiation of AML cell lines and these effects were not common to all differentiation inducers. Rapamycin enhanced the expression of CD11b in response to ATRA [10-12] and vitamin D3 [65], and had no effects in cells treated with ATO [66] or differentiated in the presence of PMA [10] or DMSO [12]. In typical APL cells, the enhancing effects of rapamycin on cell differentiation has been ascribed to rapamycin-mediated induction of autophagy, which degrades the fusion PML/RARα protein [67], but these effects cannot be responsible for enhancing effects in non-APL cell lines. In non-APL AML cells, cellular mechanisms leading to differentiation induction are poorly understood but seem to depend on the increase in the activity of proximal PI3K/Akt pathway, which is observed in response to ligands of the nuclear receptor family, like ATRA [9,57] and vitamin D3 [68, 69]. Therefore, although combined inhibition of PI3K and mTOR exerted synergistic antiproliferative effects, our previous study showed that combined inhibition diminished differentiative properties of rapamycin in AML cells [10].

3. AMPK AS A POTENTIAL THERAPEUTIC TARGET

3.1. Regulation and Functional Role of AMPK Signaling

The kinase activity responsible for phosphorylation and inactivation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and acetyl-CoA carboxylase (ACC) was named “AMP-activated kinase” to denote the new serine/threonine kinase, which is activated by an increase in 5’-AMP [70]. AMPK is a heterotrimer composed of a catalytic subunit α and two regulatory β and γ subunits; the α-subunit contains a conserved threonine residue (Thr 172) within the kinase domain, phosphorylation of which usually serves as a marker for its activation, and the γ-regulatory subunit provides binding sites for adenine nucleotides. During metabolic stress, AMP and ADP displace ATP from γ-regulatory subunit and induce conformational change that allows phosphorylation of Thr 172 by upstream kinases. Principal upstream kinases are: liver kinase B1 (LKB1), which is mutated in Peutz-Jeghers syndrome, calcium/calmodulin dependent kinase kinase (CAMKKb) and TGFβ-activated kinase (TAK1). Once activated, AMPK regulates metabolic pathways and affects the activity of various proteins involved in aging, cell cycle regulation, cell growth and apoptosis. Among many different targets, AMPK inhibits the activity of mTOR, which provides a link between a lack of ATP and a decrease in cell growth and protein synthesis. For more in-depth discussion regarding the structure, isoforms, regulation and the functional role of AMPK in metabolism and disease, excellent reviews have been recently provided [71-76].
Table 1. Clinical trials of mTORC1, dual mTORC1/2 and PI3K/mTOR inhibitors in AML.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Drugs in Combination</th>
<th>Clinical Trial</th>
<th>Status</th>
<th>Results</th>
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</table>
| Deforolimus        | mTORC1                  | -                                                      | NCT00086125 A Phase II Study of AP23573, an mTOR Inhibitor, in Patients With Relapsed or Refractory Hematologic Malignancies | Completed, Data published   | Total: 23 patients (AML) Deforolimus was well tolerated. None of the 22 evaluable patients with AML had CR or PR.  
| Sirolimus          | mTORC1                  | MEC (mitoxantrone, etoposide, and cytarabine)         | NCT00775593 An Open Label Phase II Trial of Clofarabine and Temsirolimus in Older Patients With Relapsed or Refractory Acute Myeloid Leukemia (AML) | Completed, Data published   | Total: 53 patients Sirolimus and MEC is an active and feasible regimen, the synergy between MEC and sirolimus was not confirmed.  
| Temsirolimus       | mTORC1                  | clofarabine                                           | NCT001074086 Multicentric Study of GOELAMS Phase I Evaluation of RAD001 in Association With AraCytine and Daunorubicine in AML Treatment in Patients Less Than 65 Years in Relapse More Than One Year After First Complete Remission | Completed, Data published   | Total: 28 patients RAD001 at d1 and d7 of an induction chemotherapy regimen for AML has acceptable toxicity and may improve treatment. CR= 19/28  
