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Exploiting Cellular Pathways to Develop New Treatment Strategies for AML

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Abstract

The standard approaches to the treatment of acute myeloid leukemia (AML) have been predominantly based on cytarabine and anthracyclines. Yet, the outcomes associated with AML continue to be poor, especially for those patients who are older or carry higher-risk disease. In recent years, extensive research has led to the development and study of novel agents which target AML by diverse and varied mechanisms. Among these are targeted therapeutics such as kinase inhibitors and oligonuceotide constructs. These aim to suppress the production or activity of proteins, such as FLT3 and BCL2, among others, and thus disrupt related signaling cascades essential for leukemogenesis and proliferation. In addition, other agents like flavopiridol appear to target the myeloid blast by various mechanisms including suppression of cyclin dependent kinases and interference with nucleotide synthesis. Another class of novel therapies includes inhibitors of histone deacetylase, which cause growth arrest and apoptosis through histone acetylation and resultant conformational changes. Clinical trials are now studying these and other agents alone and in combination with traditional cytotoxic therapies, with some encouraging results. In this review, we aim to provide a summary of the preclinical and clinical investigations of selected promising agents currently under study.

Keywords

Acute myeloid leukemia; Flavopiridol; HDAC inhibitor; Targeted therapies; PARP; FLT3

Introduction

Acute myeloid leukemia (AML) is characterized by an arrest in differentiation and uncontrolled proliferation of myeloid precursors in the bone marrow. This underlying process leads to hematopoietic insufficiency, and when undifferentiated cells escape the marrow, to significant

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leukocytosis, with often devastating and life-threatening sequelae. Although the majority of patients under age 60 achieve a complete remission (CR) with traditional anthracycline- and cytarabine-based induction regimens, the long-term survival rates continue to be poor at approximately 30–40% 1, 2. The prognosis is even poorer for those with high-risk AML, such as those who are older, who had preceding myelodysplastic syndromes (MDS) or myeloproliferative disorders (MPD), or those with secondary AML from environmental exposures or prior chemotherapy. In such cases, a complete remission is achieved in less than 40% of cases, with survival rates of less than 10% 2, 3.

Novel therapies to improve these unsatisfactory outcomes are aimed at developing agents which target cell signaling and cycling, as well as those which interrupt DNA repair and replication. Some of these endeavors are in early phases of development and study, while others have shown promise in preclinical and clinical investigation. The ultimate goal will be to broaden the therapeutic potential of traditional induction regimens in AML by the rational incorporation of mechanistically novel agents. In the current review, we have selected these promising approaches to discuss below.

Flavopiridol

Flavopiridol is a semi-synthetic flavone derived from the stem bark of *Amoora rohituka* and *Dysoxylum binectariferum*, plants used in India as herbal medicine 4. It has been demonstrated to have strong activity against multiple cyclin dependent kinases, and arrests the cell cycle at the G2/M phase and delays the G1 to S phase progression 5. Flavopiridol also inactivates the cdk-9/cyclin T complex, also known as PTEF-b, resulting in inhibition of RNA polymerase II, and suppression of RNA and polypeptide synthesis. This transcriptional inhibition leads to a decrease in levels of proteins, such as cyclin D1, VEGF, MCL-1, and STAT-3, essential for cell cycling and survival 6–8. In addition, flavopiridol is active to a lesser degree on tyrosine kinases, such as the epidermal growth factor receptor (EGFR), protein kinase C (PKC)and Erk 5 (Table 1).

In preclinical studies, flavopiridol was active in diverse hematopoietic cell lines 9, 10. In AML, its novel mechanism of action and ability to target both cycling and non-cycling cells in vitro has rendered flavopiridol an intriguing candidate for combination with traditional cytotoxic therapies. When administered concomitantly with cytarabine and topotecan, S-phase dependent agents, it produces antagonistic effects through its propensity to induce cell cycle arrest 11. However, it was noted that when flavopiridol administration and withdrawal preceded cytarabine and topotecan, dormant surviving cells were allowed to re-enter the cell cycle and were thus further sensitized to the latter agents 7, 11.

Clinical trials based on the in vitro model findings are in progress. In these studies, flavopiridol is administered as an initial cytoreductive agent for 3 days, following which the remaining leukemic cells could be recruited into the cell cycle and thus be kinetically sensitized for cytotoxicity by the 72 hour continuous administration of cytarabine beginning on day 6 and mitoxantrone on day 9 12, 13. In a recent phase II study of this regimen (FLAM) in 62 patients with poor-risk AML, flavopiridol was directly cytotoxic, with 44% of patients experiencing ≥50% decrease in peripheral blasts by day 2 and 26% experiencing ≥80% decrease in blasts by day 3. CRs were achieved in 75% of patients with newly diagnosed secondary AML and those with first relapse after short CR. Rates of CR were significantly lower for those with refractory disease. Disease free survival (DFS) for all CR patients was 40% at 2 years 13. These results have recently been expanded to another cohort of 45 patients with newly diagnosed, poor-risk AML. Of these, 67% achieved CR and 40% underwent a myeloablative allogeneic bone marrow transplant (BMT) in first CR, translating into long-term survival 14.

