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Targeting mTOR signaling pathways and related negative feedback loops for the treatment of acute myeloid leukemia

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Abbreviations: AML, acute myeloid leukemia; HDAC, histone deacetylase; MNK, MAPK-interacting kinase; mTOR, mammalian target of rapamycin; PDK1, phosphoinositide-dependent protein kinase-1; PI3K, phosphatidylinositol 3-kinase

An accumulating understanding of the complex pathogenesis of acute myeloid leukemia (AML) continues to lead to promising therapeutic approaches. Among the key aberrant intracellular signaling pathways involved in AML, the phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) axis is of major interest. This axis modulates a wide array of critical cellular functions, including proliferation, metabolism, and survival. Pharmacologic inhibitors of components of this pathway have been developed over the past decade, but none has an established role in the treatment of AML. This review will discuss the preclinical data and clinical results driving ongoing attempts to exploit the PI3K/AKT/mTOR pathway in patients with AML and address issues related to negative feedback loops that account for leukemic cell survival. Targeting the PI3K/AKT/mTOR pathway is of high interest for the treatment of AML, but combination therapies with other targeted agents may be needed to block negative feedback loops in leukemia cells.

Introduction

The pathogenesis and pathophysiology of hematological malignancies involve aberrant regulation and function of cell signaling pathways, among which the phosphatidylinositol 3-kinase /AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) axis has a central role.[1,2] Dysregulation of this pathway resulting from oncogene amplification, oncogene activating mutations, inactivation of tumor suppressor genes, or upstream activation of receptor tyrosine kinases (RTK), has been demonstrated in a range of human malignancies.[1–3] As this axis is clearly an important target for developmental therapeutics, there are a number of small molecule inhibitors of elements of this network in development, including PI3K inhibitors (pan- and isoform-specific), AKT inhibitors (pan-, allosteric-, and ATP-competitive), mTOR inhibitors (rapalogs and TORC1/2 inhibitors), and dual PI3K-mTOR inhibitors.[3] Of these agents, the rapalog mTOR inhibitors, temsirolimus and everolimus, are FDA-approved for the treatment of renal cell carcinoma.[4] Everolimus is also approved for pancreatic neuroendocrine tumors and advanced hormone receptor-positive breast cancer.[5] The PI3Kδ inhibitor idelalisib is FDA-approved for the treatment of certain low-grade B-cell lymphomas.[6] None of these agents is FDA-approved for the treatment of acute myeloid leukemia (AML), a disease in which there is very strong preclinical evidence implicating this pathway in its pathogenesis and pathophysiology.[7-11] In one study, more than 90% of primary AML blasts had evidence of activation of AKT, a critical substrate of PI3K, as determined by the presence of phosphorylation or a positive kinase assay. [12] Recher et al showed that S6K and 4E-BP1, both downstream effectors of mTOR, were constitutively phosphorylated among the majority of freshly isolated AML samples from 23 patients but were not phosphorylated in normal freshly isolated CD34 control cells.[13] A number of potential mechanisms for the constitutive activation of this pathway have been implicated, including activating *RAS* mutations, *FLT3-ITD* mutations, activating *C-KIT* mutations, and PTEN phosphorylation or downregulation.[14]

AML is characterized by clonal expansion of early myeloid progenitor cells and some subtypes can be classified by distinct molecular and/or cytogenetic abnormalities. The annual incidence of AML in the United States is 30 to 40 cases per million individuals.[15] With a spectrum of failure to respond to induction therapy and relapse in the majority of adult patients who attain initial remissions, the overall prognosis is poor.[15] In some patients, leukemia-initiating stem cells may be responsible for relapse - their inherent resistance to standard cytotoxic agents and unique heterogeneity, resulting from clonal evolution involving quiescent subclones, pose a major problem for therapeutic strategies in AML.[16,17] Prognosis varies considerably according to cytogenetic and molecular abnormalities, but survival remains generally poor, emphasizing an unmet need for new effective therapies.[18-20] This review will discuss the scientific rationale for

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targeting PI3K/AKT/mTOR and related feedback pathways in AML. It will also review ongoing treatment strategies that include drugs with the ability to inhibit individual components of the pathway, combinations of these inhibitors for additive or synergist effects, and novel drugs with dual inhibitory activity. Data from clinical trials involving some of these agents for the treatment of patients with relapsed or refractory AML are also summarized.