| Everolimus (RAD001)| mTORC1                  | daunorubicin, cytarabine                              | NCT00861874 A Phase I Study of Decitabine in Combination With Decitabine and Temsirolimus in Patients With Relapsed or Refractory Acute Myeloid Leukemia | Completed, Data published   | Total: 12 patients The combination of decitabine and rapamycin can be safely administered to patients with relapsed/refractory AML.  
| Sirolimus          | mTORC1                  | decitabine                                            | ACTRN12610001031055 A Phase Ib/II Clinical Evaluation of the Safety of Combining the mTOR inhibitor Everolimus with 5-Azauridine in Acute Myeloid Leukaemia (AML) | Completed, Data published   | Total: 40 patients Everolimus in combination with azacitidine is tolerable, with promising clinical activity in advanced AML. OS= 8.5 mo, ORR= 22.5%  
| Sirolimus          | mTORC1                  | MEC (mitoxantrone, etoposide, and cytarabine)         | NCT00634244 A Phase II Randomized Trial of Carboptatin and Topotecan; Flavopiridol, Mitoxantrone and Cytosine Arabinoside; and Sirolimus, Mitoxantrone, Etoposide and Cytosine Arabinoside for the Treatment of Adults With Primary Refractory or Initial Relapse of Acute Myelogenous Leukemia (AML) | Completed, Results unpublished | Total: 20 patients CR+CRi= 15%  
<p>| Temsirolimus       | mTORC1                  | Standard chemotherapy                                 | NCT01611116 A Double-blind, Placebo-controlled, Randomized, Multicenter Phase II Trial to Assess the Efficacy of Temsirolimus Added to Standard Primary Therapy in Elderly Patients With Newly Diagnosed AML | Completed, No results posted | -                                                                      |</p>
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<th>Drugs in Combination</th>
<th>Clinical Trial</th>
<th>Status</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Sirolimus</td>
<td>mTORC1</td>
<td>decitabine, ribavirine</td>
<td>NCT02109744 A Phase I/II Study of Decitabine in Combination With Sequential Rapamycin or Ribavirin in High Risk AML Patients</td>
<td>Active</td>
<td>-</td>
</tr>
<tr>
<td>Everolimus (RAD001)</td>
<td>mTORC1</td>
<td>MEC (mitoxantrone, etoposide, and cytarabine), idarubicin</td>
<td>NCT01154439 A Phase I Study Investigating the Combination of RAD001 With Standard Induction and Consolidation Therapy in Older Patients With Newly Diagnosed Acute Myeloid Leukemia (AML)</td>
<td>Active</td>
<td>-</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>mTORC1</td>
<td>azacitidine</td>
<td>NCT01869114 A Phase II Study of Azacitidine and Sirolimus for the Treatment of High Risk Myelodysplastic Syndrome or Acute Myeloid Leukemia Refractory to or Not Eligible for Intensive Chemotherapy</td>
<td>Active</td>
<td>-</td>
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<tr>
<td>Sirolimus</td>
<td>mTORC1</td>
<td>cytarabine, idarubicin</td>
<td>NCT01822015 A Pilot, Pharmacodynamic Correlate Trial of Sirolimus in Combination With Chemotherapy (Idarubicin, Cytarabine) for the Treatment of Newly Diagnosed Acute Myelogenous Leukemia</td>
<td>Active</td>
<td>-</td>
</tr>
<tr>
<td>Everolimus (RAD001)</td>
<td>mTORC1</td>
<td>PKC412 (FLT3 inhibitor)</td>
<td>NCT00819546 A Phase I Trial of Escalating Dose of RAD001 in Combination With PKC412 in Patients With Relapsed, Refractory or Poor Prognosis AML or MDS</td>
<td>Active</td>
<td>-</td>
</tr>
<tr>
<td>BEZ235</td>
<td>PI3K/mTOR</td>
<td>-</td>
<td>NCT01756118 A Phase I, Dose-finding Study of the Oral, Dual Phosphatidylinositol 3(PI3)-Kinase / Mammalian Target of Rapamycin (mTOR) Inhibitor BEZ235 in Adult Patients With Relapsed or Refractory Acute Leukemia</td>
<td>Active</td>
<td>-</td>
</tr>
<tr>
<td>PF-05212384 (PKI-587)</td>
<td>PI3K/mTOR</td>
<td>-</td>
<td>NCT02438761 Phase II Evaluating the Efficacy of the Dual Inhibition of Phosphoinositide 3 Kinase (PI3K)/Akt /Mammalian Target Of Rapamycin (mTOR) Signaling Pathway by PF-05212384 (PKI-587) for Patients With Myeloid Neoplasm Secondary to Chemo-radiotherapy (t-AML/MDS) or de Novo Relapsed or Refractory AML</td>
<td>Active</td>
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CR – complete remission, CRi - complete remission with incomplete hematologic recovery, PR – partial remission, MS – median survival, OS – overall survival, ORR – overall response rate