Alternative dosing schedules of flavopiridol are also being studied. A “hybrid” bolus-infusion schedule of flavopiridol has been investigated in CLL with promising results. In this approach, a pharmacologically-modeled schedule of flavopiridol is administered, with a 30 minute bolus of roughly half of the total dose, followed by a 4 hr infusion of the remaining portion, in an attempt to overcome the observed effects of avid binding of flavopiridol by human plasma proteins [15, 16]. This hybrid schedule of flavopiridol administration is currently being studied in a dose-escalation, phase I trial of patients with primary refractory and relapsed AML (clinicaltrials.gov, NCT00470197). Correlative in vivo pharmacodynamic studies demonstrate flavopiridol-induced suppression of target genes, including MCL-1, VEGF, E2F1, STAT-3, cyclin D1, and RNA polymerase II [17]. Another ongoing study, a phase II trial comparing the hybrid infusion of flavopiridol with bolus administration of the drug in patients with newly diagnosed, poor-risk AML is currently recruiting (clinicaltrials.gov, NCT00795002).

Flavopiridol has been combined with other novel targeted therapies to enhance antileukemic efficacy. Among these are histone deacetylase inhibitors (HDIs), which allow for acetylation of histones with resultant conformational changes and transcription of genes that allow differentiation, growth arrest, and/or apoptosis 18. Interestingly, HDIs up-regulate the expression of MCL-1, an antiapoptotic member of the bcl-2 family 19, and p21, a cyclin dependent kinase (CDK) inhibitor 20, which together can limit the cytotoxic efficacy of these agents. Therefore, therapies that can down-regulate expression MCL-1 and p21, such as flavopiridol, may be synergistically efficacious in combination with HDIs. Indeed, the HDI-mediated decrease in induction of p21 appears to be interrupted by flavopiridol, leading to a potentiation of apoptosis in human leukemia cells 19–22. The HDI, suberoylanilide hydroxamic acid (vorinostat; SAHA), has been combined with flavopiridol in preclinical studies, with synergistic induction of apoptosis through mitochondrial damage, cell cycle dysregulation, and caspase activation 18. Currently, a phase I trial of SAHA and flavopiridol in patients with relapsed/poor prognosis acute leukemia or advanced MDS is underway and enrolling patients (clinicaltrials.gov, NCT 00278330).

Other HDI-related strategies

In view of their pleiotropic mechanisms of action, HDIs lend themselves particularly well to combination regimens involving other targeted agents, in addition to the one described above in the case of flavopiridol. HDIs have been broadly classified as pan-HDIs, such as the hydroxamates vorinostat, belinostat (PXD101), and panobinostat (LBH-589), which inhibit multiple HDAC classes (e.g. Class I and II), and those whose actions are primarily directed against a single class (e.g., Class I), such as SNDX-275 and MGCD0103. Aside from their capacity to modulate gene expression by altering chromatin structure, HDIs induce cell death through multiple other mechanisms, in some cases a consequence of acetylation of non-histone proteins. For example, in human leukemia cells, HDI lethality has been related to up-regulation of death receptors 23. Other postulated mechanisms of lethality include induction of oxidative damage 24, 25, acetylation of and interference with the function of chaperone proteins such as Hsp90 26, acetylation and disruption of the function of DNA repair proteins (e.g., Ku70) [27], up-regulation of pro-apoptotic proteins such as Bim 28, and disruption of cell cycle checkpoints 29. Finally, HDIs may act by interfering with the contribution of HDACs to co-repressor complexes responsible for the block to leukemic cell maturation 30. Initial results of clinical trials suggest that HDIs, including the HDIs vorinostat and the Class I-specific HDI MGCD0103, may have some single agent activity in refractory AML [31, 32].

However, because of their diverse mechanisms of action, attention has begun to focus on the capacity of HDIs to potentiate the antileukemic activity of other targeted agents. For example, mutant tyrosine kinases, including those implicated in AML such as FLT3 (see below), appear to be particularly dependent upon intact chaperone function for their maintenance. This raises

the possibility that HDIs, at least those capable of inhibiting deacetylation of Hsp90, might enhance the activity of clinically relevant FLT3 inhibitors by down-regulating the expression of Hsp90. Indeed, the results of preclinical studies suggest that co-administration of pan-HDIs with FLT3 inhibitors results in a pronounced increase in antileukemic activity [33]. Such findings support the concept of combining HDIs with tyrosine kinase inhibitors such as FLT3 inhibitors in refractory AML.