PI3K/AKT/mTOR Pathway

PI3K (phosphatidylinositol 3′-kinase)

The important role of constitutive activation of the PI3K pathway in the pathogenesis of AML has been extensively documented.[12,21-24] While activating mutations in PI3K p85 regulatory subunit and p110 catalytic subunit have been described in Hodgkin lymphoma and various solid tumors, they are rarely seen in AML.[25,26] However, *RAS* mutations have been identified in 10–15% of AML and 25% of juvenile myelomonocytic leukemia cases and they can activate the PI3K/AKT/mTOR pathway with potential implications beyond promoting cell survival and proliferation, including remodeling of tumor microenvironment and modulation of tumor-induced immune suppression.[20,27-29] Nevertheless, there is no strong or definitive evidence of clinical benefit with single agent PI3K inhibitors in AML, but they hold promise when used in combination with inhibitors of other pathways, as described below.

Both inhibitors of class I PI3K isoforms (pan-PI3K) and isoform specific compounds are being investigated. A phase I trial is evaluating the pan-PI3K inhibitor BKM120 (buparlisib) in advanced acute leukemias based on preclinical evidence showing promising activity in acute lymphoblastic leukemia (ALL) (NCT01396499).[30] Isoform specific inhibitors of PI3K such as idelalisib have shown remarkable activity in lymphoid malignancies based on the importance of PI3Kδ in B-cell receptor signaling. Of note, idelalisib, a PI3Kδ-specific inhibitor, was recently FDA-approved for the treatment of relapsed chronic lymphocytic leukemia, follicular lymphoma, and small lymphocytic lymphoma. Despite frequent expression of PI3K-δ in AML cells and preclinical results showing activity of selective inhibitors, there is a lack of evidence of clinical efficacy of PI3K-δ inhibition in AML.[21,31] A dose-escalation trial involving idelalisib for the treatment of various hematologic malignancies, including relapsed/refractory AML, has been completed (NCT00710528) – the data has not yet been reported. The combinatorial approach of p110α specific inhibition with MEK inhibition in *RAS*-mutated AML seems promising as evidenced by the remarkable anti-tumor activity of BYL719 in combination with MEK162 in *RAS*-mutated AML cell lines and xenograft models.[32]

Another combinatorial strategy of specific interest in AML includes the blockade of both PI3K and downstream mTOR to block the escape mechanism of mTORC2 upregulation that can result in phosphorylation of AKT on Ser473, followed by activation and anti-apoptotic responses.[33] The similarities between the PI3K p110 subunit and mTOR catalytic domains have allowed the development of dual PI3K/mTOR inhibitors including BEZ-235.[34] BEZ-235 was found to induce significant apoptosis of primary AML blasts to a greater magnitude than the selective AKT and mTOR inhibitors, MK-2206 and rapamycin, respectively.[34] Notably, there was particular sensitivity among the AML blasts with MLL translocations, a subset in which 52% contained an *NRAS* or *KRAS* mutation as compared to 28% in cytogenetically normal AML blasts.[34] These results are in agreement with reports showing that *RAS* mutations can activate the PI3K/AKT/mTOR pathway.[35] As another example of the regulatory complexity of this pathway, treatment with BEZ-235 resulted in increased phosphorylation of ERK (extracellular regulated kinase) suggesting an escape mechanism for PI3K/AKT/mTOR inhibition. In turn, the combination of BEZ-235 with the MEK inhibitor AZD6244 demonstrated synergistic pro-apoptotic effects, providing additional rationale for the clinical development of BEZ-235.[34]

A phase I trial evaluated BEZ-235 in a cohort of 22 patients with refractory acute leukemia has been conducted.[36] The most frequent non-hematologic drug-related adverse events (AE) were stomatitis and GI toxicities. One patient with AML had stable disease for 4 months, and 3 responses were documented among patients with ALL (NCT01756118).[36] BEZ-235 is also being investigated in combination with nanoparticle formulations of chemotherapeutic agents (i.e. 5-fluorouracil) in other diseases, providing an innovative strategy that may be attractive in AML, especially if the nanoparticle platform can enhance drug delivery to the blasts or bone marrow microenvironment.[37] Based on extensive preclinical data, other dual inhibitors are in clinical development.[38-40]