g) https://clinicaltrials.gov/ct2/show/NCT00634244

Most of the functions of AMPK have been described on the basis of treatment of cell lines and organ cultures with pharmacological activators of AMPK, such as antidiabetic biguanides, metformin and phenformin, or 5-amino-4-imidazolecarboxamide ribonucleoside (AICAr). Although numerous drugs and xeno-
biotics, including barbiturates, 2-deoxyglucose and resveratrol, have been used as AMPK agonists, based on their ability to phosphorylate AMPK on Thr 172, their mechanisms of action are mostly indirect and only partially elucidated [77]. In addition, an increasing number of studies suggest that the majority of the effects of most commonly used AMPK agonists, metformin and AICAr, on cell cycle, metabolism and viability are actually AMPK-independent [78, 79].

More reliable way to assess the functional role of AMPK in vivo could be provided by mice knockouts. Data provided by transgenic mice models demonstrated that both AMPK [80] and LKB1 [81] are important for glucose uptake into muscle cells, but have lesser role in physiological regulation of glucose metabolism in cardiac muscle cells [82-83]. However, AMPK allows for the increase in glucose uptake and lactate production in post-ischemic cardiomyocyte [84], while LKB1 deletion does not change the rate of glucose and palmitate oxidation after reperfusion of the ischemic heart [83]. Although liver-specific LKB1 knockouts show an increase in blood glucose level [85], tissue-specific AMPK knockouts are normoglycemic [86]. The difference in phenotype of AMPK and LKB1 knockouts could be explained by the presence of different AMPK isoforms in different tissues and/or the fact that LKB1 has at least 12 different downstream targets, not including AMPK [as reviewed in 87].

The possible role of AMPK in cancer was suggested as soon as LKB1, a known tumor suppressor, was identified as an upstream kinase [88-90]. In addition, AMPK activates another tumor suppressor complex, TSC2 [91], and thus inhibits both cell growth, through mTOR inhibition, and cell proliferation, through phosphorylation and activation of p53, which is often mutated or its activity disabled in many tumors [92]. The more direct evidence for the role of AMPK as a tumor suppressor was provided by a study on transgenic mice overexpressing c-Myc in B cells in which genetic ablation of the α1 catalytic subunit of AMPK accelerates Myc-induced lymphomagenesis [93]. In addition, both direct and indirect AMPK-activators, A769662 and biguanides, delay the onset of tumorigenesis in mice that are prone to the development of tumors due to heterozygous loss of PTEN combined with reduced expression of LKB1 [94]. However, although phenformin protected against initial tumor development [94], once tumor has been already established, phenformin was more efficient in apoptosis induction in genetically engineered mouse model of lung cancer baring LKB1 mutations [95]. In a murine model of AML, AMPK was shown to confer metabolic stress resistance to leukemia initiating cells and to promote leukemogenesis [96]. Furthermore, in a mouse model of T cell acute lymphoblastic leukemia (T-ALL), AMPK deficiency led to leukemia cell death and increased animal survival [97]. Importantly, although these results may suggest that AMPK activation may be useful only for cancer prevention and not for cancer treatment, phenformin treatment reduced the number and percentage of T-ALL cells in the treated mice and resulted in a significant increase in overall animal survival further confirming the hypothesis that majority of antineoplastic effects of AMPK agonists may be AMPK-independent and related to profound metabolic changes.

In AML patients, the functional LKB1/AMPK axis was first demonstrated in primary samples pharmacologically treated with metformin in which AMPK activation fully inhibited mTORC1 and reduced oncogenic protein synthesis [98]. Tumor suppressor role of AMPK was further confirmed in a study using targeted knockdown screen of AML cell lines [99]. Of note, mutations of AMPK [100], or its predominant upstream regulator LKB1 [101], are very rare in AML patients and our understanding of the role of AMPK in AML is mostly based on studies using pharmacological modulators. In a recent study using GSK621, a more specific AMPK agonist, cytotoxic effect of AMPK activation was not dependent on mTORC1 inhibition but indeed required mTORC1 activation that was unique to AML cells and involved the eIF2α/ATF4 signaling pathway [102]. The activity of mTOR in AML cells may depend on several other factors, including microenvironment or hypoxia as HIF-1 controls myeloid hematopoietic cell function by maintaining mTOR phosphorylation through AMPK [103]. Irrespective of mTOR involvement, AMPK has many other downstream targets important in metabolism such as fatty acid metabolism that has been recently described to be activated in acute monocytic leukemia cells co-cultured with bone marrow adipocytes [104].

