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Update on rational targeted therapy in AML

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Abstract

Acute myeloid leukemia (AML) remains a challenge to both patients and clinicians. Despite improvements in our understanding of the disease, treatment has changed minimally and outcomes remain poor for the majority of patients. Within the last decade, there have been an increasing number of potential targets and pathways identified for development in AML. The classes of agents described in this review include but are not limited to epigenetic modifiers such as IDH inhibitors, BET inhibitors, and HDAC inhibitors as well as cell cycle and signaling inhibitors such as Aurora kinase inhibitors and CDK inhibitors. While the developments are encouraging, it is unlikely that targeting a single pathway will result in long-term disease control. Accordingly, we will also highlight potential rational partners for the novel agents described herein.

Keywords

High-risk AML; IDH; BET; HDAC inhibitors; LSD1; DOT1L; Flavopiridol; Palbociclib; Volasertib; Wee1; Idasanutlin; Aurora kinase; Rigosertib; Hedgehog; BH3-mimetic; Venetoclax; Pevonedistat; Tosedostat

1. Introduction

Acute myeloid leukemia (AML) is a clonal hematologic disorder marked by clinical and biological heterogeneity. Despite advances in our understanding of AML pathogenesis, classification, genomic landscape, and prognostic factors, treatment has changed little in the last 40 years. Standard therapy consists of induction with 7 days of cytarabine plus 3 days of an anthracycline (7 + 3) followed by consolidation with additional chemotherapy or stem-cell transplantation. Despite intensive therapy, many patients relapse with poor prognosis. To date, no drug is currently approved for the treatment of relapsed AML. Gemtuzumab ozogamicin (GO), an anti-CD33 immunoconjugate, was initially approved by the FDA in 2000 for older patients with AML in first relapse not candidates for standard cytotoxic chemotherapy [1]. The drug was subsequently voluntarily withdrawn from the US market in June 2010 after a randomized study which added GO to standard 7 + 3 in untreated AML patients <60 years found an increase in 30-day mortality without improvements in complete response (CR) or overall survival (OS) [2]. Additional data have since been released

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Conflict of interest statement

None.

suggesting a benefit in favorable-risk AML [3]. A recently published phase III study in relapsed AML compared the novel drug elacytarabine, an elaidic acid ester of cytarabine, with investigator's choice consisting of one of seven commonly used AML salvage regimens [4]. There were no differences in overall survival (3.5 versus 3.3 months) between the elacytarabine and control arms. The results reinforced the general perception of poor outcomes for this unfortunate patient population, as well as the urgent need for novel and more effective treatment strategies.

Cytogenetic analysis has become an integral part of the study of AML as recurrent chromosomal variations represent established diagnostic and prognostic markers. However, nearly half of AML patients have a normal karyotype. As there is significant heterogeneity in this group, it is clear that a better understanding of genetic and epigenetic changes relevant to AML pathogenesis is necessary. During genome sequencing of 200 *de novo* AML patients, an average of 13 mutations was found in genes [5]. On average, 5 occurred in genes recurrently mutated in AML. Within the same project, more than 20 driver recurrent mutations were identified. Driver mutations confer growth advantages to the leukemic cell but are not necessary for the ultimate maintenance of the leukemia [6]. Common mutations in AML that are also driver mutations represent potential therapeutic targets.

Despite progress in identification of novel targets in AML, many of which represent driver mutations, there is increasing recognition that rational combinations will likely be necessary to target the redundancy of survival pathways in tumor cells. A wide variety of genes and pathways not inherently oncogenic are necessary for maintenance of the tumor (e.g., by overcoming the otherwise lethal effects of oncogenic stress to which malignant cells are generally exposed). Although a single targeted drug may reverse the effect of a mutation, multiple new abnormalities may evolve in AML that serve as drivers of disease progression. Additionally, there may be multiple clones or subclones with alternative oncogenic pathways. Two therapies are considered orthogonal if they act synergistically to attack cancer in two distinct ways (e.g., inhibitors of "driver" tyrosine kinases and agents that promote oncogenic stress) [7].

There are numerous potential pathways and targets for development in AML. A review of all emerging agents is beyond the scope of this article [8]. Antibody-based therapies are rapidly expanding in multiple arenas in oncology, including AML. Antibody-drug conjugates, bispecific antibodies, and chimeric antigen receptor T cells represent only a few of the growth areas in AML and have recently been extensively described in other reviews [9,10]. Internal tandem duplication mutations in FLT3 have been identified in about 20% of AML patients and are associated with poor outcomes. Given the revolutionary nature of tyrosine kinase inhibitors in CML, there was initially great enthusiasm for their use in AML. Early results involving FLT3 inhibitors were largely disappointing and primarily led to transient reduction in blast counts. Studies involving second-generation FLT3 inhibitors suggest greater potency. There are multiple recent reviews in the literature detailing the success and failures of these agents [11,12]. Traditional cytotoxic therapy, the backbone of treatment for AML in most cases, continues to evolve. CPX-351, a liposomal formulation of cytarabine:daunorubicin, demonstrated clinical benefit in older AML patients [13]. Additional agents such as clofarabine, cladribine, sapacitabine, and vosaroxin remain under

investigation and will not be discussed here. The goal of the present review is to highlight some of the more promising novel approaches and agents that have entered the therapeutic armamentarium for relapsed/refractory AML (Table 1). A select number of new agents are being studied as single agents, but many are being examined in combination with cytotoxic chemotherapy or a hypomethylating agent such as azacitidine or decitabine.

