Novel Targeted Therapeutics in Acute Myeloid Leukemia: An Embarrassment of Riches

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Abstract

Purpose of review: Acute myeloid leukemia (AML) is an aggressive malignancy of the bone marrow that has a poor prognosis with traditional cytotoxic chemotherapy, especially in elderly patients. In recent years, small molecule inhibitors targeting AML associated *IDH1*, *IDH2* and *FLT3* mutations have been FDA approved. However, the majority of AML cases do not have a targetable mutation. A variety of novel agents targeting both previously untargetable mutations and general pathways in AML are currently being investigated. Herein, we review selected new targeted therapies currently in early phase clinical investigation in AML.

Recent Findings: The DOT1L inhibitor pinometostat in KMT2A rearranged AML, the menin inhibitors KO-539 and SYNDX-5613 in KMT2Ar and *NPM1* mutated AML and the mutant TP53 inhibitor APR-246 are examples of novel agents targeting specific mutations in AML. In addition, BET inhibitors, polo-like kinase inhibitors and MDM2 inhibitors are promising new drug classes for AML which do not depend on the presence of a particular mutation.

Summary: AML remains in incurable disease for many patients but advances in genomics, epigenetics and drug discovery have led to the development of many potential novel therapeutic agents, many of which are being investigated in on-going clinical trials. Additional studies will be necessary to determine how best to incorporate these novel agents into routine clinical treatment of AML.

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Keywords

Acute Myeloid Leukemia; Targeted Therapy; DOT1L; MDM2 inhibitors; bromodomain inhibitors; polo-like kinase inhibitors; menin inhibitors

Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignancy of hematopoietic stem and progenitor cells that has a poor prognosis with standard cytotoxic chemotherapy (1). In the last decade, significant progress has been made in defining the cytogenetic, molecular and epigenetic alterations underlying leukemogenesis (2–4). Response to cytotoxic chemotherapy and therefore prognosis is highly dependent upon cytogenetic and mutational aberrations present. The identification of AML associated mutations has led to the development of new classes of targeted inhibitors including the mutant IDH inhibitors enasidenib (5, 6) and ivosidenib (7, 8) and the FLT3 inhibitors midostaurin (9) and gilteritinib (10). However, only a minority of AML patients have targetable mutations. Targeted inhibitors that block pathways broadly involved in AML and that are not dependent upon specific mutations are another strategy; two such drugs have recently been approved. The BCL2 inhibitor venetoclax has been approved in combination with hypomethylating agents or low dose cytarabine (LDAC) for older (75 years) and/or unfit AML patients.(11, 12). The smoothened inhibitor glasdegib is similarly approved to be given in combination with LDAC (13). While these new agents have improved outcomes in AML, none are considered curative. There remains an unmet need for additional novel targeted therapeutics, particularly in patients with adverse risk disease. In this article, we will review selected targeted inhibitor classes currently under development for the treatment of AML.

BET Inhibitors

The epigenetic reader bromodomain and extraterminal domain (BET) protein BRD4 was initially identified as an essential gene for the survival of MLL-AF9 / NRAS driven murine AML cells (14) and has been shown to have a crucial role in maintaining survival in human AML leukemia stem cells (LSC) (15). BRD4, especially the short isoform, is over-expressed in human AML and high risk myelodysplastic syndrome (MDS) (16, 17). Collectively, evidence suggests that BRD4 serves to maintain core transcriptional programs in LSCs (18). It should be noted that little is known about the role of the other ubiquitously expressed BET family members BRD2 and BRD3 in leukemogenesis.

Pharmacologic inhibitors of BRD4 have been developed which function via displacing BRD4 as well as BRD2 and BRD3 from acetylated lysines on histones and transcription factors (19, 20). BET inhibitor treatment of AML cells results in differentiation and loss of LSC associated gene expression signatures (14, 19, 21). In addition to their initially reported activity in mixed-lineage leukemia 1 / lysine-specific methyltransferase 2A (*MLL1/ KMT2A)* rearranged (KMT2Ar) AML, these drugs have been shown to have pre-clinical activity against multiple sub-types of AML, including *NPM1* mutated (21), *IDH2* mutated (22), *ASXL1* mutated (23), t(8;21) positive core binding factor (CBF) AML (24) and *JAK2*

mutated secondary AML (25). These pre-clinical data suggest that BET inhibitors may have broad activity in AML that is not limited specific mutations or cytogenetic abnormalities.

Multiple BET inhibitors are currently in pre-clinical and early clinical development, with significant differences in selectivity, half-life and other pharmacologic properties. Early BET inhibitors were panselective, with relatively indiscriminant binding between both different BET proteins and BD1/BD2 domains within a given BET protein (26). Subsequent work has shown that BD1 may be more critical for continued chromatin binding, while the role of BD2 may be limited to initiating binding and interactions with transcription factors (27). Additionally, BD2 seems to have more relevance for inflammatory responses while transcriptional networks in AML may be BD1 dependent (28). There still is much to be learned regarding the relative roles of different BET proteins and specific bromodomains in AML, and it remains unclear which bromodomain should be pharmacologically targeted for optimal responses.