Another rational HDI combination strategy of potential relevance to AML involves the use of proteasome inhibitors. Preclinical studies indicate that HDIs interact synergistically with proteasome inhibitors such as bortezomib in diverse malignant hematopoietic cell types, including myeloid leukemia, CLL, and myeloma [34–36]. The mechanisms underlying such interactions may be multi-factorial, including inhibition of NF-κB activation as well as disruption of aggresome formation, leading to ER stress [26]. Notably, a regimen combining vorinostat with bortezomib has shown significant activity in patients with refractory multiple myeloma [37]. Although proteasome inhibitors have relatively modest single agent activity in AML [38], the possibility that co-adminstration of proteasome and deactylase inhibitors may yield superior activity seems to be a plausible one. Consequently, Phase I trials of HDIs in combination with bortezomib are underway.

By promoting a more open chromatin structure, HDIs render transformed cells more susceptible to agents that interfere with DNA function and integrity. For example, pretreatment of breast cancer cells with vorinostat significantly potentiated the lethal effects of topoisomerase II inhibitors 39. Analogously, pretreatment of human leukemia cells with vorinostat sensitized them to the lethal effects of VP-16 and ara-C [40]. A clinical trial combining vorinostat with cytotoxic chemotherapy (e.g., idarubicin and ara-C) is underway.

Over the last several years, attention has focused on a strategy combining HDIs with hypomethylating agents for the treatment of various malignancies, including AML. This is based on the concept that silencing of genes implicated in leukemogenesis may be overcome by hypomethylating agents such as the DNA methyltransferease inhibitors (DNMTIs) 5-azacytidine or deoxyazacytidine. Furthermore, reversal of silencing of such genes by DNMTIs combined with disruption of the activity of HDAC-associated co-repressor complexes (by HDIs) may allow full expression of genes responsible for cell differentiation and death. Multiple preclinical studies have shown synergistic induction of cell death by regimens combining HDIs and DNMTIs 41, including those involving leukemia cells 42. Based upon this rationale, multiple HDI/DNMTI trials are underway in AML and MDs e.g., 5-azacytidine and SNDX-275 or 5-deoxyazacytine and valproic acid), and initial results appear potentially promising, particularly in patients who present with high-risk disease 43. One key question remaining to be resolved is whether such regimens act through de-repression of cell death or differentiation-related genes, or more directly through cytotoxic actions.

New anti-FLT3 Targeted Agents

Despite an exciting rationale for the use of tyrosine kinase inhibitors (TKIs) in AML, the clinical results have so far been modest. The most advanced studies involve inhibitors of the FMS-like tyrosine kinase-3 (FLT3) receptor. Approximately a third of patients with a diagnosis of AML carry a FLT3 internal tandem duplication (ITD) mutation, which renders the kinase constitutively active in driving the proliferation of the leukemic blast 44. The preponderance of current data suggests that an ITD mutation is a significant, independent, negative prognostic predictor in AML, with disease-free and overall survival severely and adversely affected 45–47. Development of targeted therapy against FLT3 is rapidly evolving. A number of small molecule FLT3 inhibitors have been studied beyond phase I investigation in patients with AML, including two indolocarbazole derivatives, midostaurin (PKC412), and lestaurtinib, and

have been reviewed elsewhere 48–57. In this review, we will focus on promising FLT3 inhibitors in earlier phases of clinical development.

Sorafenib, a multi-kinase inhibitor, was initially developed to inhibit the Raf-1 kinase pathway. It has since been demonstrated to be a potent inhibitor of multiple receptor tyrosine kinases, including FLT3 [58, 59]. Sorafenib has been approved for use in advanced renal cell and hepatocellular carcinomas, after improving survival parameters in clinical trials [60, 61]. Targets of sorafenib, such as FLT3, c-KIT, NRAS, and Raf kinase, are frequently mutated in AML. Together, these mutations seem to promote proliferation and arrest of differentiation in hematopoietic progenitor cells [62]. Preclinical studies in FLT3-driven leukemic cell lines, primary samples, and xenograft models have revealed that sorafenib suppresses FLT3 signaling and promotes apoptosis [63, 64].