2. Epigenetic modifiers

With the advent of targeted sequencing, exome sequencing, and whole-genome sequencing, a number of recurrently mutated genes encoding proteins involved in epigenetic regulation of transcription have been identified including *TET2*, *IDH1*, *IDH2*, *DNMT3a*, and *EZH2*. In the Cancer Genome Atlas, 44% of AML patients displayed mutations in genes involved in genomic DNA methylation [5]. *DNMT3a* mutations are present in 18–22% of AML patients [14]. The incidence appears to increase with age and appears associated with poor outcome. *DNMT3a* mutations have been associated with both *NPM1* and *FLT3*-ITD mutations. *EZH2* mutations are rare in AML, but potentially oncogenic; in MDS, they are associated with inferior survival [15,16]. Mutations in *TET2* have been recognized in 7–23% of AML patients. Controversy exists regarding the prognostic impact of *TET2* mutations. The *TET2* mutation appears to be mutually exclusive with the *IDH1/IDH2* mutations [17]. *IDH* mutations have been identified in 15–30% of *de novo* AML and secondary AML, primarily cytogenetically normal AML. Similar to *TET2*, the prognostic significance of *IDH* mutations remains controversial. Currently, these mutations represent a major focus of interest with regard to therapeutic intervention in AML. The hypomethylating agents, azacitidine and decitabine, are the most extensively studied DNA methyltransferase inhibitors. While approved for use in MDS in the US, they have also demonstrated utility in elderly AML patients [18,19]. Many of the combination studies with novel agents in elderly AML patients utilize a hypomethylating agent backbone.

2.1. IDH Inhibitors

Isocitrate dehydrogenase (*IDH1* and *IDH2*) is an enzyme critical for the conversion of isocitrate to α -ketoglutarate (α -KG). The mutant forms of *IDH* are not inactive, but rather possess novel activity catalyzing the conversion of α -KG to the oncometabolite 2-hydroxyglutarate (2-HG). Elevated 2-HG levels have been identified in multiple tumor types including AML with *IDH* mutations. Accumulation of 2-HG is purported to block normal cell differentiation and promote tumorigenesis via competitive inhibition of α -KG-dependent enzymatic activities. AML with *IDH* mutations is characterized by a hypermethylated signature leading to impaired hematopoietic differentiation [20]. The hypothesis is that this abnormality in differentiation can be reversed via *IDH* inhibition. AG-221 is an oral first in class inhibitor of the *IDH2* mutant protein. Preliminary results of a phase 1 study with once or twice daily continuous dosing (28-day cycle) have been reported [21]. Of the 48 enrolled patients, 32 were evaluable for efficacy (day 28 bone marrow). Investigator-assessed responses were observed in 20 patients (8 CR, 3 CRi, and 8 PR). Identified responses appeared to be durable in select patients. Five patients with CR were able to go onto transplant. As proof of principle, investigators noted greater than 90% reduction in 2-HG levels. The drug was generally well tolerated. An ongoing phase 3 study

compares the efficacy of AG-221 versus conventional care regimens in relapsed/refractory AML patients older than 60 with *IDH2* mutations (NCT02577406). AG-120, an oral, first-in-class IDH1 inhibitor, has shown similar efficacy as a single agent in patients with IDH1-mutant hematologic malignancies [22]. Seven of the 14 efficacy evaluable patients had objective responses with 4 CRs, 2 marrow CRs, and 1 PR with durable responses of up to 5.7 months. Another six patients had stable disease. An ongoing phase 1 study is evaluating the safety of AG-120 or AG-221 in combination with induction and consolidation in patients with newly diagnosed AML (NCT02632708). Finally, recent studies suggest that AML cells bearing IDH mutations may be particularly dependent upon the anti-apoptotic protein Bcl-2 for their survival, raising the possibility that such cells may be susceptible to BH3-mimetics [23].

2.2. BET inhibitors

BET inhibitors reversibly bind the bromodomains of Bromodomain and Extra-Terminal proteins BRD2, BRD3, BRD4, and BRDt. The BET proteins bind to acetylated lysines in histones and control gene expression. In a variety of human AML cell lines, suppression of BRD4 was able to suppress *MYC* effectively suggesting a potential role in cancer treatment [24]. OTX015, a thienotriazolodiazepine, is a small molecule oral inhibitor of BRD 2/3/4 demonstrated to induce apoptosis in a variety of leukemia cell lines and human AML samples [25]. In a phase 1 3 + 3 dose escalation trial, OTX015 was investigated as monotherapy in relapsed/refractory leukemia [26]. In the 33 AML patients, there was 1 CR, 1 CRp, 2 with partial blast clearance and one with resolution of gum hypertrophy. Four of the 5 responders had secondary or therapy-related AML; responses occurred at multiple dose levels. OTX015 was well tolerated except for GI toxicity. Investigation in an expansion cohort for AML continues (NCT01713582). There is preclinical evidence BET antagonists synergize with the HDACi panobinostat and FLT3 inhibitors to overcome FLT3 resistance [27,28].