To date, the final results of only one clinical trial of BET inhibitors in AML has been fully reported (29). In this study OTX015 / MK-8628 was given in phase 1 dose escalation study in relapsed and refractory (r/r) acute leukemia patients. On the basis of dose limiting toxicities (DLTs), including gastrointestinal (GI) and thrombocytopenia, when given continuously in a separate study in lymphoma and multiple myeloma (MM) patients (30), OTX015 was given for 14 days followed by 7 days off drug. Dose escalation was hampered by poor tolerability secondary to GI toxicities, fatigue and cytopenias, particularly at doses higher than 80 mg daily. Responses included 2 complete remissions (CR) and 1 CR with incomplete count recovery (CRi) with no apparent relationship between dose level and responses. Duration of response was short, with all 3 responding patients relapsing within 5 months or less. OTX015 is no longer being clinically developed; however multiple trials of second and third generation BET inhibitors, both as single agents and in combination with hypomethylating agents or venetoclax are currently on-going as summarized in Table 1.

DOT1L inhibitors

Approximately 5–10% of AML cases harbor 11q23 chromosomal rearrangements involving the *MLL1/KMT2A* histone methyltransferase (2, 3). KMT2Ar are disproportionately common in pediatric and young adult patients (31) and in patients with prior exposure to topoisomerase II inhibitors (32). These patients have an aggressive course with inferior responses to chemotherapy (33). KMT2A fusion proteins inappropriately recruit disruptor of telomeric signaling −1 (DOT1L), a H3K79 methyltransferase, to KMT2A target genes, in particular the HOXA cluster and MEIS1, resulting in deposition of activating histone methylation marks at these promoters and ultimately overexpression (34). Genetic screens demonstrated that KMT2A fusions require DOT1L activity for leukemic transformation (35), making DOT1L an attractive pharmacologic target. Subsequently, EPZ004777, a potent and selective inhibitor of DOT1L, was developed (36). EPZ004777 has little effect on leukemia cells without KMT2Ar, but decreases proliferation and induces apoptosis and differentiation in KMT2Ar cells. Importantly, given that greater than 60 types of 11q23 translocation partners have been described to date (37), EPZ004777 has activity against multiple KMT2A fusion proteins (33, 36). However, EPZ004777 has unsuitable

pharmacokinetic (PK) properties for in vivo use, prompting the development of an optimized compound, pinometostat (EPZ-5676), for clinical development (38). Pinometostat is highly potent DOT1L inhibitor with >37,000 fold selectivity over other protein methyltransferases. In subcutaneous (SQ) MV4–11 xenograft models, pinometostat treatment resulted in tumor regression (38). In pre-clinical models, continuous drug exposure was required for efficacy, with decreased responses seen with intermittent dosing. Therefore, pinometostat was given as a continuous IV infusion for 21 days of a 28 day cycle in the initial early phase clinical studies. In a multi-center dose escalation study in 51 r/r acute leukemia patients, of which 37 were KMT2Ar, pinometostat was well tolerated, with the most common adverse events being fatigue, nausea, febrile neutropenia and electrolyte abnormalities (39). Serious (>grade 3) treatment related adverse events (AEs) included leukocytosis possibly related to differentiation, anemia, prolonged QTc, cardiac failure and liver abnormalities. Two patients had objective CRs and two patients had clearance of leukemia cutis lesions without regression of bone marrow disease. Additionally, seven patients had leukocytosis with morphologic changes on bone marrow examination consistent with differentiation. A similar safety profile was seen in a dose escalation study in pediatric KMT2Ar acute leukemia patients (40), with the exception of cardiac toxicity, which was only observed in adult patients. In contrast to the initial study in adult patients, pinometostat was dosed continuously until progression or unacceptable toxicity. No objective responses were obtained, but 7/18 patients had transient decreases in blood or bone marrow blast percentage.

On the basis of these results in heavily pre-treated r/r patients, pinometostat is currently being investigated in two phase 1b/2 studies in newly diagnosed KMT2Ar AML patients. In NCT03724084, pinometostat is given in combination with daunorubicin and cytarabine-based induction chemotherapy in patients ages 14 or older who are candidates for intensive chemotherapy. In NCT03701295, either 54 mg or 90 mg/m²/day pinometostat is given continuously in combination with azacitidine either in newly diagnosed KMT2Ar AML patients ineligible for induction chemotherapy or in r/r KMT2Ar AML. Recruitment for these trials is on-going and results have not yet been reported.

Polo-like kinase inhibitors

Polo-like kinases are a family of serine/threonine kinases with roles in cell cycle regulation and characterized by the presence of a conserved C-terminal polo-box domain that mediates protein-protein interactions with substrates (41). The best studied family member, polo-like kinase 1 (PLK1) is required for cells to progress from G2 to M phase in the cell cycle and is characteristically overexpressed in rapidly dividing cells including during fetal growth and in various malignancies (42) including AML (43). Additionally, PLK1 interacts with the PI3K/AKT/MTOR pathway, particularly in leukemia cells (44). There is little to no PLK1 expression in normal, adult tissues (43) including CD34+ progenitors and PBMCs (43, 45). Genetic or pharmacologic inhibition of PLK1 in leukemia cells results in cell cycle arrest in pro-metaphase leading to apoptosis and decreased colony formation (43, 46), with little effect on normal CD34+ cells. Importantly, the effects of PLK1 depletion or inhibition do not appear dependent upon the presence of TP53 (42). Recent pre-clinical data suggests that complex karyotype AML (47), aneuploid AML (48) and secondary AML (49) may be particularly dependent upon PLK1 and therefore especially sensitive to PLK1 inhibition.