Emerging data suggest that sorafenib is well tolerated as a single agent in high-risk AML, with some patients experiencing impressive clinical responses. Earlier studies revealed transient, but significant, decreases in bone marrow blasts, particularly in patients with FLT3-ITD mutations 65, 66. Sorafenib was subsequently employed on a compassionate use-basis in a limited number of FLT3-ITD AML patients both prior to and after allogeneic stem cell transplantation. Two of three patients with refractory disease, who were given sorafenib, were able to proceed to transplant after remissions, suggesting that sorafenib can effectively reduce leukemic burdens in patients awaiting stem cell transplantation. Additionally, prolonged complete molecular remissions were noted in the few patients given sorafenib after transplant in this study [67]. A phase I/II trial in patients with newly diagnosed AML found that sorafenib, when combined with cyrtarabine- and idarubicin-based induction, produced complete remissions in the majority, 22 of 25 evaluated patients (88%). Eight of these patients had FLT3-ITD mutations, and the drug was noted to effectively suppress FLT3-phosphorylation in correlative studies [68]. Other ongoing clinical trials are evaluating the safety and efficacy of sorafenib in combination with clofarabine, vorinostat, and various induction regimens (clinicaltrials.gov, NCT00516828, NCT00908167, NCT00893373, NCT00875745).

KW-2449, a promising multi-kinase inhibitor that effectively suppresses FLT3 phosphorylation, inhibited growth of leukemia cell lines and suppressed phosphorylation of FLT3 and its downstream target, STAT5. A phase I trial of KW-2449 demonstrated modest single agent clinical activity in 8 of 31 AML patients (26%), including 5 with FLT3 mutations [69]. These responses were often transient decreases in blasts, likely due to transitory FLT3 inhibition. Correlative studies are defining optimal administration schedules to achieve the sustained target inhibition necessary for ideal clinical responses 70. KW-2449 is also an aurora kinase inhibitor 71, and it is possible that this action may contribute to the antileukemic activity of this compound.

AC220 is a receptor tyrosine kinase inhibitor (TKI), demonstrated to have potent and specific in vitro and in vivo activity against the FLT3 tyrosine kinase. A phase I study in relapsed or refractory AML is currently under way, with promising preliminary results. Eleven of 45 patients (24%) have experienced transient clinical responses, with 4 achieving CRs (2 patients with incomplete platelet recovery and 2 with incomplete platelet and neutrophil recovery). An additional 7 patients had partial responses. Of note, three of the responders were FLT3 mutants [72]. These very promising results may be due to the exceptional potency and selectivity of AC220 when compared to other TKIs, as well as its ability to effectively suppress both wild-type and mutated FLT3 tyrosine kinases 73, 74.

Studies of AML cell lines have further identified an up-regulation of the serine/threonine kinase PIM (proviral integration site for Moloney murine leukemia), a downstream target of FLT3. PIM, currently under extensive investigation, appears to play an important mediating role in

signaling cascades and is felt to directly suppress the pro-apoptotic BAD [75, 76]. More recent investigation has revealed that PIM may be an integral component of FLT-3 signaling complex in FLT3-ITD cell lines, and that inhibitors of PIM appear to be preferentially cytotoxic to FLT3-ITD AML cell lines and primary patient samples. Furthermore, PIM inhibition appears to lead to a suppression of phosphorylation of STAT5 as well as Akt, and therefore may affect cell survival through these signaling pathways, in addition to its affect on BAD phosphorylation [77]. Targeted agents against PIM are in early stages of development and study [78] (clinicaltrials.gov, NCT00848601), but may play an important role for the treatment in AML in the future.

Inhibitors of the PI3-K/Akt/mTOR Signal Transduction Pathways

The phosphatidylinositol 3-kinase (PI3-K)/Akt/mammalian target of rapamycin (mTOR) signal transduction pathways are vital intracellular cascades which regulate translation, ribosomal biogenesis, cell cycling, and apoptosis. Its intricacies have been extensively reviewed elsewhere [79]. In brief, PI3-K is activated when bound by a variety of receptor tyrosine kinases, such as FLT3, EGFR, and HER-2/neu (Figure 1). PI3-K converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the inner surface of the membrane. Phosphoinositide-dependent kinase (PDK1) and Akt are then recruited to the membrane by PIP3. Akt, an important mediator in the intracellular cascade, is subsequently activated by PDK1 and acts on down-stream enzymes to stimulate proliferation and inhibit pro-apoptotic signals [80]. As examples, it suppresses p27Kip1, a direct inhibitor of cdk-2, which is then free and able to promote transcription and resultant cell proliferation [81], and inhibits the pro-apoptotic bcl-2 antagonist of cell death (BAD) [82]. Another target enzyme is tuberous sclerosis protein 2 (TSC2), which when phosphorylated, releases the protein Rheb to interact with and activate the mTOR kinase. mTOR, an important mediator, is involved in the progression from G1 phase to S when essential factors are available for cell division [80]. mTOR's targets include p70S6K, an activator of the ribosomal machinery and protein synthesis, and 4E-BP1, which promotes translation of RNA. Activation of these enzymes leads to enhanced synthesis of essential proteins in cell cycling and survival [80]. Recent studies have also linked nucleophosmin (NPM) as an important mediator of mTOR dependent proliferation in oncogenesis [83].