AKT

The protein kinase B family of serine/threonine kinases (AKT1, AKT2, and AKT3) is a key effector of the PI3K pathway. PI3K phosphorylates phosphatidylinositol 4,5-biphosphate, generating phosphatidylinositol 3,4,5-triphosphate that in turn recruits proteins containing pleckstrin homology domains, such as AKT, to the cell membrane.[41,42] While in the cell membrane, AKT is phosphorylated at Thr308 and Ser473, events that lead to its activation and generation of downstream biological responses, including promotion of cell proliferation and anti-apoptotic signaling via several effectors.[41-48]

The importance of AKT in leukemogenesis was highlighted by the demonstration of constitutive activation of AKT in 70–86% of primary AML patient samples tested and the correlation of AKT activation with inferior survival.[9,49] The mechanisms of constitutive activation of AKT in AML seem to rely partially upon active upstream FLT3 as opposed to activating mutations in *PI3K* and *AKT*.[49] In fact, AKT is critical for the proliferative phenotype induced by FLT3-ITD.[49] AKT also contributes to chemotherapy resistance of AML blasts, while specific phosphorylation at Thr308 correlates with a high-risk cytogenetic profile.[50,51] Taken together, these results provide a firm rationale for the development of AKT inhibitors as therapies for AML.

The purine nucleoside analog triciribine (TCN-PM) inhibits AKT phosphorylation by interfering with AKT's PH domain and preventing its membrane localization.[52] Based on preclinical evidence of AKT inhibition, triciribine was evaluated among 41 patients with advanced hematological malignancies, including 36 patients with AML. The treatment had an acceptable toxicity profile and correlative studies demonstrated a significant reduction in the levels of pAKT (Ser473) and pBAD (Ser112).[52] While no objective responses were documented, stable disease among 17 patients suggested a potential role for this inhibitor.[52] The phospholipid analog, perifosine, inhibits AKT by altering lipid rafts and preventing the membrane localization of AKT, resulting in apoptosis of AML cell lines and enhancement of etoposide cytotoxicity.[53] These results provided the impetus for ongoing clinical studies of perifosine in refractory AML and other malignancies (NCT00391560).[54-57]

Another strategy utilized to block AKT has been the development of small molecule inhibitors such as MK-2206 and GSK690693.[58,59] MK-2206 is an oral non-ATP competitive allosteric inhibitor of AKT1, 2, and 3 and induces apoptosis and cell cycle arrest of AML cell lines. However, it demonstrated limited efficacy during a phase I study with only one response among 18 patients, leading to early termination of the trial.[60] The adverse AE profile was notable for grade 3/4 rash in 33% of patients.[60] Correlative studies suggested that the lack of efficacy resulted from limited inhibition of AKT and downstream targets (i.e., pFOX3A, pS6K, p4EBP1), coupled with upregulation of escape mechanisms including Bcl^{-}2, Smad3, and STAT-3.[60] Nevertheless, MK-2206 might have a role in combinations with other pathway inhibitors and/or chemotherapy, as suggested by preclinical evidence in other diseases.[58,61-63] GSK690693 has shown promising preclinical activity in acute lymphoblastic leukemia, but a phase I trial in advanced hematological malignancies was cancelled prior to enrollment of patients, and no data have been published in AML (NCT00666081).[64] Another pan-AKT small molecule inhibitor, GSK2110183, is being investigated in clinical trials for patients with multiple myeloma or refractory chronic lymphocytic leukemia (NCT01532700; NCT01428492).

Novel strategies attempting to enhance the efficacy of AKT inhibitors include use of nanoparticle formulations, combinations with other agents, and the development of AKT isoform-specific inhibitors.[65] In addition, testing these compounds specifically in patients with increased levels of pAKT or activating AKT mutations may lead to improved efficacy outcomes than would be observed among an unselected population.