The listing of clinical trials currently underway using AMPK modulators for the treatment of hematologic malignancies is presented in Table 2.

### 3.2. Metformin

Metformin or 1,1-dimethylbiguanide is the first-line medication in the treatment of type 2 diabetes mellitus (T2DM). By chemical structure, biguanides are derivatives of guanidines, which gained popularity in 1950s due to their good oral bioavailability and the ability to reduce blood glucose levels without inducing hypoglycemia.
Table 2. AMPK modulators in clinical trials for hematologic malignancies.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Disease</th>
<th>Clinical Trial</th>
<th>Results</th>
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<tbody>
<tr>
<td>metformin</td>
<td>Acute lymphoblastic leukemia</td>
<td>NCT03118128 Effect of the Addition of Metformin Hydrochloride on the</td>
<td>Completed, no results posted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prognosis of Patients With B-cell Precursor (Ph+ Negative)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acute Lymphoblastic Leukemia With High Expression of ABCB1 Gene</td>
<td></td>
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<tr>
<td>metformin</td>
<td>Relapsed chronic lymphocytic leukemia</td>
<td>NCT01750567 A Phase II Pilot Study of Metformin Therapy in Patients</td>
<td>Active study</td>
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<tr>
<td></td>
<td></td>
<td>With Relapsed Chronic Lymphocytic Leukemia and Untreated CLL Patients With Genomic Deletion 11q</td>
<td></td>
</tr>
<tr>
<td>metformin</td>
<td>Childhood acute lymphoblastic leukemia</td>
<td>NCT01324180 A Phase I Window, Dose Escalating and Safety Trial of Metformin in Combination With Induction Chemotherapy in Relapsed Refractory Acute Lymphoblastic Leukemia: Metformin With Induction Chemotherapy of Vincristine, Dexamethasone, Doxorubicin, and PEG-asparaginase (VPLD)</td>
<td>Completed, no results posted</td>
</tr>
<tr>
<td>metformin</td>
<td>Recurrent/refractory plasma cell myeloma and chronic lymphocytic leukemia</td>
<td>NCT02948283 A Pilot Feasibility Study of Metformin/Ritonavir Combination Treatment in Patients With Relapsed/Refractory Multiple Myeloma or Chronic Lymphocytic Leukemia</td>
<td>Active study</td>
</tr>
<tr>
<td>metformin</td>
<td>Acute myeloid leukemia</td>
<td>NCT01849276 A Phase I Study of Metformin and Cytarabine for the Treatment of Relapsed/Refractory Acute Myeloid Leukemia</td>
<td>Active study</td>
</tr>
<tr>
<td>metformin</td>
<td>Lymphoma</td>
<td>NCT00659568 A Phase I Study of Temsirolimus in Combination With Metformin in Advanced Solid Tumours</td>
<td>Completed, no results posted</td>
</tr>
<tr>
<td>metformin</td>
<td>Diffuse large B cell lymphoma</td>
<td>NCT03200015 Effect of Metformin in Combination With R-CHOP for the First Line Treatment of Patients With Diffuse Large B-cell Lymphoma</td>
<td>Active study</td>
</tr>
<tr>
<td>metformin</td>
<td>Lymphoma</td>
<td>NCT02145559 A Pharmacodynamic Study of Sirolimus and Metformin in Patients With Advanced Solid Tumors</td>
<td>Active study</td>
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<tr>
<td>AICAR</td>
<td>Chronic lymphocytic leukemia</td>
<td>NCT00559624 A Phase I/II Open Label Dose Escalation Study to Investigate the Safety and Tolerability of Acaadesine in Patients With B-cell Chronic Lymphocytic Leukemia</td>
<td>Completed, MTD (single dose)= 210 mg/kg; acceptable safety profile a)</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>Leukemia, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Neoplasms</td>
<td>NCT00004245 A Phase I Study of Salicylate for Adult Patients With Advanced Myelodysplastic Disorders or Acute Myelogenous Leukemia</td>
<td>Completed, acceptable safety profile b)</td>
</tr>
</tbody>
</table>

MTD – maximum tolerated dose

However, the more potent biguanides phenformin and buformin have not been in clinical use since 1970s because of their lower safety profile mostly due to the increased risk of lactic acidosis. Metformin demonstrated less toxicity and therefore has become the most commonly prescribed oral antidiabetic drug worldwide [105, 106].