The first PLK1 inhibitor studied clinically, BI-2536, (46) was investigated in elderly r/r AML patients in a multi-center phase 1 dose escalation study (50). The toxicity profile was typical of AML patients, with the majority of adverse events being hematologic and infectious in nature. Encouragingly, 2 CRs and 3 partial responses (PRs) were observed in a heavily pretreated population. Of patients whose response was initially classified as a PR, all three eventually achieved a CR with additional cycles of treatment. One of these patients maintained a CR for 21 months. Similar to pre-clinical data, bone marrow cells 24 hours after BI-2536 infusion exhibited mitotic arrest and apoptosis. Despite these encouraging results, development of BI-2536 was discontinued due to a multi-compartmental PK behavior thought to be unfavorable for clinical use.

Several PLK1 inhibitors are currently being studied in early phase clinical trials (Table 2). Of these, volasertib, a derivative of BI-2536, has the most data. Volasertib is potent competitive inhibitor of PLK1 with a sub-nanomolar IC50, little activity on unrelated kinases (51) and improved pharmacologic properties compared to pre-clinical PLK1 inhibitors (52). It has additional inhibitor activity on BRD4 (53) though the relevance of this in vivo is unclear. In the first-in-human phase 1 dose escalation study of volasertib given as a 1 hour IV infusion every 3 weeks in advanced or metastatic solid tumors, volasertib was well tolerated, with the main AEs being cytopenias and nausea (54). Neutropenia and thrombocytopenia were dose limiting but reversible. The maximum tolerated dose (MTD) was found to be 400 mg. PRs were seen in 3 of 65 patients and stable disease (SD) was observed in an additional 26 subjects. A similar toxicity profile was seen in two additional phase 1 studies in advanced solid tumors (55, 56) and in r/r AML patients receiving either volasertib monotherapy or volasertib in combination with LDAC (57). The MTD in combination with LDAC was determined to be 350 mg. Subsequently, volasertib was investigated in a phase 2 study that randomized 89 older AML patients unsuitable for induction chemotherapy to LDAC (20 mg SQ twice daily on days 1–10) with or without volasertib (350 mg on days 1 and 15) (58). The composite CR/CRi rate was significantly higher in patients receiving LDAC + volasertib (12/42, 31%) compared to LDAC alone (6/45, 13.3%). Combination arm patients also had longer event-free survival (EFS) (median 5.6 vs 2.3 months) and overall survival (OS) (median 8.0 vs 5.2 months). Responses were not correlated with cytogenetic risk classification. Unfortunately, the POLO-AML2 phase 3 study of LDAC + volasertib versus LDAC + placebo did not meet its primary endpoint of improved CR/CRi (59). The combination arm did have a higher CR/CRi rate (25.2 vs 16.8%) which was not statistically significant. The initial analysis showed an increased incidence of fatal infections in the volasertib arm, which was related to total dose density. Subsequently, a phase 1 study of volasertib in combination with decitabine was conducted in 13 elderly AML patients (60). The MTD of volasertib when given in combination with decitabine was 400 mg on days 1 and 5. Two patients had DLTs, including grade 3 asthenia and grade 3 mucositis, at the 400 mg dose level. Additionally, treatment related grade 5 anemia occurred in one patient; this was not considered a DLT due to the study protocol defining only non-hematologic toxicities as DLTs. Three objective responses, 2 CRi and 1 PR, were reported. An additional study of volasertib in combination with azacitidine in MDS (NCT02721875) has been terminated but final results have not yet been reported.

At this time, further development of volasertib in AML has been discontinued. The toxicities and limited clinical efficacy of volasertib was thought to be potentially related to off-target effects on other polo-like kinase family members PLK2 and PLK3. Less is known about the physiologic role of PLK2 and PLK3 in hematopoiesis. Some studies suggest that PLK2 may have tumor suppressor activity in hematologic malignancies including AML (61) and its suppression may increase senescence and apoptosis in normal marrow progenitors (62). Onvansertib/NMS-P37/PCM-075 is a potent reversible, competitive orally bioavailable selective PLK1 inhibitor that has limited off target activity on PLK2 and PLK3 at concentrations up to 10 μM (63, 64). Additionally, onvansertib has a shorter half-life than volasertib, which may also contribute to decreased toxicity.