2.3. HDAC inhibitors

Histone deacetylase inhibitors (HDACIs) represent another class of agents under investigation in AML. The HDAC inhibitors romidepsin, vorinostat, and belinostat were initially approved as a single agent for the treatment of relapsed/refractory T cell lymphomas. More recently, panobinostat was approved for relapsed multiple myeloma in combination with bortezomib and dexamethasone. The early rationale for HDACIs involved their role in epigenetic modulation. Post-translational acetylation of the amino acid residues on the histone tails change the secondary structure of the histone protein tails in relation to the DNA strands. The conformational changes in chromatin induced by HDACIs increase the distance between DNA and histones, thereby increasing accessibility of transcription factors to gene promotor regions. Conversely, deacetylation of the histones decreases access of transcription factors to the promoter regions. Normal differentiation and death programs are influenced by histone modification. Histone acetylation is mediated by histone acetyl transferases; acetyl groups are removed from the histones by histone deacetylases (HDACs). There are at least 18 humans HDACs with varying function, location and substrates categorized into 4 classes by their homology to yeast proteins. HDACs represent a plausible therapeutic target given the common occurrence of increased expression in cancer cells. In

this context, HDAC expression is frequently deregulated in AML cells [29]. Distinct HDAC binding patterns have been identified in certain AML subtypes [30]. Many of the affected genes are involved in hematopoiesis, transcriptional regulation, and signal transduction. In recent years, additional mechanisms of action for HDACIs have been identified leading to recognition of their pleiotropic activity through a wide variety of disparate and mutually interactive mechanisms (Table 2). For example, recent studies have highlighted the finding that HDACIs disrupt the DNA damage response (DDR) through multiple mechanisms, including disruption of both homologous recombination and non-homologous end-joining, and interfere with DNA damage checkpoints [31].

The response rates for single-agent HDACIs in AML have been relatively low (Table 3). As a result, most investigations of HDACIs have involved combination with other agents, particularly the hypomethylating agents, decitabine, and azacitidine, given evidence of synergism between demethylation and histone deacetylase inhibition [32]. Valproic acid was one of the first HDACIs investigated in AML. In a phase 1/2 study of 54 patients with high-risk MDS/AML (untreated older than 60 years in phase 2), valproic acid was administered concomitantly with decitabine 15 mg/m² IV for 10 days. There were 10 CRs and 2 CRps (RR 22%) with a remission duration of 7.2 months [33]. In a larger phase 2 study, 149 patients with high-risk MDS/AML aged >60 were randomized to decitabine 20 mg/m² IV for 5 days with or without valproic acid. The outcomes were similar in the two groups with an overall CR rate of 34% [34]. In a phase 1 study, 81 patients with relapsed/refractory AML/MDS were administered decitabine 20 mg/m² IV for 5 days with sequential or concurrent vorinostat [35]. More responses were identified in the concurrent schedule. In the relapsed refractory group, two of 13 patients had a CR in the concurrent group as compared to 0/15 in the sequential group. Entinostat, a more potent HDACI, was investigated in a randomized phase 2 study for patients with AML/MDS [36]. One hundred forty-nine patients were randomized to azacitidine 50 mg/m² for 10 days with or without entinostat. There was no difference in the overall hematologic response rate, 44% versus 46%, respectively. Diminished hypomethylation was noted in the combination. Pracinostat, a potent oral HDACI selective for class I, II, and IV isoforms was combined with azacitidine 75 mg/m² IV/SQ daily for 7 days in previously untreated AML patients 65 deemed inappropriate for intensive induction or with other high-risk features [37]. At the initial assessment, 8 of the 14 patients evaluable for efficacy had a CR/Cri without unexpected toxicities. Based on single agent activity (1 CR and 1 PR with durations of 206 and 362 days, respectively), pracinostat was granted Orphan Drug Status by the FDA in 2014 [38]. In a phase 1b/2 study, panobinostat, an oral pan-HDACI, was administered semi-sequentially with azacitidine 75 mg/m² daily for 5 days to previously untreated AML or poor-risk MDS patients [39]. Among the 29 patients with AML, there were 3 CR/CRis (10%) and 6 PRs. In an attempt to better ascertain which patients may benefit from the addition of panobinostat to azacitidine, qualitative flow cytometry was employed. Dose-dependent increases in histone H3 and H4 acetylation after panobinostat was given were identified. Additionally, there was a strong correlation between acetylation increases >50% from baseline and clinical response (44 vs 0%).

2.4. LSD1 inhibitors

Lysine-specific demethylase 1 (LSD1) is another histone-modifying enzyme expressed in several leukemia cell lines. Pharmacologic inhibition or genetic knockdown of LSD1 in human leukemia cells induces differentiation [40]. GSK2879552, an oral LSD1 inhibitor, is currently being investigated as a monotherapy in a phase 1 study for patients with relapsed/refractory AML (NCT02177812). In leukemia cell lines, there appears to be synergism between HDAC and LSD1 inhibitors which supports a clinical trial for further exploration [41].