The anti-diabetic properties of metformin are commonly explained by the reduction of hepatic gluconeogenesis, the increase in cellular glucose uptake and increased insulin sensitivity [reviewed in 107,108]. These effects of metformin were first associated with the activation of AMPK [109], but later studies in transgenic mice demonstrated that both metformin-mediated decrease of gluconeogenesis [86], and increase in glucose uptake were mediated by an AMPK-independent mechanism [110].
In 2005, Evans et al. reported the results of the case-control study showing that the use of metformin reduced the risk of cancer in a large cohort of patients with T2DM [111]. The finding was further confirmed by several studies and meta-analyses [reviewed in 112], even though the beneficial effects of metformin on cancer incidence appear to be much smaller than first reported, especially in randomized controlled trials and after adjustment for time-related biases. First reports of prospective randomized control trials with a clinical endpoint in which metformin was applied for an oncological indication demonstrated that the addition of metformin to a standard systemic therapy did not improve outcome in patients with advanced pancreatic cancer [113,114]. At the moment, there are more than 120 ongoing clinical trials, 8 out of them for hematologic malignancies, which aim at repurposing metformin for anti-cancer use [115].

The anticancer effects of metformin were first associated with AMPK since the activation of AMPK, as measured by phosphorylation of Thr 172, have been observed in many models in vitro [116] and in vivo [94]. Metformin was first proposed to activate AMPK indirectly, through inhibition of complex I of mitochondrial respiratory chain and subsequent decrease in energy production and increase in AMP/ATP ratio [117]. However, further studies showed that metformin can activate AMPK in two different cell lines without any detectable change in cellular ADP-to-ATP ratio [118], and that metformin-mediated activation may employ a more complex mechanism involving reactive nitrogen species, c-Src, phosphatidylinositol 3-kinase [119] and/or protein kinase C-ζ-mediated phosphorylation of LKB1 [120]. To make the story even more complicated, numerous studies in various cancer cell lines [78,121,122], and mouse models of tumorigenesis [95] demonstrated that antitumor effects of biguanides are actually AMPK-independent.

Irrespective of AMPK involvement in metformin action, the fact remains that metformin exerts inhibitory effects on cancer cells growth in vitro, but does not improve outcome in patients with pancreatic cancer when added as an adjuvant to standard chemotherapy in randomized clinical trials [113,114]. The simplest explanation for the difference may be that the doses of metformin used in vitro were approximately 1000 times higher than the peak plasma concentrations after oral intake. However, the concentration in plasma was the same as the one achieved in T2DM patients in which epidemiological studies reported beneficial effects of metformin in cancer prevention initially raising the possibility that the effectiveness of metformin in diabetic patients is maybe due to indirect effects on plasma levels of insulin and insulin-like growth factor 1, which are all known to act in a pro-oncogenic manner [123]. This mechanism is supported by a recent meta-analysis that showed a decrease in cancer incidence in T2DM patients who were taking metformin or thiazolidinediones but an increase in patients who were taking insulin or insulin secretagogues [124]. However, according to data obtained with high concentrations of metformin in vitro, there might be a rationale for the use of phenformin as more potent biguanide in the treatment of cancer since in these settings potential beneficial effects of the drug on cancer might outweigh its toxic side effects [108].