In a phase 1 dose escalation study of onvansertib given days 1–5 of 21 day cycles to advanced/metastatic solid tumor patients, no DLTs were observed up to 24 mg/m^2/day (65). Drug-related grade 4 neutropenia and thrombocytopenia were seen at higher doses. SD was the best response and was achieved in 5 of 16 evaluable patients. *KRAS* mutations appeared to be a biomarker of response in metastatic colon cancer and onvansertib has been granted Fast Track designation by the United States Food and Drug Administration (FDA) for further development in this patient population. In AML, onvansertib was investigated in a phase 1b dose escalation study in r/r patients in combination with LDAC or decitabine, with a starting dose of 12 mg/m^2 on days 1–5 of a 28 cycle (66). Overall, ovanasertib was well tolerated, with the most common adverse events being hematologic toxicities. Two DLTs, grade 3 mucositis and grade 4 rash, occurred at 90 mg/m^2 in the decitabine arm. The MTD for onvansertib was therefore established as 60 mg/m^2. CR or CRi was reported in 5 patients (5/23, 24%) on the decitabine arm and 1 patient (1/15, 7%) on the LDAC arm, with a median remission duration of 5.5 months. Notably, while the study was not powered to detect associations between different mutational subgroups, 4 of the 6 patients who attained CR or CRi had spliceosome mutations. An update of the phase 2 expansion study of 60 mg/m^{2}onvansertib in combination with decitabine was recently reported at the 2020 American Society of Hematology meeting (67). The toxicities were similar to those seen in the phase 1b study. The combined CR/CRi rate was 17%, with half of responders having a spliceosome mutation.

Rigosertib is a small molecule with inhibitory activity on polo-like kinases, RAF and PI3K (68) that has been investigated in AML and MDS. It is not yet clear to what extent its activity its activity depends upon PLK versus RAS pathway inhibition. Due to space constraints, we will not discuss rigosertib further in this review.

Mutant TP53 inhibitors

TP53 is a tumor suppressor protein with roles in DNA damage response, cell cycle arrest, apoptosis and senescence (69). Approximately 5–10 percent of AML cases have *TP53* mutations (2, 3), with an increased prevalence in therapy related-AML (70), secondary AML (71) and complex-karyotype AML (72). *TP53* mutated AML has a poor prognosis, with inferior responses to traditional cytotoxic chemotherapy (73–75). Interestingly, AML with subclonal*TP53* mutations has a similarly poor prognosis compared to cases in which the dominant clone is TP53 mutated (76). Responses may be more favorable with decitabine-

based therapy (77), though this may be altered by the effect of concurrent karyotype abnormalities on responses in this patient population (78, 79).

A novel therapeutic strategy is to target mutated TP53, a protein which had previously been considered undruggable. APR-246, a PRIMA-1 analog, binds covalently to TP53 and induces a conformational shift in mutated TP53 to a wildtype-like structure which is able to bind specific DNA sequences and activate target gene transcription, thereby ultimately inducing apoptosis (80, 81). In addition, APR-246 has TP53-independent activity that leads to increased oxidative stress and cell death (82), providing a plausible mechanism for activity in TP53 wildtype cells and in cells without detectable TP53 expression. In preclinical models of AML, APR-246 has synergistic effects with azacitidine (83).

In a first-in-human phase 1/2 dose escalation study in patients with refractory hematologic malignancies and advanced prostate cancer, APR-246 was given as a 2 hour IV infusion for 4 consecutive days, followed by a 17 day safety monitoring period (84). Retreatment was only allowed if disease burden was judged to have decreased by 25% or greater after initial treatment. APR-246 was well tolerated, with the most common AEs being fatigue, dizziness, headache, confusion and sensory disturbances. Neurologic side effects were infusion-related and resolved after the end of infusion. The MTD was determined to be 60 mg/kg. One AML patient had a reduction in bone marrow blasts from 46% to 26% and one non-Hodgkin Lymphoma patient had a minor response on computed tomography; both of these individuals had *TP53* mutated disease. An extension of this study in 8 AML and 2 chronic lymphocytic leukemia (CLL) patients sought to improve safety by extending the infusion time to 6 hours and additionally allowed for retreatment in 21 day cycles (85). Using this strategy, the MTD was increased to 105 mg/kg. Two AML patients met response criteria. One patient achieved a CR, though interpretation of this observation is complicated by use of hydrea and sorafenib within 4 weeks of treatment. *TP53* mutational status was not determined for this patient. A second *TP53* mutated AML patient had a reduction in peripheral blood blast count >25% at 21 days but was taken off study and did not have additional response assessment or treatment due to AEs thought unrelated to the study drug. A third patient with *TP53* mutated CLL had a transient >25% decrease in total lymphocyte count and lymph node size after 1 cycle of treatment but progressed during cycle 2. Notably in both of these studies, clinical activity in hematologic malignancies was concentrated in *TP53* mutated patients

Based on these results, APR-246, now known as eprenetapopt, was then studied in combination with azacitidine in two early phase studies in *TP53* mutated AML and MDS patients. Sallman et al reported the results of a phase 1b/2 of APR-246 alone or in combination with azacitidine in *TP53* mutated myeloid malignancies including 40 MDS, 11 AML and 4 chronic myelomonocytic leukemia patients (86). In the phase 1b study, dose escalation up to 100 mg/kg eprenetapopt given IV on days 1–4 was well tolerated without DLTs either as monotherapy or in combination with azacitidine given on days 4–10. The RP2D of 100 mg/kg was determined to be equivalent to a fixed dose of 4500 mg daily on the basis of PK data; this dosing was used in the phase 2 expansion. The AEs were similar to those associated with each drug as a single agent and included cytopenias, febrile neutropenia, nausea/vomiting, diarrhea and transient neurologic toxicities during eprenetapopt infusion. Grade 3/4 toxicities were limited to cytopenias; there were no

reported serious neurologic toxicities. Of 45 evaluable patients, 36 achieved CR or marrow CR, including 6 of 8 evaluable AML patients. The median duration of CR was 7.3 months; this was not significantly different between MDS and AML patients. Notably, 19 patients discontinued study treatment in order to undergo allogeneic stem cell transplant.