2.5. DOT1L inhibitors

Leukemias with recurrent translocation at the 11q23 locus are referred to as MLL-rearranged (MLL-r) and are generally associated with a poor prognosis. MLL is rearranged in approximately 5–10% of AML. The MLL protein has over 70 potential fusion partners. MLL loses its catalytic domain as the MLL-r associated protein. However, many of the common MLL fusion partner proteins are able to bind another histone methyltransferase (HMT) known as DOT1L. Via transcriptional activation, it is speculated that DOT1L enzymatic activity represents an oncogenic driver of MLL-r leukemia. EPZ-5676 is a small molecule inhibitor of DOT1L that has been studied in patients with relapsed refractory leukemia [42]. The phase 1 expansion stage was limited to patients with MLL-r or partial tandem duplications of MLL. In the 28 evaluable patients, there was one morphologic CR, one cytogenetic response, and resolution of leukemia cutis in 2. EPZ-5676 was well-tolerated in the initial study. Continued investigation of EPZ-5676 in patients with MLL gene rearrangements is warranted. In addition, other agents potentially capable of disrupting the MLL complex are at various stages of pre-clinical investigation, including menin antagonists, CDK inhibitors, and BET inhibitors, among others [43].

3. Cell cycle and signaling inhibitors

3.1. CDK inhibitors

Cyclin-dependent kinases (CDKs), critical for the regulation of cell cycle progression, represent a rationale therapeutic target in cancer. CDK-cyclin complexes are overactive in many cancers and their inhibition causes cell cycle arrest and induces apoptosis [44]. Flavopiridol, alvocidib, a semi-synthetic flavonoid derived from rohitukine, was the first CDK inhibitor (CKDI) to enter clinical trials in humans. It is a pan-CDKI with inhibition of CDKs 1, 2, 4/6, 7, and 9 [45]. In addition to cell cycle arrest and apoptosis, inactivation of CDK9/Cyclin T complex leads to blockade of cellular transcription and subsequently blocks production of anti-apoptotic proteins such as Mcl-1 (Myeloid cell leukemia-1). In pre-clinical studies, administration of flavopiridol to marrow leukemic blasts followed sequentially by cytarabine synergistically increased cytarabine-induced apoptosis [46]. Such synergism led to development of FLAM (flavopiridol, cytarabine, mitoxantrone), a timed-sequential regimen for the treatment of AML. In a phase 2 study of 45 adults with newly diagnosed high-risk leukemia, 30 patients (67%) achieved a CR [4]. In a recently completed randomized phase 2 study, 165 patients with poor risk newly diagnosed AML were randomized in a 2:1 fashion to FLAM versus standard 7 + 3 [47]. The cycle 1 CR rate in the FLAM group was 70% compared to 46% in the standard 7 + 3 group. Re-induction (i.e., 5

+ 2) was permissible in those patients with residual disease on day 14 after 7 + 3; FLAM is not intensified based on early bone marrow biopsy results. The CR rate of FLAM versus 7 + 3/5 + 2 was 70% versus 57%, $P = 0.08$, suggesting improved efficacy. Early recognition of tumor lysis syndrome is essential with the use of flavopiridol in light of the 7–10% grade 3 toxicity in other phase 2 studies. Additionally, older patients appeared to have increased risk for FLAM toxicity with 8/11 deaths on the FLAM arm occurring in patients ≥ 60 years. FLAM appears to have encouraging efficacy in *de novo* and secondary AML with CR rates of 60% which exceeded historical data. Despite the apparent increase in efficacy, there was no difference in OS between the 2 groups. It is notable that the study was not powered to detect an OS difference. The lack of OS benefit was further confounded by inconsistent application of additional induction in the 7 + 3 arm. Given the promising activity of FLAM, a phase 3 study is necessary to eliminate any potential biases.

Multiple preclinical studies demonstrated synergy with pan-CDKIs and HDACIs via several mechanisms including down-regulation of XIAP and Mcl-1 as well as blockade by CDKIs of HDACI-induced up-regulation of p21 [48,49]. Unfortunately, the preclinical studies did not translate into a clinical benefit. In a phase 1 study of alvocidib and vorinostat in patients with relapsed/refractory acute leukemia and MDS, there were no objective responses in 26 evaluable patients [50].

Palbociclib, an oral CDK4/CDK6 inhibitor, is currently approved for the treatment of ER+/Her2-neu negative breast cancer in combination with letrozole. The down-regulation of CDK6 confirmed that MLL-rearranged human leukemia cells rely on CDK6 [51]. In MLL-rearranged AML cell lines, palbociclib decreases the growth and increases the differentiation of the leukemia cells. The study provides a sound rationale for investigating the agent in MLL-rearranged acute leukemias (NCT02310243). As is the case with other targeted agents, it is unlikely monotherapy alone will be effective; additional preclinical studies may help in selecting a rational partner.