In the context of hematologic malignancies, metformin was reported to activate AMPK and inhibit growth of AML cell lines and primary AML cells while sparing normal hematopoiesis ex vivo [98], inhibit BCR-ABL-expressing cell lines and primary CML cells [125], interfere with the growth and survival of murine PTEN-deficient T cell lymphomas and human T-ALL/T-LL cancer cells [126], and enhance the anti-myeloma effect of bortezomib [127], but the functional role of AMPK in metformin effects has not been tested. In lymphoma cell lines, AMPK siRNA experiments proved that metformin-mediated inhibition is AMPK-dependent [128], shRNA-mediated down-regulation in T-ALL cell line prevented metformin-induced apoptosis [129] and knockdown of AMPKζ1 desensitizes metformin-mediated enhancements of vincristine-induced apoptosis in leukemia cells [130]. However, further studies in AML cell lines again showed that metformin mediated effects on apoptosis and proliferation are preserved even in cells with siRNA-downregulated AMPK suggesting AMPK-independent effects [131].

In a recent retrospective study aimed to evaluate whether metformin related cancer benefits reported in solid tumors are also present in AML patients, baseline metformin use provided no significant benefit in AML overall ad disease free survival. Lack of metformin benefit in AML could be ascribed to advanced age but also to the practice of metformin substitution with insulin which further confirmed the hypothesis that at least part of the beneficial effects of metformin in cancer prevention is indirect and due to a decrease in plasma insulin level [132].

Apart from having pro-apoptotic effects, metformin has been reported to enhance differentiation in APL cell line NB4 through activation of ERK signaling.
pathway [133]. In this study, enhancing effects of metformin were found to be restricted to APL cells, and only pro-apoptotic effects of metformin were observed in several non-M3 AML cell lines. The effects of metformin may depend on the basal activity of mTOR, as a downstream target of AMPK [14, 78], or the activity of ERK, which is known to regulate AMPK [134] and increase during differentiation [14, 135]. In monocytic U937 cells with a high basal level of mTOR, no increase in the level of differentiation was observed in response to metformin [14], and inhibitory effects of metformin were observed in PMA-mediated differentiation of acute monocytic leukemia cell line THP-1 [136]. There is increasing evidence that differential effects of metformin on the differentiation of hematologic and immunologic cells with its subsequent immunomodulatory effects could be another mechanism of the antineoplastic activity of metformin [137, 138]. In mouse models of normal hematopoiesis, the addition of metformin to the culture of Lin- Sca-1- c-Kit- bone marrow cells decreases differentiation and helps maintain highly repopulating hematopoietic stem cells in culture [139]. In a preclinical murine model of Fanconi anemia, metformin increased the size of the hematopoietic stem cell compartment, enhanced quiescence in hematopoietic stem and progenitor cells and delayed the tumor formation [140].

3.3. AICAR

The abbreviation AICAR has been widely used for 5-amino-4-imidazolecarboxamide (AICA) ribonucleoside or acadesine, although it should be reserved for AICA-ribonucleotide or ribotide, which is the phosphorylated form of AICA-ribonucleoside or riboside (Fig. 2). Therefore, AICA-riboside should be properly abbreviated as AICAr to denote an exogenous substance which, after entering cells, becomes phosphorylated by adenosine kinase into AICA-ribotide or AICAr [141]. From yeast to man, AICAr (also termed ZMP) is a normal cellular metabolic intermediate in de novo purine synthesis [142]. In humans, AICAr can be found to accumulate in various purine synthesis disorders, including Lesch-Nyhan disease [143].

As a cell-permeable nucleoside that shares some structural similarities with adenosine, AICAr was first developed to block adenosine reuptake in the ischemic heart [144]. In 1995, AICA riboside was reported to activate AMPK kinase [145, 146] and since then has been used in numerous studies related to metabolism, insulin signaling pathways and diabetes. Another surge of interest in AICAr was raised when it was shown to act as an “exercise in a pill” by increasing endurance in sedentary mice [147] and to prevent heat-induced sudden death in mice carrying mutations of type I ryanodine receptors [148]. Finally, the most recent interest in another possible use of AICAr was initiated by data demonstrating anti-tumor effects of metformin and various AMPK-agonists in cancer [111, 149].

AICAr inhibits fatty acid synthesis [145], increases fatty acid oxidation [150] and induces hypoglycemic effects in vivo [151-153], which are probably due to an increase of glucose uptake into muscle cells through GLUT4 translocation [154] and an AICA-riboside mediated inhibition of gluconeogenesis in the liver [151]. Some of these AICAr-mediated metabolic effects are AMPK-independent [155, 156], but may be of relevance for the potential treatment of type 2 diabetes since the inhibition of fat synthesis and an increase in lipolysis may reduce fat storage and decrease peripheral resistance to insulin action [74]. However, poor oral bioavailability of AICAr renders it quite unsuitable for the treatment of metabolic disorders like diabetes [157].