PDK1 (phosphoinositide-dependent protein kinase-1)

PDK1 is part of the large AGC (protein kinase A, protein kinase G and protein kinase C) kinase family and regulates several other downstream effector kinases, including S6K (p70 ribosomal S6 kinase), SGK (serum- and glucocorticoid-induced protein kinase), PKC and AKT.[66] PDK1 is constitutively active and also has a PH domain that recruits this kinase to the cell membrane upon binding to PIP_3.[67] Upregulation of PDK1 downstream kinases is frequently observed in cancers with *PTEN* and *PIK3CA* mutations.[66,68] Hence, there is growing interest in the development of small molecule PDK1 inhibitors for the treatment of several malignancies including AML.[69-71]

Overexpression of PDK1 was observed in approximately 45% of AML patient samples and correlated strongly with protein kinase C (PKC) activation.[72] The highest levels of PDK1 were seen among the monocytic subtype of AML in agreement with the important role of PKC pathways in monocytic differentiation.[73] PDK1 overexpression was detected among 42% of patients with myelomonocytic AML and it was associated with worse overall survival (OS).[74] Furthermore, overexpression of PDK1 promoted survival of AML blasts by a PKC-dependent mechanism and these cells were sensitive to the PDK1 inhibitor BX-795.[74] The clinical-translational implications of these results, however, are limited by the lack of specificity of BX-795.[75,76] Lack of specificity has been a major drawback of several PDK1 inhibitors.[70] Nevertheless, other PDK1 inhibitors have shown preclinical efficacy in AML cell lines and xenograft models and more specific and potent PDK1 inhibitors might be incorporated into clinical trials for hematological malignancies in the near future.[71,77]

mTORC1/mTORC2

The mTOR pathway controls a number of critically important cell processes.[78] The mTOR kinase is present in 2 unique multiprotein complexes, mTOR complex 1 (mTORC1) and 2 (mTORC2).[79] In addition to mTOR kinase itself, mTORC1 consists of the following proteins/elements: RAPTOR, PRAS40, mLST8, and DEPTOR.[79] In general, mTORC1 promotes mRNA translation, inhibits autophagy, and acts as a signal integrator for various incoming signals. S6K1 and 4E-BP1 are known downstream mTORC1 effectors involved in the initiation of mRNA translation.[80] The mTORC2 complex is comprised of mTOR, RICTOR, PROTOR1, PROTOR2, mSIN1, mLST8, and DEPTOR and acts as a mediator of actin cytoskeletal organization, cell polarization, and pro-survival signals.[79]

The rapamycin analogs (rapalogs) act by selectively targeting mTORC1 by binding to its immunophilin, FK506 binding protein 12 (FKBP12), leading to inhibition of downstream mTOR signals.[14] Rapalogs have been shown to have antileukemic activity in AML, in the preclinical and clinical settings, as monotherapy and in combination with chemotherapy or other inhibitors of the PI3K/AKT/mTOR axis.[13,81-84] In general, the rapalogs' clinical activity in AML is modest at best (**Table 1**).[83,85-87] In a phase I trial that assessed the rapalog deferolimus among 23 AML patients, none achieved a response.[88] A phase I/II study of everolimus in 9 AML patients also resulted in no bone marrow responses.[81] However, the combination of everolimus with chemotherapy in the first relapse setting resulted in 68% complete response and median OS of 19 months.[89] Similarly, the combination of temsirolimus with clofarabine in elderly patients with AML showed encouraging results with an overall remission rate of 21% and median disease free survival of 3.5 months.[90]

Interest in mTOR inhibition in AML has shifted to using mTOR catalytic inhibitors (also referred to as active site inhibitors) or agents that have dual PI3K/mTOR or AKT/mTOR