3.2. Plk inhibitors

Volasertib, a cell cycle inhibitor, was granted Breakthrough Therapy status by the FDA in 2013 for use with low-dose cytarabine in patients with high-risk AML ineligible for standard therapy. The Polo-like kinases (Plks) play essential roles in many important cellular processes including mitosis, DNA replication, stress response to DNA damage and recovery. Plk is overexpressed in various malignancies including AML [52]. Volasertib is a dihydropteridinone derivative of small molecule ATP-competitive kinase inhibitors. Volasertib blocks spindle formation and induces cell cycle arrest in M phase. In a phase 1 study in relapsed/refractory AML, volasertib was given in a dose escalation fashion alone or in combination with fixed low-dose cytarabine 20 mg SQ twice daily for 10 days [53]. As monotherapy with escalating doses, 4 of 29 patients achieved a CRi. In the combination arm, 7 of 32 patients achieved CR or CRi. A subsequent phase 2 study of low-dose cytarabine with or without volasertib in AML patients not suitable for intensive induction has also been reported [54]. Eighty-seven patients received low-dose cytarabine 20 mg twice daily SQ for 10 days with or without volasertib 350 mg IV days 1 and 15 every 4 weeks. The response rate (CR + CRi) was higher in the combination arm (31.0% versus 13.3%). Responses were

identified across all cytogenetic groups. Median event-free survival (5.6 versus 2.3 months) and OS (8.0 versus 5.2 months) were also longer in the combination arm. Of note, the study was not powered for a survival benefit. There is an ongoing phase 3 study of low-dose cytarabine versus low-dose cytarabine + volasertib with results expected in early 2016 (NCT01721876). Volasertib is also being studied in combination with hypomethylating agents and standard induction chemotherapy.

3.3. Wee1 inhibitors

The Wee1 kinase represents another emerging target in the treatment of AML. Wee1 is a kinase that is a key regulator of cell cycle progression. Phosphorylation of CDK1/cyclin B results in G2 cell cycle arrest in response to DNA damage. In the presence of DNA damaging agents, inhibition of the checkpoint results in perpetuation of the damage with cell death as a result of irreparable genetic lesions. Significantly, integrated genomic analysis identified Wee1 as a critical mediator of cell fate and a novel therapeutic target in AML [55]. A phase 1 trial of AZD1775 and belinostat is ongoing in patients with relapsed/refractory myeloid malignancies (NCT02381548). The preclinical use in AML has largely focused on circumventing cytarabine resistance [56]. However, given preclinical studies in combination with vorinostat, which demonstrated reciprocal interactions between HDAC and Wee1 inhibitors in disrupting the DNA damage response, it is rational to combine the latter agents with a HDACI [57].

3.4. MDM2 inhibitors

MDM2 is a critical negative regulator of the p53 tumor suppressor. Small molecule antagonists of MDM2 are able to effectively induce p53 resulting in activation of the p53 pathway leading to cell cycle arrest, apoptosis, and growth inhibition in cancer cell lines and xenografts in nude mice [58,59]. As the fraction of AML with mutated *p53* is small, MDM2 inhibition appears to be a rational target for investigation. Idasanutlin (RS7388) is a potent, oral nutlin-class MDM2 antagonist. In a phase 1/1b dose escalation study, idasanutlin was evaluated as monotherapy and in combination with cytarabine 1 g/m² for 6 days every 28 days [60]. Extension 1 at the recommended phase 2 dose (RP2D) included older AML patients (idasanutlin monotherapy); extension 2 (idasanutlin with or without cytarabine) included relapsed/refractory AML patients. In the part 1 extension, 9 patients were treated at the RP2D with 1 CRi/morphologic leukemia-free state (MLFS) and 1 PR. Further enrollment was discontinued secondary to prolonged myelosuppression increasing the risk of infection and early deaths (3 deaths in first 30 days). In the part 2 extension cohort, 34 of 40 planned patients had been enrolled at last report. Twenty-three patients were evaluable for responses with 4 CRs, 1 CRi, and 1 PR. The CRs were seen irrespective of p53 status and in all groups including therapy-related, relapsed/refractory, and *de novo*. One patient on monotherapy and two on combination therapy were relapse-free for >400 days and >200 days. Preclinical data suggest Flt3-ITD patients may have particular benefit from MDM2 inhibitor treatment [61]. A phase 3 study of cytarabine with or without idasanutlin in patients with relapsed/refractory AML is planned (NCT02545283).

3.5. Aurora kinase inhibitors

Aurora kinases are serine/threonine kinases that have a key role in several stages of mitosis. There are 3 classes (Aurora A, B, and C) of the kinase which have generated interest in cancer related to their increased expression. Several aurora kinases inhibitors have been developed which have implication in AML. It is notable that many are multi-kinase inhibitors and also potentially inhibit the Flt3 kinase. Pre-clinical and clinical data suggest the aurora kinase inhibitors may be more active in Flt3-mutated AML [62,63]. Alisertib (MLN8237), an inhibitor of Aurora kinase A, was investigated as monotherapy in 57 patients with advanced AML or high-risk MDS in a phase 2 study [64]. In the 35 response-evaluable patients, there was 1 CR and 5 PRs with acceptable toxicity. Additionally, 49% of the patients had stable disease. Alisertib was combined with standard 7 + 3 induction in a phase I study [65]. Thirteen of the 14 treated patients had marrow ablation at the mid-induction point of therapy. As a result, a phase 2 study of alisertib with induction chemotherapy in high-risk AML is planned (NCT02560025). In a phase 2 study, barasertib (AZD1152), an Aurora B kinase inhibitor, was compared with low-dose cytarabine 20 mg SC twice daily for 10 days in elderly patients with newly diagnosed AML [66]. The CR/Cri rate in the barasertib arm was 35% (17/48) as compared to 12% (3/26) in the low-dose cytarabine arm. Responses to barasertib were sustained a median of 82 days (range, 28–321 days). Additionally, responses occurred across all cytogenetic risk groups. The study was not formally powered to detect a survival benefit (8.2 versus 4.5 months) which was not statistically significant. AMG900, an oral potent pan Aurora kinase inhibitor, is under evaluation in adults with relapsed/refractory AML (NCT01380756). In terms of combination therapy, both barasertib and alisertib demonstrate synergy with cytarabine in pre-clinical models [67,68]. Additionally, there are reports of synergy with MDM2 and Aurora kinase inhibition [69]. MK0457, a small molecule pan-Aurora kinase inhibitor, activates p53; Nutlin-3, a small molecule antagonist of MDM2, increases p53 levels to induce p53-mediated apoptosis. Concomitant inhibition synergistically induced apoptosis in AML cells with wild-type p53 suggesting further investigation in the combination is warranted.