On the other hand, clinical trials involving more than 4000 patients with coronary artery bypass graft (CABG) surgery proved that AICAr (or acadesine) was safe and well-tolerated when used as an intravenous agent for the prevention of ischemia-reperfusion injury associated with CABG [158-160]. In the treatment of cardiovascular disorders, AICAr is classified as an adenosine regulating agents since the proposed mechanism of AICAr-effects includes the inhibition of adenosine deaminase and an increase in adenosine concentrations during ischemic conditions [161]. In the list of prohibited substances of The World Anti-Doping Agency (WADA) from year 2011 AICAr was classified as an AMPK agonist, but then moved to the class of “Hormone and metabolic modulators” in the list 2012 [162]. Endurance athletes could obviously benefit from the use of the substance, but it is difficult to estimate how widespread is the usage of AICAr as a doping agent since the detection of the abuse of AICAr, as an endogenous substance, in sports is a complex problem [163].

The possible clinical efficacy of AICAr as an anti-cancer agent has been recently tested in hematological malignancies. In year 2003, AICAr was first reported to induce apoptosis of B cell chronic lymphocytic leukemia (CLL) cells in vitro at doses that are well tolerated when achieved in plasma after intravenous injection [164]. Ten years later, results of the first clinical study testing the effects of acadesine in CLL demonstrated that AICAr had an acceptable safety profile and
antileukemic activity in patients with poor prognosis [165]. In other hematological malignancies, cytotoxic effects of AICAr in vitro have been demonstrated on B cells from mantle cell lymphoma and splenic marginal zone lymphoma [166], childhood ALL cells [167] and CML [125].

The cellular mechanisms of beneficial effects of AICAr are still partially understood. As previously mentioned, the principal mechanism of action was long assumed to be the activation of AMPK, but more and more studies show that both AICAr and metformin display AMPK-independent effects on cell proliferation, metabolism and differentiation [14, 78, 79, 168]. AICAr-mediated apoptosis in CLL cells is not mimicked by phenformin or A-769662, a direct AMPK agonist, and AICAr also potently induce apoptosis in B lymphocytes from mice lacking α subunit of AMPK [169]. In CML cells, AMPK knockdown by shRNA failed to prevent the effect of acadesine, and acadesine exerted a potent anti-leukemic effect through protein kinase C-dependent autophagic cell death [168]. In mantle cell lymphoma, acadesine effects may be potentiated in combination with the anti-CD20 monoclonal antibody rituximab [170] or Bcl-2 inhibitors [171].

The possible role of AICAr in differentiation therapy has been less investigated, although differentiative properties of AICAr have been described in several cell systems [172, 173], including AICAR-mediated differentiation of mouse embryonic stem cells along erythroid lineage [174]. Our recent study demonstrated that AICAr induced apoptosis, reduced proliferation and enhanced ATRA-mediated differentiated of HL-60 and NB4 cells. In monocytc U937 cells, a non-APL AML cell line, AICAR alone induced the expression of cell surface markers associated with mature monocytes and macrophages. Although we detected time and dose-dependent increase in the level of Thr 172-phosphorylated AMPK, a significant decrease in AMPK expression that was achieved by using commercially available siRNA sequences in U937 cells had no significant effects on the AICAr-mediated effects on the number of viable cells or the expression of differentiation markers [14]. Of note, no differentiative effects of metformin were observed although both agents exerted similar growth-arresting properties in all cell lines and reduced the phosphorylation of p70 S6K as a downstream target to the similar level.

To conclude, the results obtained with AICAr in various preclinical in vitro models, as well as the results of the first clinical studies testing AICAr for CML, suggest that the strategy using AICAr may improve therapy of hematological malignancies, including differentiation therapy for AML.