Alterations in one or more components of the PI3-K/Akt/mTOR pathway have been noted in diverse neoplasms, including AML. Mutations of key enzymes can lead to increased constitutive signaling, with resultant survival and proliferation of malignant cells, and resistance to chemotherapy [84, 85]. This survival can be suppressed by inhibiting the activity of PI3-K cascade, leading to the dephosphrylation of BAD and subsequent apoptosis [86, 87]. Constitutive activation of the PI3-K/Akt signaling cascade is readily detectable in 50 to 70% of patients with AML [82, 88]. Additionally, FLT3-ITD mutations lead to constitutive activation of the PI3-K/Akt cascade, promoting cell survival and proliferation [89]. The mTOR pathway is also up-regulated, with targets, such as p70S6K and 4E-BP1, constitutively phosphorylated in the majority of AML samples [90]. Dysfunction and down-regulation of the TSC1/TSC2 complex, a protein suppressor up-stream of mTOR, has also been linked to increased mTOR activity [91]. Given the above observations, there is a burgeoning rationale for the therapeutic targeting of one or more members of the PI3-K/Akt/mTOR cascade for diverse malignancies, including AML (Table 2).

One member, the mTOR protein, is being extensively investigated for therapeutic potential in AML. Rapamycin (sirolimus), an antibiotic derived from the bacterial species *streptococcus hygroscopicus*, was initially approved, and has been extensively used as an immunosuppressant [92]. However, it has been shown to also effectively inhibit mTOR when complexed with the FK506 binding protein 12 (FKBP12) [79]. As a result, it has been employed to target the PI3-

K/Akt/mTOR pathway in malignancies. An ester derivative of sirolimus, temsirolimus, and other mTOR inhibitors, such as everolimus (RAD001) and deforolimus, have also been studied as antineoplastic agents 93. These appear to have complex effects on the PI3-K/Akt/mTOR cascade. For example, some have found that temsirolimus and everolimus, in addition to their effects on mTOR signaling, additionally block the activity of Akt. This appears to be mediated through suppression of the newly discovered rictor/mTOR protein complex (mTORC2), which phosphorylates and activates Akt 94, 95.

Rapamycin has been demonstrated to effectively suppress leukemic cell lines and arrest the cell cycle at the G1 phase, which correlates with an up-regulation of the cdk inhibitor, p27kip1. The constitutive phosphorylation of down-stream targets of mTOR, p70S6K and 4E-BP1, was suppressed with the administration of rapamycin. A pilot clinical study of daily rapamycin in nine patients with refractory or relapsed AML produced 4 partial responses [90]. Another small study of rapamycin in MDS-derived secondary AML in patients over the age of 65 demonstrated no clinical responses [96]. A phase I/II study of temsirolimus in patients with hematologic malignancies included nine patients with AML and five with MDS. Of the latter, two patients achieved minor hematologic responses. The study also demonstrated that the phosphorylation of downstream targets of mTOR were effectively suppressed [97].

mTOR inhibitors are also being studied in combination with traditional cytotoxic therapies. In preclinical investigation, sirolimus dramatically increased the cytotoxicity of cytarabine and etoposide against AML blasts [85, 98]. Multiple clinical trials are now under way to evaluate mTOR inhibitors in combination with traditional AML therapies for patients with poor risk AML (clinicaltrials.gov, NCT00235560, NCT00780104). Of these, the Eastern Cooperative Oncology Group is recruiting patients into a phase II randomized trial comparing three combination chemotherapy regimens for relapsed/refractory AML. One arm of this multi-center study will investigate the combination of sirolimus, mitoxantrone, etoposide, and cytarabine (clinicaltrials.gov, NCT00634244).

Bcl-2 Targeted Agents

Bcl-2, often up-regulated in AML, is a mitochondrial protein that impedes apoptosis. Patients with higher levels of bcl-2 expression have poorer prognoses, with lower rates of complete remission and worse survival, possibly due to the contribution of bcl-2 to chemotherapy resistance [99, 100]. Therefore, suppressing bcl-2 has been pursued as a therapeutic approach, leading to the development of multiple potential therapeutic agents (Table 3).

Antisense oligonucleotides are short sequences of single-stranded deoxyribonucleotides that complement and bind specific coding regions on mRNA, forming DNA-mRNA complexes which are subsequently degraded. In this manner, the ultimate translation of the targeted protein is prevented. Oblimersen (Genasense), a phosphorothioate, 18-base oligonucleotide, was found in preclinical studies to effectively suppress bcl-2 mRNA expression [101]. A Phase I trial of oblimersen combined with FLAG (fludarabine, cytarabine, and GCSF) salvage therapy in relapsed/refractory AML yielded a 29% CR rate, as well as evidence of decreased Bcl-2 mRNA and protein expression 102. In the setting of newly diagnosed AML in older patients, the combination of oblimersen with traditional cytarabine/anthracycline based regimens yielded a 48% CR rate 103. These results affirmed the safety of combining this agent with traditional regimens. Unfortunately, a randomized, phase III trial of older patients failed to show improved outcomes for those receiving the combination with oblimersen 104.