3.6. Rigosertib

Rigosertib (ON01910.Na), a non-ATP competitive small molecule inhibitor of both the phosphoinositide 3-kinase (PI3K) and Plk signaling pathways, induces cell cycle arrest in the G2-M phase with subsequent mitotic catastrophe. Although toxic against malignant cells, it appears to have very little effect on non-malignant cells. In a phase 1/2 study, single agent rigosertib was administered in 2 dosing schedules to 26 patients with relapsed/refractory AML or transformed myeloproliferative neoplasms (MPN) [70]. The best response was disease stabilization (>50% absolute reduction of peripheral blasts) in 2/25 patients. A phase 1/2 study of oral rigosertib and azacitidine 75 mg/m² for 7 days for relapsed/refractory AML and MDS patients is currently accruing patients (NCT01926587). In the phase 1 portion, there was one CR and one CRi in 18 treated patients.

3.7. Hedgehog pathway

The hedgehog pathway is aberrantly activated in a variety of cancers. During embryonic development, the Hedgehog signaling pathway regulates proliferation and differentiation. In

cancer, the pathway has been shown to increase tumor invasiveness and regular cancer stem cell proliferation. In pre-clinical models, aberrant activation of the Hedgehog pathway is necessary for maintenance of some CD34+ leukemia stem cells [71]. PF-04449913 is a novel oral small molecule inhibitor that binds Smoothened (SMO), a membrane protein in the Hedgehog pathway. In AML cell lines and human primary AML cell, PF-04449913 appeared to improve sensitivity to cytarabine in the dormant leukemia stem cell [72]. Of note, *GLI2*, a transcription factor in the Hedgehog pathway, is increased in FLT3-ITD AML. Combined inhibition of FLT3 with sorafenib and IPI-926, a Hedgehog pathway inhibitor, reduced the burden of leukemic cells in the blood and bone marrow in mice models suggesting a possible therapeutic strategy to improve the therapy of FLT3 inhibitors [73]. Multiple phase 1 studies in AML are ongoing with PF-04449913 monotherapy or in combination with low-dose cytarabine or the hypomethylating agents. Vismodegib, an SMO antagonist approved for the treatment of basal-cell carcinoma, in combination with ribavirin with or without decitabine, is also under investigation in relapsed/refractory AML (NCT02073838).

4. Other agents

4.1. BH3-mimetics

Dysregulation of apoptosis is prevalent in many malignancies including AML. One common defect stems from overexpression of the anti-apoptotic protein Bcl-2 and related family members. Obatoclax mesylate, a small molecule binds to the BH3 binding site of Bcl-2 and related family members Bcl-xL, Mcl-1, Bcl-w, A1, and Bcl-b. In a phase 1 study, 44 patients with refractory leukemia or MDS received drug via continuous infusion without DLT [74]. One AML patient with MLL-r achieved a CR of 8 months duration. In a phase 1/2 study of obatoclax in untreated AML, there were no responses in the 15 patients [75]. Further development was halted secondary to occurrence of severe neurologic toxicity (e.g., somnolence). Navitoclax (ABT-263) is a second-generation BH3 mimetic with high affinity for Bcl2 and Bcl-xL [76]. In preclinical models, single-agent navitoclax exhibited activity in tumors dependent on Bcl-2 and Bcl-XL for survival. The development of navitoclax was initially deferred due to dose-dependent thrombocytopenia related to on-target inhibition of Bcl-xL, although clinical investigation has subsequently resumed. The expression of Mcl-1 appears to confer resistance suggesting agents such as PI3K/mTor inhibitors, able to down-regulate or neutralize Mcl-1, may have possible synergy with navitoclax. The PI3K/Akt/mTor and Ras/Raf/MEK/ERK pathways are critical to AML growth, proliferation and survival via multiple mechanisms including regulation of Bcl-2 family proteins. The combination of a PI3K/mTor inhibitor and BH3 mimetic enhanced leukemia cell death in AML cell lines, patient-derived blasts, and xenograft models [77, 78]. Venetoclax (ABT-199) is a selective oral small molecular bcl-2 inhibitor. In a phase 2 study, 32 patients with relapsed/refractory AML or frontline unfit for standard therapy were evaluated for efficacy in an intra-patient dose escalation study [79]. Of the 28 patients evaluable for response at first assessment, there was one CR and 4 CRis. Of the CR/CRi patients, 3 had IDH mutations (11 of the total had IDH1/IDH2 mutations). Unfortunately, all 3 relapsed before week 12. The most common adverse events (AEs) were nausea, diarrhea, and fatigue. There were no AEs leading to death. In this heavily pre-treated group (30 with relapsed/

refractory AML) with few therapeutic options, venetoclax had some evidence of activity, particularly in patients with IDH mutations. Six of the 32 patients had at least a 51% reduction in bone marrow blasts at first assessment. Additional studies in AML continue with venetoclax and low-dose cytarabine (NCT02287233) as well with hypomethylating agents (NCT02203773).