Table 1. Clinical trials involving inhibitors of PI3K/AKT/mTOR pathway in AML

Target	Agent(s)	Reference or clinicaltrials.gov registry ID	Phase	n	Clinical setting	Efficacy outcome
mTORC1 (rapalog)	Rapamycin (Sirolimus)	Recher et al, 2005	pilot	9		4 w/ at least PR, DOR 28d; 1 SD
		Callera et al, 2008	I	7	Elderly pts with AML transformed from MDS	No responses
		Perl et al, 2009	I	29	c/w MEC	4 CR, 2 PR
		Boehm et al, 2009	pilot	5	5 patients	2 w/ WBC response
		Liesveld et al, 2013	I	13	c/w decitabine	After cycle 1, 4 w/ decline in blasts (1 CR), 4 SD, med OS 4 mo
	Deferolimus	Rizzieri et al, 2008	II	55	Multiple hem CAs (n: 23 AML, 2 MDS)	No AML/MDS responses but SD in 5 pts for 1–3 cycles
	Everolimus	Yee et al, 2006	I/II	27	Multiple hem CAs (n: 9 AML, 5 MDS)	1 pt had hem response
		Park et al, 2013	Ib	28	c/w '3+7'; pts in first relapse	19 (68%) CR, med OS 19.5 mo
		NCT00819546	I	—	w/ PKC-412 (FLT3i)	Results awaited
	Temsirolimus	Amadori et al, 2011	II	53	c/w clofarabine	CR/CRi 21%, DFS 3.5 mo, OS 4 mo
AKT	Triciribine	Sampath et al, 2013	I	43	Multiple hem CAs (n: 39 AML, 3 MDS/CMML)	No responses but SD in 17 pts after cycle 1
	MK-2206	Konopleva et al, 2014	II	19	2nd salvage for R/R AML	No responses except for 1 CRi
	GSK-2141795	NCT01907815	II	—	w/ trametinib (MEKi)	Ongoing trial
	GSK-2141795	NCT00881946	I	—	Multiple hem CAs	Results awaited
	BKM-120	NCT01396499	I	—		Ongoing trial
Dual PI3K/mTOR	BEZ-235	Wunderle et al, 2013	I	22	Multiple hem CAs (n: 11 AML)	SD in 4 AML pts, w/ DOR 4 mo
	BEZ-235	NCT01756118	I	—		Ongoing trial
PI3Kδ	Idelalisib	NCT00710528	I	—	Multiple hem CAs	Results awaited

AML, acute myeloid leukemia; CAs, cancers; CR, complete remission; CRi, complete remission with incomplete blood count recovery; c/w, combined with; DFS, disease free survival; DOR, duration of remission; FLT3i, inhibitor of fms-related tyrosine kinase 3; hem, hematologic; MDS, myelodysplastic syndrome; MEC, mitoxantrone/ etoposide/ cytarabine; mo, months; mTORC, mammalian target of rapamycin complex; n, number of patients; OS, overall survival; PI3K, phosphoinositide 3-kinase; PR, partial remission; pts, patients; R/R, relapsed or refractory; SD, stable disease; w/, with

activity and has been matched with more promising results.[7,91] The dual catalytic mTORC1/2 inhibitor OSI-027 has been shown to block mTORC1 and mTORC2 activities and to suppress mRNA translation of genes that mediate proliferation in AML cells, resulting in more potent antileukemic properties in vitro than rapamycin.[91] PP242, another dual catalytic mTORC1/2 inhibitor, led to apoptosis in primary AML samples and suppressed chemokine receptor CXCR4 expression in primary leukemic cells and in stromal cells co-cultured with leukemic cells.[11] In a mouse model, PP242 inhibited mTOR signaling in leukemic cells and reduced leukemia burden to a greater degree than that of rapamycin.[11] Promising findings were also observed with agent AZD8055, supporting its role as an mTORC1/2 inhibitor.[92] Among bone marrow samples from AML patients, it fully inhibited phosphorylation of 4EBP1, prevented the mTORC1-dependent PI3K/AKT feedback activation that has been observed with rapamycin, and promoted potent antileukemic effect in *in vitro* and *in vivo* preclinical models.[93] Decreased proliferation and induction of apoptosis among AML stem cells with high AKT/mTOR activity by another mTORC1/2 inhibitor, MLN0128, was recently reported.[94] MLN0128 was shown to selectively target mTORC1/2 downstream signaling in these cells with minimal effects on other pathways and without affecting non-leukemic stem cells. Compensatory activation of multiple pro-survival proteins was noted, among which was HDAC3. The use of the combination of MLN0128 and the histone deacetylase (HDAC) inhibitor vorinostat led to increased apoptosis as compared to that associated with single-agent MLN0128.[94]

Despite the promising results seen thus far with the dual catalytic mTORC1/2 inhibitors, there is concern that over time, resistance will develop by activation of other pathways. Therefore, there is interest in concomitantly inhibiting multiple pathways within the PI3K/AKT/mTOR axis and other escape mechanisms including autophagy, RAS/RAF/MEK/ERK pathway, MAPK-interacting kinases (MNKs), HDAC pathways, and PIM kinases.[95-99]