Another anti-apoptotic protein is XIAP (X-linked suppressor of apoptosis), which binds and inhibits the caspases 3, 7 and 9, essential down-stream mediators of the apoptotic cascade. Like bcl-2, XIAP is over-expressed in AML, may be involved in leukemic cell survival and drug resistance, and when highly expressed, linked to poor clinical outcomes 105. Inhibitors of XIAP

have been shown to activate downstream caspases and promote apoptosis in AML cell lines 106. AEG35156 is a 19-base, antisense phosphorothioate, which effectively suppressed XIAP mRNA and protein levels in preclinical models 107. A phase I/II trial of AEG35156 in combination with re-induction therapy was recently completed in refractory/relapsed AML patients. In the phase I portion of the study, 24 patients were treated with escalating doses of AEG35156 and one achieved a CR. In the subsequent phase II trial, 32 patients were treated with the highest planned dose, and of these, 15 (47%) achieved a CR/CRp. Importantly, this regimen was not efficacious in patients with multi-refractory AML. However, of 11 patients who were refractory to single induction regimen, 10 (91%) experienced a CR/CRp. XIAP mRNA levels from patient blasts were quantified by RT-PCR, and their suppression was detected 108, 109.

PARP Inhibitors

Poly ADP-ribosylation is known to occur after single or double-stranded DNA damage, a process of post-translational modification of histones and other nuclear proteins by PARP (poly ADP ribosylation polymerase). The PARP superfamily consists of multiple nuclear proteins, of which PARP-1 and PARP-2 appear to play a central role in repairing DNA damage. PARP binds DNA by the zinc-finger motif of its N-terminal, recruiting other essential enzymes, and bringing about base excision repair (BER) [110–112]. Increased PARP activity is one of the mechanisms by which tumor cells avoid apoptosis caused by DNA damaging agents 113, 114, and thus has been considered as a target for anti-neoplastic therapy. Inhibition of PARP sensitizes tumor cells to cytotoxic agents which induce DNA damage that would be normally repaired through the BER system [115, 116].

The promise of clinical activity for PARP inhibitors was increased by the recent demonstration of prolonged survival in breast cancer patients with metastatic triple-negative disease [117]. Although in earlier phases of investigation and development, PARP inhibition is also being actively investigated in AML [118]. One agent, ABT-888, a potent inhibitor of PARP-1 and -2, has been demonstrated to potentiate the cytotoxic effects of temozolamide, platinum agents, cyclophosphamide, and radiation [119]. ABT-888 has since been studied in an early phase study, and demonstrated proof of target inhibition of PARP in tumor biopsies and peripheral blood samples [120]. A phase I clinical trial of ABT-888 in combination with topotecan and carboplatin in patients with high-risk MDS or relapsed/refractory AML is currently recruiting patients (clinicaltrials.gov, NCT 00588991).

MEK1/2 Inhibitors

The Ras/Raf/MEK1/2/ERK1/2 pathway, referred to as the mitogen-activated protein kinase (MAPK) pathway is frequently dysregulated in cancer, including hematologic malignancies such as AML 121, 122. The Raf family (Raf-1, A-Raf, B-Raf) signals downstream to phosphorylate the mitogen-associated/extracellular regulated kinases 1/2 (MEK1/2), which in turn phosphorylate extracellular regulated kinases 1 and 2 (ERK1/2) on threonine and tyrosine residues. ERK1/2 is involved in phosphorylation of multiple substrates implicated in cell survival and proliferation. These include p90RSK1, which activates the CREB transcription factor, and, following nuclear translocation, the Fos and Elk1 transcription factors 123. In addition, ERK1/2 modulates the expression, in some cases through phosphorylation, of multiple Bcl-2 family members and components of the apoptotic apparatus, including Bcl-2, Bim, Bad, survivin, and caspase-9 124. Thus, this pathway has become a major target for therapeutic intervention. In addition to inhibitors of upstream components of the pathway, including Ras and Raf, attention has recently focused on inhibitors of MEK1/2.