4.2. Neddylation inhibitors

Pevonedistat (MLN4924) is an additional promising new agent in AML treatment. It is a first-in-class inhibitor of protein “neddylation,” a post-translational protein modification loosely analogous to ubiquitination, in which proteins are targeted for proteasomal destruction. The primary proteins targeted for degradation are the Cullen-RING E3 ubiquitin ligases which include substrates with role in cell cycle progress, DNA damage, and the stress response. Notably, pevonedistat has been shown to block degradation of I κ B α , leading to inhibition of NF- κ B, upon which leukemia stem cells are known to depend [80]. In a phase 1 study, pevonedistat was administered in two different dosing schedules to 50 patients with relapsed/refractory AML [81]. For the 23 patients treated at or below the maximum tolerated dose (MTD), there were 2 CRs and 4 PRs with 2 responses maintained at 12 and 10 months. Additionally, prolonged stable disease defined as treatment 5 cycles (21 days) without evidence for progression or response, was reported in 6 patients. Across all dose levels, confirmation of induction of 8 target genes attributed to “neddylation” inhibition was identified. An ongoing phase 1b study combines pevonedistat and azacitidine 75 mg/m² for 7 days in AML patients older than 60 [82]. In a preliminary report, there were 6 CRs in 18 response-evaluable patients.

4.3. Aminopeptidase inhibitors

Tosedostat (CHR-2797) is an aminopeptidase inhibitor. This relatively new class of cancer drugs possesses anti-proliferative, antiangiogenic, and pro-apoptotic activity via disruption of protein synthesis and the protein cell cycle. Aminopeptidases catalyze the cleavage of amino acids from the N-terminus of peptide/protein substrates leading to protein degradation and release of free amino acids. Inhibition of the aminopeptidases disrupts normal protein turnover leading to peptide accumulation and decrease in free amino acids which leads to a stress response, amino acid deprivation response, including activation of stress-related pathways such NF- κ B and pro-apoptotic regulators. Additionally, there is inhibition of mTor which turns off protein synthesis. In rapidly proliferative malignancies such as acute leukemia, such activation leads to apoptosis. In a phase 1/2 study, tosedostat was evaluated in elderly and/or relapsed AML/MDS patients. In the 51 AML patients, there were 3 CRs, 1 CRp, and 3 MLFS [83]. In the OPAL phase 2 study, patients >60 years with relapsed/refractory AML were assigned to 2 different tosedostat dosing regimens [84]. In the 73 patients, there was one CR and 6 CRis. In a phase 1/2 study, tosedostat was combined with azacitidine or low-dose cytarabine in older patients with high-risk MDS or AML [85]. In the phase 1 portion, the ORR was 33% (CR/CRp/MLFS). The phase 2 portion of the study continues (NCT01636609).

5. Combination approaches

As noted previously, it is unlikely that any single approach employing the agents described above, including the most targeted, will be successful in eradicating AML cells due to the related problems of intrinsic or acquired forms of resistance. These can include loss of target inhibition due to the development of mutations, in which case the leukemic cell remains dependent upon activation of the target gene (e.g., FLT3 inhibitor mutations). Alternatively, cells may be resistant to an inhibitor by bypassing the site of inhibition e.g., through activation of downstream targets, or alternatively, by activating parallel survival pathways that relieve the cell of its addiction to the original pathway [86]. One approach to circumventing this problem involves combining agents that act in complementary ways to promote cell death. The most rational of these approaches involves blocking the activation of a parallel pathway that confers resistance to the initial inhibitor e.g., the use of MEK1/2 inhibitors in combination with BRAF inhibitors in melanoma [87]. A review of rational combination approaches involving targeted agents in AML has recently appeared and will not be repeated here [88]. In general, such strategies take two principal forms: 1) combining novel agents with standard-of-care chemotherapy; 2) combining targeted agents with each other to avoid resistance or to induce a form of synthetic lethality. Several of the novel agents discussed here potentially lend themselves very well to this approach. For example, HDACIs have been shown to interact synergistically with BET inhibitors in human leukemia cells, and given recent breakthrough status of HDACIs in AML along with encouraging single-agent activity of BET inhibitors in AML, such a combination strategy warrants consideration in this disease [27]. Similarly, the dependence of IDH1/2-mutated cells on Bcl-2 for their survival and encouraging early results for BH3-mimetics such as venetoclax in AML raises the possibility of a BH3-mimetic/IDH1/2 inhibitor strategy in IDH1/2 mutant disease [89]. Many more such rational combinations involving new agents are possible and await pre-clinical validation prior to potential clinical implementation. A select number of promising single agents and combinations are described in Table 4.