Combinatorial approaches to overcome escape mechanisms of resistance

As study of the PI3K/AKT/mTOR pathway has intensified, multiple regulatory escape mechanisms have been identified (**Fig. 1**). For instance, treatment with the dual mTORC1/2 inhibitors OSI-027 and AZD-2014 results in induction of autophagy in AML cell lines, as suggested by increased expression of LC3II, suppression p62/SQSTM1 proteins, and formation of autophagic structures, documented by electron microscopy.[99] Induction of autophagy in response to mTORC1/2 inhibition was also noted among samples from patients with AML. The addition of chloroquine, a known inhibitor of autophagy, potentiated the effects of mTORC1/2 inhibitors in reducing colony formation of primary AML cell lines. Additional experiments

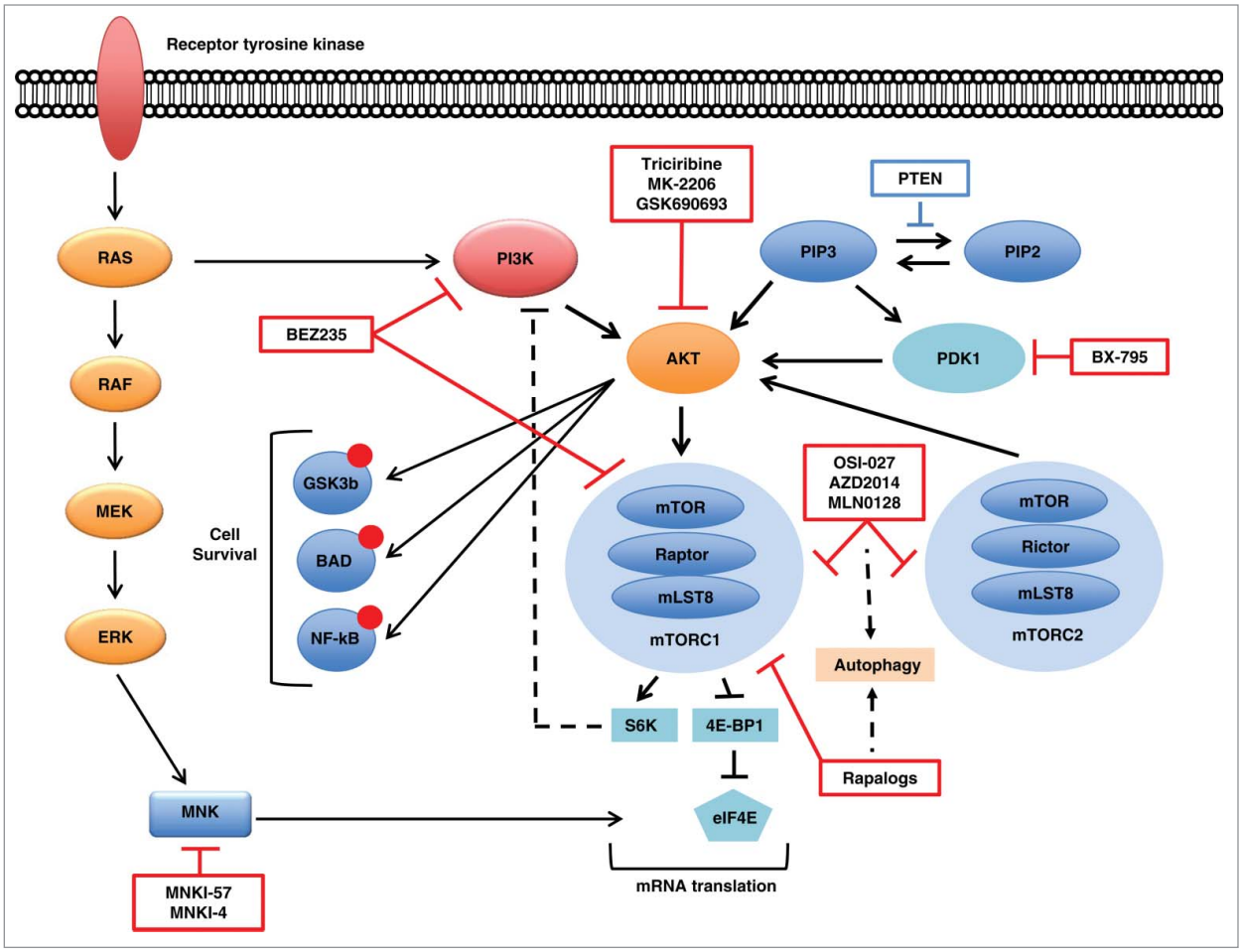

Figure 1. PIK3/AKT/mTOR signaling pathway in acute myeloid leukemia. Red boxes depict several inhibitors of specific pathway components undergoing preclinical or clinical studies. Dashed lines represent escape mechanisms of cell survival emerging after blockade of mTORC1 and/or mTORC2.