A Groupe Francophone des Myélodysplasies phase 2 study of APR-246 4500 mg IV days 1–4 followed by azacitidine given days 4–10 of a 28 day cycle was published concurrently (87). Fifty-two patients, including 34 MDS and 18 AML patients, were evaluable for response. The overall response rate (ORR) was 52% including 37% CR. The reported CR rate in MDS patients was 53%. AML patients had lower CR/CRirates, with a trend towards decreased efficacy in patients with >30% marrow blasts (14% vs 45% for patients with 20–30% marrow blasts). Adverse events were similar to those reported in the phase 1b/2 study (86). Two reversible grade 3 neurologic toxicities were observed in patients with baseline lower renal function. In both of these studies, TP53 mutations VAF clearance was associated with CR. There was no clear relationship between co-occurring mutations and response.

On the basis of these results, the FDA granted APR-246 and azacitidine breakthrough designation for *TP53* mutated MDS in April 2020. However, the randomized phase 3 study of APR-246 and azacitidine compared to azacitidine (NCT03745716) was recently reported to not meet the primary endpoint of CR. However, further analysis and full report of the study is still pending at the publication of this manuscript. APR-246 is also being investigated as maintenance therapy in combination with azacitidine following alloSCT in *TP53* mutated AML or MDS (NCT03931291) and in combination with azacitidine/venetoclax for newly diagnosed *TP53* mutated myeloid malignancies (NCT04214860). In addition, Aprea Therapeutics is currently developing an orally bioavailable APR-246 derivative, APR-548. A phase 1 study of APR-548 in combination with azacitidine in TP53 mutated MDS is planned but not yet recruiting (NCT04638309).

MDM2 Inhibitors

TP53 activity is inhibited by binding its negative regulators MDM2 and MDM4, which target it for degradation via ubiquitination. MDM2 is overexpressed in AML so even cells without TP53 mutation or deletion may have low functional TP53 activity. Small molecule inhibitors of the MDM2/p53 interaction reduce p53 destruction leading to increased cellular p53 levels and ultimately apoptosis (88). The first inhibitor of this class, nutlin-3a, was not studied clinically due to poor bioavailability and toxicity in preclinical models. Subsequently, two nutlin-3a analogues, RG7112 and RG7388, were developed. RG7112 was studied in a phase 1 trial in r/r acute and chronic leukemia patients, with modest efficacy (89) including 3 CR/CRi out of 30 AML patients. However, its further development was also limited by GI toxicity (90) and unfavorable PK findings.

RG7388/idasanutlin is an orally bioavailable MDM2 inhibitor with increased potency and selectivity compared to both RG7112 and nutlin-3a (91). In a phase 1 dose escalation study of r/r AML, idasanutlin was given either as a single agent on days 1–5 of a 28 cycle or in combination with cytarabine 1 g/m^2 IV on days 1–6 of a 28 day cycle (92). Enrollment was not limited to *TP53* wild-type AML. Myelosuppression and diarrhea were identified as DLTs, with both occurring at the 800 mg twice daily dose level. Two different formulations

of idasanutlin were studied, with different recommended phase 2 dosing determined for each: 600 mg twice daily for the initial formation and 300 mg twice daily (BID) for the optimized formulation used in an expansion study. Seven of 37 evaluable patients (19%) receiving monotherapy achieved a CR or CRi, while 21 of 59 (36%) evaluable combination treatment patients obtained a CR or CRi. The median duration of response was 7.7 months with monotherapy and 8.5 months with combination therapy. Six patients proceeded to alloSCT. Notably, only 1 of 25 patients with *TP53* mutations attained CR, compared to 22 of 71 *TP53* wild-type patients. Responses were additionally correlated with MDM2 expression in AML blasts (92, 93). Idasanutlin was additionally studied at a lower dose in polycythemia vera and essential thombocythemia patients refractory to standard of care treatments (94), where it was well tolerated. GI toxicities including nausea, vomiting and diarrhea were the most common AEs but these were generally low grade. Of 16 enrolled patients, responses were observed in 9, including 5 CRs and 4 PRs. Unfortunately, despite these promising results, the phase III MIRROS study of idasanutlin in combination with cytarabine (NCT02545283) in r/r AML (95) was terminated early when it failed to meet its primary endpoint of improved overall survival compared to cytarabine alone. It is important to note that the bioavailability and PKs of idasanutlin are heavily influenced by formulation (96–98). It is therefore difficult to directly compare results from initial studies to later trials using an optimized formulation. Currently idasanutlin is being investigated in combination with venetoclax in r/r AML (NCT02670044 and NCT04029688) and in combination with cytarabine and daunorubicin based induction chemotherapy in newly diagnosed AML (NCT03850535). Preliminary results of the phase 1 study investigated idasanutlin and venetoclax in elderly r/r AML and secondary AML were reported at the 2019 American Society of Hematology annual meeting (99). Adverse events were consistent with known toxicities of both drugs as single agent therapy. The RP2D was determined to be 600 mg venetoclax given continuously in combination with either 150 mg or 200 mg of idasanutlin on days 1–5 of 28 day cycles. Across all dose levels, CR or CRi were recorded in 11 of 49 patients.