In preclinical studies, MEK1/2 inhibitors such as PD98059 and PD184352 have been shown to inhibit the growth and survival of AML cells, and to sensitize them to retinoids and standard

chemotherapeutic agents 125. MEK1/2 inhibitors have also been shown to enhance the antileukemic activities of other targeted agents, including Mdm2 126 and Bcl-2 antagonists [127]. The first MEK1/2 inhibitor to enter the clinic, PD325901 (Pfizer), has not been tested in AML, but plans are underway to evaluate several newer MEK1/2 inhibitors in this disease, including AZD6244 (Astra Zeneca), AS703026 (EMD Serono), and GSK1120212 (Glaxo-Smith-Kline). Finally, in view of evidence that simultaneous interruption of the Ras/Raf/MEK1/2/ERK1/2 and PI3K/Akt/mTOR pathways markedly increases transformed cell lethality [128], combination of MEK1/2 with PI3K or mTOR inhibitors represents an intriguing future possibility for the treatment of AML.

Conclusion and Future Directions

AML therapy continues to be a daunting challenge. Survival has not changed significantly for years, and new strategies are needed. Over the last decade, investigators have evaluated multiple approaches in targeting the survival, cycling, and proliferation of AML blasts. Attempts at impeding DNA repair, interrupting up-regulated signaling cascades, and targeting epigenetic modulation are ongoing as investigational approaches. Some agents, such as flavopiridol have already demonstrated promise in serially designed clinical trials. Others, such as those targeting individual signaling proteins, are in earlier phases of investigation and development. Additionally, in this review, we have chosen not to include discussion on certain emerging therapies in AML, such as hypomethylating agents and tipifarnib. These promising approaches merit detailed and wide-ranging discussion beyond the scope of our review, and we refer the reader to extensive reviews in the literature [129–132]. Future directions for therapeutic exploitation in AML may include immuno-modulation with vaccines, investigating the leukemic microenvironment, targeting leukemic stem cells, and targeting oncogenic fusion proteins or transcription factors implicated in leukemogenesis (e.g. AML-ETO, MLL etc).

It is now clear that mutation or upregulation in one pathway does not account for AML transformation. Blasts rely on multiple dysregulated pathways to emerge and survive, and to ultimately develop resistance to therapy. Therefore, pursuing several molecular lesions in a concurrent or serial fashion may be a promising approach to targeted therapy. This pursuit has been advanced by a better understanding of the nature of defects underlying AML. These have been described as either class I mutations, compromising of alterations in genes for integral components of signal transduction and promoting increased survival and proliferation, or class II inactivating mutations, leading to chromosomal aberrations which target core binding factors with resultant disruption of differentiation [133, 134]. Finally, targeted agents should also be considered for and could be incorporated into maintenance regimens after induction therapy, particularly for those patients with minimal residual disease. All in all, it is hoped that the ongoing progress in expanding novel therapies will soon yield useful adjuncts to the therapy of AML and significantly improve its currently poor prognosis.

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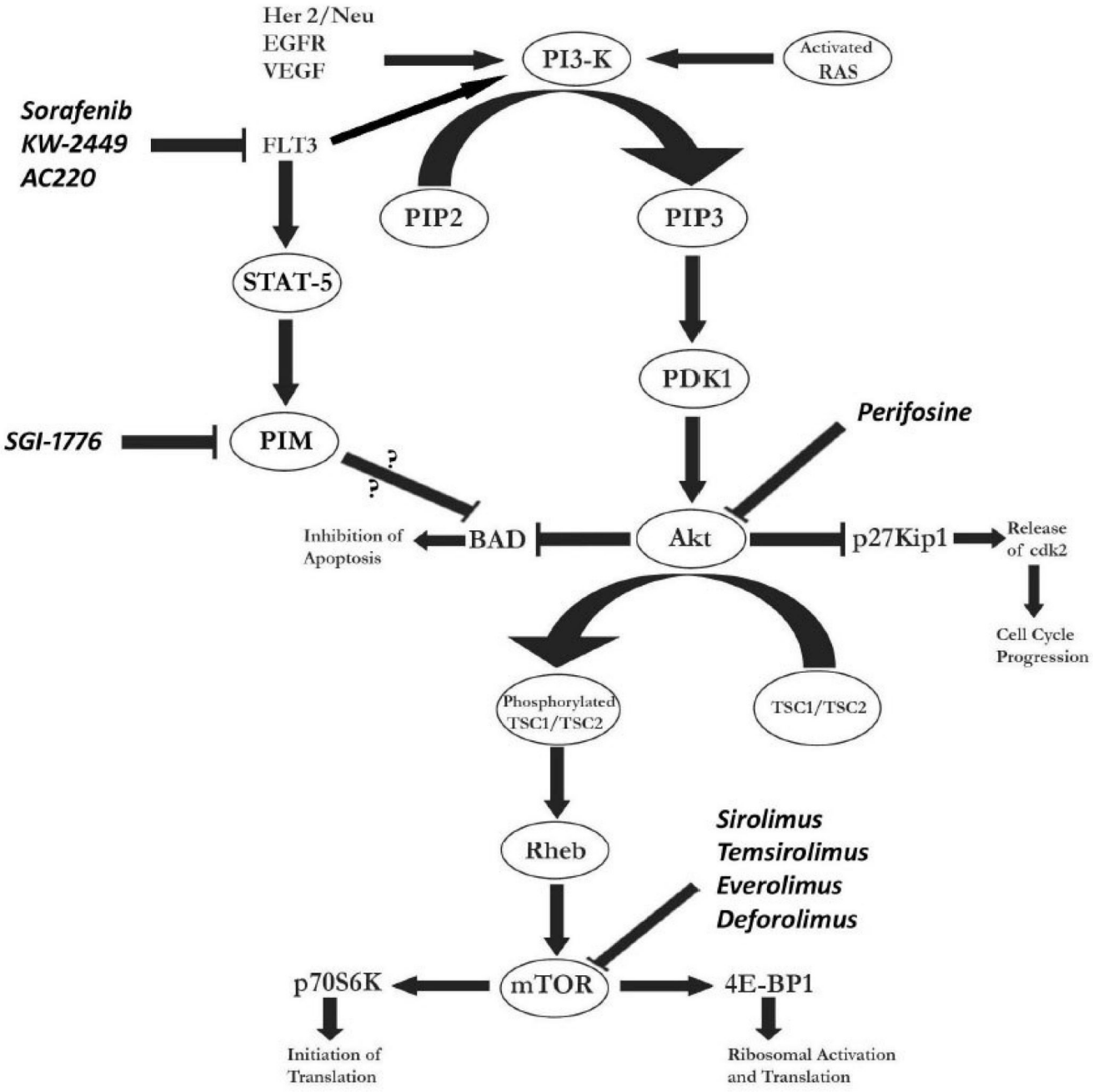