also suggested that induction of autophagy resulted during treatment with OSI-027 or AZD-2014 by preventing the inhibitory phosphorylation of ULK1 kinase (Ser757) that regulates autophagy.[100] These results have significant clinical implications in light of evidence showing the role of autophagy in chemotherapy resistance in AML models and might explain the limited efficacy of mTORC1 inhibitors in AML.[81,101,102] Furthermore, they provide support to develop combinations of autophagy inhibitors and mTORC1/mTORC2 blocking agents and/or chemotherapy for treatment of AML. The availability of chloroquine and more potent autophagy inhibitors (i.e., Lys05) coupled with encouraging preliminary results from trials in solid tumors will hopefully stimulate the conduct of related clinical studies in AML.[103-106]

Recent results have demonstrated paradoxical AKT phosphorylation in AML cells treated with AKT or PI3K inhibitors for 24 hours compared to the shorter duration treatment of 4 hours.[10] These inhibitors also increased the expression of IGF-1R, PDGFR and insulin receptors and induced their autophosphorylation. These effects ultimately result in sustained activation of the PI3K/AKT/mTOR pathway by a mechanism involving upregulation of the adaptor protein insulin receptor substrate 1. This feedback loop was successfully blocked by the combination of an AKT inhibitor with the PDGFR inhibitor sunitinib, the IGF-1R/IR inhibitor linsitinib, or the FLT3 inhibitor quizartinib.[10] Hence, these findings suggest that turning off the PI3K/AKT/mTOR axis goes beyond combining individual inhibitors of axis nodes and may require strategies accounting for the plasticity of feedback loops that emerge after exposure to certain drugs.[107] Altogether, these results epitomize the complexity of PI3K/AKT/mTOR pathway regulatory network and highlight the potential importance of incorporating receptor tyrosine kinase inhibitors into genomic-based treatment plans for AML patients.

Another important escape mechanism emerging from mTORC1 inhibition consists of activation of MAPK-interacting kinases (MNK1 and MNK2) that increase eIF4E phosphorylation and trigger protein synthesis and pro-survival signals.[108] Based on the convergence of RAS/RAF/MAPK and PI3K/AKT/mTOR pathways in oncogenesis and the evolving role of MNKs in hematological malignancies, the effect of MNK inhibitors alone or in combination with mTOR inhibitors is being investigated.[97,98,109] MNK2-specific and dual MNK1/2 inhibitors (MNKI-57 and MNKI-4) displayed potent anti-proliferative activity in chronic myeloid leukemia (CML) and AML cells.

Combination of rapamycin and MNK inhibitors led to a significant reduction of rapamycin-induced eIF4E phosphorylation and synergistically reduced proliferation of CML blasts (KYO-1 cells).[97] These findings are consistent with the emerging role of MNKs in CML blastic phase disease.[110]

Another example of the close relationship between these pathways is provided by ERK upregulation in AML cells treated with the PI3K/mTOR inhibitor BEZ-235.[34] In agreement with this result, the combination of BEZ-235 with a MEK inhibitor (AZD6244) demonstrated a synergistic pro-apoptotic effect. In fact, the limited efficacy of single agent selumetinib (MEK inhibitor) in refractory AML observed in a phase II trial might be explained by the concomitant activation of PI3K/AKT/mTOR as suggested by preclinical studies.[95,111] Lastly, combined treatment of AML cells with PIM (AZD1897) and AKT (AZD5363) inhibitors potentiated the blockade of the mTOR axis and resulted in marked anti-leukemic effects and induction of apoptosis, providing a blueprint for clinical development of this combination.[96] Another PIM inhibitor (LGH447) is currently undergoing a phase I trial for refractory AML and high-risk myelodysplastic syndrome as single agent (NCT02078609), but we are not aware of any trials exploring the combination of PIM and AKT inhibitors.