The MDM2 inhibitor AMG-232 is structurally distinct and interacts with the glycine shelf region of the TP53 binding pocket on the surface MDM2 (100), a property not found in nutlin derivatives. Like idasanutlin, it is orally bioavailable. In a phase 1 dose-escalation study in advanced solid malignancies and MM, AMG-232 was well tolerated, with the most common adverse events being GI toxicities and anemia (101). No objective response were reported. In a second study, AMG-232 was given alone or in combination with the mitogen-activated protein kinase (MEK) inhibitor trametinib in r/r AML (102). Of note compared to the first-in-man study in solid tumor patients, this study had increased dose frequency with AMG-232 being given for 7 days every 2 weeks rather than 7 days every 3 weeks. Despite the increased dosing, the toxicity profile was similar. As expected, AML patients had a higher incidence of grade 3 and 4 cytopenias. Dose escalation in the monotherapy arm was halted due to GI toxicities. However, the protocol did not allow prophylactic medications to manage nausea or diarrhea and the authors note that tolerability may be improved with prophylactic anti-emetics. Responses included 4 morphological leukemia free states (MLFS) in the monotherapy arm and 1 CR and 1 PR in the combination arm out of a total 30 evaluable patients. As expected based on the mechanism of action,

no responses were seen in patients with *TP53* mutations. Most responses were short lived. However, the patient who attained a CR maintained this response for over 550 days. Trametinib was discontinued after cycle 2 due to trametinib-related ocular toxicity and AMG-232 was continued as a single agent until disease progression 650 days after initiating study treatment. AMG-232 is currently being investigated in combination with decitabine (NCT03041688 and NCT04113616), LDAC (NCT04113616) and with induction chemotherapy (NCT04190550) in AML

One limitation of MDM2 inhibitors is that most compounds have limited effects on other MDM family members such as MDMX, which may be a source of resistance. AML cells have high MDMX expression (102); this may present difficulties for the development of MDM2 inhibitors. ALRN-6924 is a stapled peptide with dual inhibition of both MDM2 and MDM4 (103). Notably it is the first stapled peptide to be given in man. In a first-in-human phase 1 study in advanced solid tumorand lymphoma patients, ALRN-6924 was generally well tolerated with the most common AEs being cytopenias, GI toxicity, liver function test abnormalities and infusion reactions, all of which were reversible. ALRN-6924 has been investigated in a phase 1 study in r/r AML and MDS, both as a single agent and in combination with cytarabine. Final results have not yet been reported but in preliminary results presented in abstract form (104), no DLTs had been observed and the MTD had not yet been reached. Marrow CR was reported in 2 MDS patients.

Menin Inhibitors

Several genetic subgroups of AML are characterized by overexpression of the homeobox (HOX) A family of transcription factors, including KMT2Ar (105), KMT2A partial tandem duplication (PTD) mutated (106) and *NPM1* mutated AMLs (107–109). As discussed above, KMT2Ar AML is a particularly aggressive subtype of AML. KMT2A-PTD occurs in 10% of adult AML cases (110) and is also associated with poor prognosis (111). Mutations in the nuclear chaperone protein nucleophosmin (NPM1), which result in mis-localization of NPM1 to the cytoplasm (112), occur in 25–30% percent of adult AML cases (2–4) and up to 10% of pediatric and young adult AML (113).

HOX genes serve as master regulators of hematopoiesis, controlling both self-renewal in normal hematopoietic stem cells and differentiation to committed myeloid progenitors (114, 115). The understanding of how KMT2A fusions and KMT2A-PTD proteins deregulate HOX gene expression has been elegantly investigated by multiple groups over the last twenty years. In normal hematopoiesis, KMT2A is a crucial transcriptional regulator of HOX genes (116). Similarly, KMT2A fusion and KMT2A-PTD proteins bind to the promoters of HOXA genes and activate transcription (105, 117, 118) via recruitment of a complex of chromatin remodeling proteins comprising the adaptor protein menin (119–121) in addition to DOT1L (34, 35) and BET proteins (14) as discussed above. The interaction of menin with both wild type KMT2A (119) and KMT2A fusion proteins (120) is necessary for HOX gene expression (121, 122). The mechanism by which cytoplasmic NPM1 induces HOX expression is less clear, but it seems to be a direct consequence of the *NPM1* mutation since expression of a mutated NPM1 allele in mice also results in increased expression of HOXA genes (123–125). In addition, genetic or pharmacologic abrogation of cytoplasmic

NPM1 reduced HOX gene expression and produced cell cycle arrest and differentiation in *NPM1* mutated cell lines, patient derived xenografts and genetically engineered human HSCs (126, 127). Like KMT2Ar and KMT2A-PTD, AML *NPM1* mutated leukemia cell lines require wildtype KMT2A and menin to maintain HOX expression (128).