6. Conclusion

AML remains a challenging disease despite advances in our understanding. The only therapy with some utility in high-risk patients is allogeneic stem cell transplant. However, transplant portends its own challenges and does not obviate the risk of relapse. Additionally, many patients are not eligible for transplant secondary to age, co-morbidities or lack of available donor. There is a strong impetus for development of new agents in AML. As understanding of pathogenesis increases, potential targets are realized. However, it is becoming clear that targeting a single aberrant pathway is unlikely to result in long-term disease control. With an average of 13 coding mutations in *de novo* AML and on average 5 of these occurring in genes recurrently mutated, it is increasingly evident that the complex genetic make-up characteristic of AML requires a more comprehensive approach. Furthermore, targeting the limited number of driver mutations ignores observations that multiple new abnormalities may occur during AML evolution which can subsequently drive disease progression. Rational combinations which modulate distinct or “orthogonal” pathways seem to offer the greatest opportunity for more sustained responses. To date, intensive chemotherapy has afforded the greatest benefit in AML. At this juncture, it is

unlikely targeted agents will replace standard drugs such as cytarabine. However, targeted agents with limited single agent activity may offer benefit when combined with either chemotherapy or potentially other novel agents. In the case of agents demonstrating potential efficacy, multiple questions arise including identification of optimal patient populations, disease state (e.g., treatment-naïve versus relapsed/refractory disease), and the timing (e.g., scheduling or sequence) of therapy. In addition, there may be additional roles for targeted agents in the setting of post-transplant maintenance. The development of more effective targeted agents in AML will hopefully alter outcomes and ultimately effect cure in a disease that has been lethal to far too many patients.

6.1. Practice points

- Consider clinical trials in patients with relapsed or refractory AML.
- Select targeted agents, particularly IDH1/2 inhibitors, have significant single agent activity.

6.2. Research agenda

- Identification of patients who will derive greatest benefit from novel agents.
- Identification of combinations, chemotherapy with a novel agent or multiple novel agents, which are of greatest benefit.

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Table 1

Selected rational AML targets.

Drug class and action	Drug	Single/combination	Phase of development	Reference
<i>Epigenetic modifiers</i>				
IDH inhibitors	AG-221, AG-120	Single agent	3	21, 22
BET inhibitors	OTX015	Single agent	1	26
HDAC inhibitors	Pracinostat	Single agent/With azacitidine	2	37, 38
LSD1 inhibitors	GSK2879552	Single agent	1	-
DOT1L inhibitors	EPZ-2676	Single agent	1	42
<i>Cell cycle and signaling inhibitors</i>				
CDK inhibitors	Flavopiridol	With chemotherapy	2	4, 47
	Palbociclib	Single agent	1	-
Plk inhibitors	Volasertib	Single agent/With cytarabine	3	53, 54
Wee1 inhibitors	AZD1775	With belinostat	1	-
MDM2 inhibitors	Idasanutlin	Single agent +/- cytarabine	3	60
Aurora kinase inhibitors	Alisertib, barasertib	Single agent	2	65, 66
Rigosertib	-	With azacitidine	1/2	70
Hedgehog	PF-04449913	Single agent +/- cytarabine or hypomethylating agent	1	-
<i>Other agents</i>				
BH3-mimetics	Venetoclax	Single agent	2	79
Neddylation inhibitors	Pevonedistat	Single agent	1	81, 82
Aminopeptidase inhibitors	Tosedostat	Single agent/with azacitidine or cytarabine	2	83, 84, 85

Table 2

Key mechanisms of HDACI lethality.

Down-regulation of anti-apoptotic proteins
Up-regulation of pro-apoptotic proteins
Activation of the death receptor pathway
Induction of Bid cleavage and activation
Induction of endogenous cyclin-dependent kinase inhibitor p21
ROS generation and induction of DNA damage
Disruption of chaperone protein function
Inhibition of DNA repair

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Table 3

Single agent activity for selected HDACIs.

Agent	HDAC Target	Number of AML patients	Activity (%)	Development phase in AML
Valproic acid	Classes I, IIa	58	1 CR, 1 CRi, 1 PR (5%) CR duration 16 mo	2
Vorinostat	Classes I, II	31	2 CR, 2 CRi, 3 HI (17%) Median duration of response 6 weeks	3
		37	1 CR – 398 days (3%)	
Entinostat	Class I	38	0	2
Mocetinostat	Class I	22	2 MLFS (9%)	2
Belinostat	Classes I, II	12	0	2
Pracinostat	Classes I, II, IV	12	1 CR, 1 PR, duration 206 and 362 days	2
Panobinostat	Classes I, II	13	0	2

Table 4

Select promising agents/combinations.

Agent(s)	Class	Responses	Reference
AG-221	IDH2 inhibitor	20/32 (8 CR, 3 CRi, 8 PR)	21
AG-120	IDH1 inhibitor	7/14 (4 CR, 2CRi, 1 PR)	22
Pracinostat	HDAC inhibitor	2/12 (1 CR, 1 PR)	38
Pracinostat/Azacitidine	HDAC inhibitor	8/14 CR/CRi	37
Volasertib	Plk inhibitor	4/29 CRi	53
Volasertib/low-dose cytarabine	Plk inhibitor	13/42 CR/CRi	54
Barasertib	Aurora kinase inhibitor	17/48 CR/CRi	66
Venetoclax	BH3-mimetic	5/28 CR/CRi	79
Pevonedistat	Neddylation inhibitor	6/23 (2 CR, 4 PR)	81