Figure 1.

The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and FMS-like tyrosine kinase 3 (FLT3) cascades in acute myeloid leukemia, and relevant targeted therapies. BAD—bcl-2 antagonist of cell death; CDK2—cyclin-dependent kinase 2; EGFR—epidermal growth factor receptor; PDK1—phosphoinositide-dependent kinase 1; PIP2—phosphatidylinositol-4,5-bisphosphate; PIP3—phosphatidylinositol-3,4,5-trisphosphate; TSC1, TSC2—tuberous sclerosis proteins 1 and 2; VEGF—vascular endothelial growth factor; PIM—Proviral integration site for Moloney murine leukemia virus.

Table 1

Mechanistic Targets of Flavopiridol [5–8]

Action of Flavopiridol	Impact on cell survival and proliferation
Inhibition of serine-threonine CDKs through non-cell cycle dependent and cycle dependent mechanisms	Cell cycle arrest at the G1-S and G2-M checkpoints.
Decrease in the activty of VEGF	Inhibition of angiogenesis and cell growth.
Binding and inactivation of the CDK9/Cyclin T1 complex (PTEFb)	Inhibition of the RNA polymerase II complex and resultant blockade of transcriptional elongation.
Binding to DNA and disruption of transcription	Disruption of DNA binding to key transcription factors such as STAT3, leading to a decrease in the expression of the target proteins like Mcl-1.
Inhibition of tyrosine kinases e.g EGFR, Erk, etc.	Inhibition of constitutive activation of receptors and downstream kinases, leading to a decrease in proliferation and survival.

Table 2

New Therapies in AML: Targeting the PI3-K/mTOR Cascade

Target	Compound(s)	Mechanism
PI3-K	CAL-101	Small molecule inhibitor of the delta isoform of the 110 kDa catalytic subunit of class IA PI3-K (clinicaltrials.gov, NCT00710528)
	PI-103	Small molecule inhibitor of PI3-K and mTOR [135].
Akt	Perifosine	Decreases plasma membrane localization of Akt and its phosphorylation [136] (clinicaltrials.gov, NCT00391560).
	GSK21110183	Oral small molecule Akt inhibitor (clinicaltrials.gov, NCT00881946).
mTOR	Sirolimus (Rapamycin) Temsirolimus Everolimus (RAD 001) Deforolimus	Directly suppresses mTOR when bound to FKBP12 [79, 90, 93, 96, 137].
PIM	SGI-1776	Small molecule inhibitor of tyrosine kinase (clinicaltrials.gov, NCT00848601).

Table 3

New Therapies in AML: Targeting BCL-2 and Anti-Apoptotic Pathways

Target	Compound(s)	Mechanism
BCL-2	Oblimersen	Antisense oligonucleotide which binds to BCL-2 mRNA, leading to degradation of the complex [101, 104].
	Obatoclax ABT-263 AT-101	Small molecule inhibitors which suppress BCL-2 by binding to its BH3-binding groove [138–140]. (clinicaltrials.gov, NCT00684918).
XIAP	AEG-35156	Antisense oligonucleotide which binds to XIAP mRNA, leading to degradation of the complex [107, 108].