The role of menin in maintaining self-renewal in multiple subtypes of AML made it an attractive pharmacologic target. However, disrupting large protein-protein interactions pharmacologically was technically challenging and for many years, this was thought to be undruggable. Recent developments have challenged those assumptions. Detailed structural studies that accurately modeled the interaction between menin and KMT2A, especially the KMT2A binding pocket on menin were a pre-requisite to successful drug development (129). The first pre-clinical small molecule inhibitor of menin / KMT2A1 interaction was MI2–2, a thienopyrimidine compound developed at the University of Michigan (130). Unlike DOT1L inhibitors, which inhibit the H3K79 methyltransferase enzymatic activity of the KMT2A fusion protein complex, menin inhibitors disrupt formation of the complex (131). MI2–2 and related compounds had encouraging pre-clinical in vitro activity, inducing cell cycle arrest, apoptosis, differentiation and reduction in KMT2A fusion protein target gene expression, including HOX genes (130, 132). Importantly, activity was independent of the KMT2A fusion partner (132). However, poor pharmacokinetic properties limited in vivo studies. Therefore, a series of optimized derivative compounds with improved potency, reduced off-target effects and oral bioavailability was developed (133). MI-3454, the most recently reported of this family of compounds, induced complete remissions in both KMT2Ar and *NPM1* mutated patient derived xenograft (PDX) mice with little effect on normal hematopoiesis (134). Similarly, KO-539, a structurally related analog of MI-3454, induced in vivo differentiation, complete remissions and long lasting survival in KMT2Ar cell line xenografts (135, 136) and *NPM1* mutated PDX models (137).

KO-539 is currently being investigated in phase 1/2a study in r/r AML (NCT04067336) and has been granted orphan drug designation by the FDA. Notably, the phase 1 study does not include a requirement for *NPM1* mutations or KMT2Ar. In preliminary results reported at the 2020 ASH annual meeting (138), 12 patients had been enrolled at doses ranging from 50–400 mg once daily dosed continuously. Importantly, no dose limiting toxicities have been identified and no patients discontinued treatment due to adverse events. The most common toxicities included nausea, diarrhea and rash, all of which were grade 1 or 2. KO-539 pharmacokinetics were not altered by co-administration of CYP3A4 inhibitors, such as azole antifungals. Of 8 evaluated patients, 6 had evidence of clinical activity. These included a *NPM1*, *DNMT3A* mutated AML that attained MRD negative complete remission, a MRD positive CR in a *SETD*, *RUNX1* mutated AML and a MLFS in a *NPM1* mutated, *FLT3*-ITD AML. Additionally a patient with KMT2Ar AML had a decrease in hydrea requirements when treated with the lowest dose, 50 mg daily. Other reported responses were decreasing peripheral blasts and SD in one patient each. A CR occurring in a patient without *NPM1* mutations or KMT2A rearrangement raises the possibility that KO-539 may have broader applications in the treatment of AML. This is in line with pre-clinical data showing menin's role in maintenance of transcriptional programs in other subtypes of AML, including *MN1-ETV6* translocations (139), *FLT3* mutated (140), and *CEBPA* mutated (141). Currently, enrollment in the phase 1 dose escalation study is on-going; a phase 2 expansion study

in r/r *NPM1* mutated and KMT2Ar AML is planned following the determination of the recommended phase 2 dose.

A second structurally unrelated menin inhibitor, VTP50469, was discovered via iterative structure based design (142). Similar to the MI series of compounds, VTP50469 binds to the KMT2A binding pocket of menin but with a distinct binding mode that engages somewhat different amino acids. VTP50469 inhibits the growth of KMT2Ar leukemia cells, reduces KMT2A fusion protein target gene expression, improved survival in a murine MLL-AF9 leukemia model and in KMT2Ar PDX, with some PDX mice surviving over 1 year (142). Subsequently, VTP50469 was also shown to reduce HOX gene expression and inhibit in vivo growth in *NPM1* mutated murine cells as well as *NPM1* mutated AML PDX mice (125). An analog of VTP50469, SYNDX-5613, is currently being investigated in a phase 1/2 study in r/r AML (NCT04065399). The phase 1 dose escalation study does not require any specific mutation or chromosome re-arrangement, while the planned phase 2 expansion will include KMT2Ar AML, KMT2Ar acute lymphoblastic leukemia (ALL) and *NPM1* mutated AML. Preliminary results were reported at the 2020 AACR meeting (143). Of 6 evaluable patients, two had objective responses, including a CRi in a t(10;11), *FLT3*-ITD AMl patient and a partial response after 28 days of therapy in a t(9;11) AML patient. Of note, this patient continues on study. No DLTs have been identified but two patients had grade 1–2 QTc prolongation that resolved with dose reductions. In addition, unlike KO-539, the pharmacokinetics of SYNDX-5613 appeared affected by CYP3A4 inhibitors, with patients taking concurrent CYP3A4 inhibitors more likely to reach target plasma concentrations greater than the predicted IC95 for the menin-KMT2A interaction. Based on these preliminary results, SYNDX-5613 was granted orphan drug designation by the FDA in April 2020.