CONCLUSION

Numerous studies support the hypothesis that the inhibition of mTOR and activation of AMPK may have beneficial effects in promoting differentiation and blocking proliferation of acute myeloid leukemia cells. An advantage of possible therapeutic approach based
on the modulation of AMPK/mTOR signaling pathway relies on the fact that majority of drugs that modulate the pathway are already in use for unrelated purposes; rapalogs have been used as immunosuppressive drugs for decades, biguanides are the most widely prescribed oral antidiabetics, and AICAR is used as an exercise mimetic. However, results of clinical trials using rapalogs in leukemia have led to disappointing results, and no significant improvement was observed when dual mTOR inhibitors or inhibitors that block both PI3K and mTOR component of the pathway were used. Many authors still consider mTOR a promising target in AML mostly due to the fact that the activity of mTOR is high even in those AML patients with no genetic aberrations of PI3K/Akt. Several combinatorial approaches have been proposed in order to overcome resistance mechanisms, including agents targeting RAS/RAF/MEK/ERK axis, histone deacetylase pathways, the Bcl-2 apoptotic pathways and many others [29, 30]. Recent data showing cytotoxicity of a specific AMPK activator in AML indicated a potential for AMPK-activating agents in the treatment of mTORC1-overactivated leukemia [102].

In this review, we wanted to stress that some components of this signaling pathways may also have a role in differentiation of leukemia cells. Although approaches aimed to block upstream and/or parallel signaling pathways may help to increase cytotoxicity, blocking PI3K/Akt [9, 10] or ERK pathway [14, 135] certainly decreases differentiation of the cells. The concept of differentiation therapy first emerged from the success of ATRA therapy in APL, but is recently revisited again as there are more and more novel strategies and approaches aimed to increase the ability of ATRA to induce myeloid differentiation and apoptosis in non-APL AML [175] or to transform other differentiating drugs into more efficient therapies [176]. Rapamycin-mediated inhibition of mTOR enhances differentiation of leukemia cells in response to various agents, including ATRA [10, 11, 177]. In addition, activation of AMPK with AICAR increases the expression of differentiation markers in parallel with a decrease in mTOR activity [14]. Therefore, modulation of AMPK and mTOR might have some role in new strategies to induce differentiation of leukemia cells.

Downstream targets of AMPK/mTOR in differentiation response are only partially elucidated. Autophagy is one of the mechanisms that was reported to be important for rapamycin-mediated enhancement of dasatinib-induced differentiation of leukemia cell lines [177] and ATRA-mediated differentiation of APL cells [67]. Our recent study confirmed that autophagy increased in parallel with differentiation in response to many agents, including AICAR, but differentiation was not abolished by down-regulation of key components of classical or canonical autophagy pathway [178]. Another possibility is that both rapamycin and AICAR, as pharmacological modulators of AMPK/mTOR, have profound effects on cell metabolism, and there are more and more data pointing to the role of metabolic changes during both proliferation and differentiation of leukemia cells. A recent study that examined metabolic changes in leukemia cell lines (including HL-60 and U937) in response to metformin revealed that AMPK-independent proapoptotic effects of metformin depend on the ability to produce Pasteur effect, i.e. switch to glucose consumption, glycolysis and lactate production [131]. It is possible that the induction of apoptosis in response to metformin might be directly due to its action on mitochondria because several agents that inhibit mitochondrial electron transport have been recently shown to act synergistically with Bcl-2 inhibitor and inducer of intrinsic apoptosis pathway in AML and ALL cell lines and primary cells [179]. The role of mitochondrial metabolism and glycolysis in leukemia cell differentiation in response to AICAr and other differentiation agents remains to be determined [180], but several studies pointed to the role of fatty acid oxidation [181], mitochondrial translation and oxidative phosphorylation [182], glycolysis [183] or glutaminolysis [184] in AML proliferation and survival. Efforts to identify new therapeutic targets have recently pointed to the possible roles of either dihydroorotate dehydrogenase [185] or one-carbon folate pathway, specifically methyleneterahydrofolate dehydrogenase-cyclohydrodrolase 2 (MTHFD2) [186] in differentiation blockade of AML cells. The most recent clinically successful proof-of-concept that drugs targeting an “oncometabolite” may be useful in differentiation therapy of leukemia was provided by studies testing the effects of enasidenib in AML patients having mutations of isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) [187, 188].

To conclude, both inhibition of mTOR by rapamycin and activation of AMPK by AICAR have beneficial effects on leukemia cell differentiation. Better understanding of their mechanism of action could help to identify new therapeutic targets to overcome myeloid differentiation blockade. As differentiation therapy of APL with ATRA still represents the most successful pharmacological therapy of AML, an important task would be to identify similar differentiation therapy strategies for the remaining 90% of AML patients.
CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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