Summary

The aberrant activity of the PI3K/AKT/mTOR axis, modulating critical cellular functions such as proliferation, metabolism, and survival, has been well established as a major component of AML pathogenesis. Deregulation of this pathway may be the result of oncogene amplification and activating mutations, inactivation of tumor suppressor genes, or activation of RTKs. A number of classes of pharmacologic inhibitors of various nodes of this pathway in AML have been evaluated in the preclinical setting, resulting in promising signals of activity. Despite these results and the number of years spent accumulating evidence of this pathway's role in AML pathogenesis, there is still no evidence of meaningful clinical effectiveness for pharmacologic inhibition of this pathway as a treatment for AML. Nonetheless, the prospect of inhibiting this pathway in combination with other novel agents, based on previously discussed data to support this approach, remains highly encouraging and might be able to overcome some of the biological challenges of AML pathogenesis.

The complexity of this pathway's multiple regulatory mechanisms and feedback loops is evident. Aside from escape mechanisms inherent to inhibition of this axis, such as cross-talk from compensatory oncogenic pathways (e.g., RAS or MYC), feedback leading to rebound activation of PI3K after mTOR inhibition, and upregulation of upstream receptor tyrosine kinases, AML stem cells may be become resistant to very targeted interventions by recruiting other signaling molecules, modifying gene expression through epigenetic mechanisms, or altering components of the stromal microenvironment. Therefore, despite the remarkable advances secondary to the knowledge acquisition from AML genetic profiling and novel targeted therapies blocking PI3K/AKT/mTOR pathway, combinatorial approaches capable of blocking multiple escape mechanisms of resistance may be required for achieving clinically meaningful benefits. A phase III study demonstrating improved OS with the combination of BRAF and MEK inhibition over BRAF inhibition alone in patients with advanced *BRAF*-mutated melanoma provides proof of principle for mechanistically rational combinations of targeted therapies.[112]

What makes the combinatorial approach a challenging endeavor for designing clinical trials for most malignancies, however, is the increasing number of potential drug combinations in the setting of limited resources. In the case of targeting PI3K/AKT/mTOR in AML, one must consider the variety of classes of inhibitors available for each node of the pathway, including pan-PI3K inhibitors, isoform-specific PI3K inhibitors (and possibly agents that selectively target just 2 or 3 out of the 4 class I isoforms of PI3K), rapalogs, catalytic dual mTORC1/2 inhibitors, pan-PI3K/mTOR inhibitors, and AKT inhibitors. When considering targeting PI3K, for example, it may seem attractive to target a specific PI3K isoform, in an attempt to improve efficacy and decrease off-target toxicity. However, out of concern for functional redundancy of all 4 class I PI3K isoforms, pan-PI3K inhibition may be more efficacious albeit possibly more toxic, especially considering the vital role of PI3K in normal cells. The success of idelalisib, a PI3Kδ isoform inhibitor, among certain B-cell neoplasms may be explained by the differential expression of PI3Kδ among haematopoietic cells as compared to cells of other origin. In regard to mTOR inhibition, the further pursuit of the use of rapalogs in AML should likely be abandoned in favor of catalytic dual mTORC1/2 inhibitors (for reasons already discussed), but the question of whether to pursue rapalog use in combination with targets of other pathways (e.g.,, autophagy or PIM inhibitors) remains to be answered. Further adding to the complexity of designing appropriate trials is deciding among the many compensatory oncogenic pathways (e.g., RAS-RAF-MEK-ERK) to concurrently target, the interindividual genetic heterogeneity of the patients in the trial, and the intraindividual genetic heterogeneity of AML cells that can result from subclonal evolution.

One could consider performing multiple phase I trials in parallel, to identify optimal dosing for combinations of PI3K/AKT/mTOR inhibitors with one another and/or with agents that target other compensatory pathways, prioritizing the selection of agents and combinations based upon biologic rationale and preexisting preclinical evidence, followed by a phase II "pick the winner" protocol involving multiple combinations. The incorporation of correlative studies, performed at baseline and after initiation of treatment, such as pS6, p4EBP1, other established pharmacodynamic markers, tumor microenvironment markers, and high-throughput whole genome sequencing (i.e., next generation sequencing) will be imperative to help identify biomarkers for predicting response to therapy and toxicity. These challenges highlight the need for novel trial designs and collaboration among multiple institutions, cooperative groups, and biopharmaceutical companies.

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