Conclusions and Future Directions

Since 2017, eight new drugs have been approved for the treatment of AML. Our understanding of the altered signaling pathways and epigenetic perturbations underlying leukemia cell survival and proliferation continues to grow. With this new knowledge, additional potential pharmacologic targets are identified and novel targeted inhibitors developed, including drugs designed to inhibit pathways previously considering un-druggable. However, our excitement is tempered by the knowledge that AML remains an incurable disease for the majority of patients, especially older patients and those with medical co-morbidities limiting treatment. We now have an "embarrassment of riches" when it comes to potential novel therapeutics for AML. As of February 2021, 497 actively recruiting clinical trials for adult AML are currently registered with clinicaltrials.gov. The challenge for the field will be to identify the most promising new drugs, impact for particular genomic subgroups and determine the most optimal combinations or sequences of novel therapeutics.

Acknowledgments

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• Of importance

•• Of major importance

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Table 1.

Current Clinical Trials with BET Inhibitors

Drug	Selectivity	Sponsor	NCT ID	Phase	Disease	Combination	Status	Reference
ABBV-075 / Mivebresib	Pan-BET	AbbVie	NCT02391480	1	AML	Venetoclax	Completed	144
ABBV-075 / Mivebresib	Pan-BET	AbbVie	NCT04480086	1	MF	Ruxolitinib or navitoclax	Recruiting	145
ABBV-744	BD2	AbbVie	NCT03360006	1	AML	None	Recruiting	
ABBV-744	BD2	AbbVie	NCT04454658	1	MF	Ruxolitinib or navitoclax	Recruiting	145
AZD5153	Bivalent	107	NCT03013998	1/2	AML	Venetoclax	Recruiting	
CPI0610	Pan-BET	Constellation Pharmaceuticals	NCT02158858	2	MF	Ruxolitinib	Recruiting	146–148
CPI0610	Pan-BET	Constellation Pharmaceuticals	NCT04603495	3	MF	Ruxolitinib	Recruiting	149
FT-1101	Pan-BET	Forma Therapeutics	NCT02543879	1	AML, MDS, NHL	None	Completed	150
GSK525762 / Molibresib	Pan-BET	GlaxoSmithKline	NCT01943851	1	AML, NHL, MM	None	Completed	151
INCB054329	Pan-BET	Incyte Corporation	NCT02431260	1	AML, MDS/MPN, MF	None	Terminated	
INCB057643	Pan-BET	Incyte Corporation	NCT04279847	1	MF	None	Recruiting	152
PLX2853	Modestly BD2	Plexxikon	NCT03787498	1	AML, MDS	None	Recruiting	153
PLX51107	Pan-BET	MDACC/NCI	NCT04022785	1	AML, MDS	Azacitdine	Recruiting	
PLX51107	Pan-BET	Plexxikon	NCT02683395	1	AML, MDS, lymphoma	None	Terminated	154
RO6870810 / TEN-10	Pan-BET	Roche	NCT02308761	1	AML, MDS	None	Completed	155

Abbreviations: AML, acute myeloid leukemia; BET, bromodomain extraterminal domain; MDS, myelodysplastic syndrome; MF, myelofibrosis; MM, multiple myeloma; MPN, myeloproliferative neoplasm; NHL, non-hodgkin lymphoma

Table 2.

Current Clinical Trials with PLK Inhibitors

Drug	Selectivity	Sponsor	NCT ID	Phase	Disease	Combination	Status	Reference
BI-2536	Pan PLK	Boehringer Ingelheim	NCT00701766	1	AML	None	Completed	50
CFI-400945	PLK4	University Health Network Toronto, Canada	NCT03187288	1	AML, MDS	None	Recruiting	156
CFI-400945	PLK4	Treadwell Therapeutics, Inc	NCT04730258	1/2	AML, MDS, CMML	Azacitidine or decitabine	Not yet recruiting	157
CYC140	PLK1	Cyclacel Pharmaceuticals	NCT03884329	1	AML, ALL, MDS, CML, CLL	None	Recruiting	
Onvansertib	PLK1	Cardiff Oncology	NCT03303339	1/2	AML	LDAC or decitabine	Active, not recruiting	66, 67
Rigosertib	PLK/RAF/PI3K inhibitor	Onconova Therapeutics	NCT01926587	1/2	AML, MDS, CMML	Azacitidine	Active, not recruiting	158
Rigosertib	PLK/RAF/PI3K inhibitor	Onconova Therapeutics	NCT00854646	1	AML, MDS, CMML, CML, CLL	None	Completed	159
Rigosertib	PLK/RAF/PI3K inhibitor	Onconova Therapuetics	NCT01241500	3	MDS	None	Completed	160
Rigosertib	PLK/RAF/PI3K inhibitor	Onconova Therapeutics	NCT02562443	3	MDS	None	Active, not recruiting	
Volasertib	Pan PLK	Boehringer Ingelheim	NCT00804856	1	AML	LDAC	Active, not recruiting	57
Volasertib	Pan PLK	Boehringer Ingelheim	NCT00804856	2	AML	LDAC	Active, not recruiting	58
Volasertib	Pan PLK	Boehringer Ingelheim	NCT01721876	3	AML	LDAC	Active, not recruiting	59
Volasertib	Pan PLK	Boehringer Ingelheim	NCT02003573	1	AML	Decitabine	Terminated	60
Volasertib	Pan PLK	Boehringer Ingelheim	NCT02721875	1	MDS	Azacitidine	Terminated	

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; LDAC, low-dose cytarabine; MDS, myelodysplastic syndrome; PLK, polo-like kinase