New drugs in acute myeloid leukemia

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The standard therapy for acute myeloid leukemia (AML) has not changed meaningfully for the past four decades. Improvements in supportive care and modifications to the dose and schedule of existing agents have led to steady improvements in outcomes. However, developing new therapies for AML has been challenging. Although there have been advances in understanding the biology of AML, translating this knowledge to viable treatments has been slow. Active research is currently ongoing to address this important need and several promising drug candidates are currently in the pipeline. Here, we review some of the most advanced and promising compounds that are currently in clinical trials and may have the potential to be part of our future armamentarium. These drug candidates range from cytotoxic chemotherapies, targeted small-molecule inhibitors, and monoclonal antibodies.

Key words: acute myeloid leukemia, new therapies, FLT3 inhibitors, BCL2 inhibitor, monoclonal antibodies, targeted therapy

Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults and among the most lethal. In the United States, the annual incidence of AML is 19 000 new cases, and the incidence of AML-associated deaths is 10 000. Classification systems based on pretreatment karyotype and mutations allow stratification of patients by risk [1]. There have been significant research efforts aimed at improving outcomes in AML, but the standard therapy for most subtypes of newly diagnosed AML is still unchanged for the past four decades. Outside the setting of a clinical trial, most patients with newly diagnosed AML are offered the combination of standard-dose cytarabine with an anthracycline (daunorubicin or idarubicin), the so-called 7 + 3 regimen. Favorable-risk patients are offered high-dose cytarabine (HiDAC) consolidation, whereas those with adverse-risk disease are offered allogeneic stem-cell transplant (SCT) in first remission. Other than all-trans-retinoic acid in 1995 and arsenic trioxide in 2001 for the treatment of acute promyelocytic leukemia, the last drug Food and Drug Administration (FDA)-approved for AML was idarubicin in 1990. Gemtuzumab ozogamicin (GO), a CD33 monoclonal antibody bound to the toxin calicheamicin, was approved for AML salvage in 2000 and withdrawn from the market in 2010 [2, 3].

Despite advances in understanding the pathophysiology of AML and recognizing its molecular heterogeneity, developing viable therapeutics for patients with AML has proved a daunting challenge. With improvements in supportive care, long-term survival has improved in younger patients with AML, with 5-year overall survival (OS) in the range of 40%–50%. However, among patients over the age of 60 years, who make up the bulk of AML cases, the long-term outlook is dismal, with a 5-year OS of 10%–20% [4, 5]. There remains a clear need for newer therapies and a more individualized approach for the treatment of AML. In parallel with newer therapies, newer end points beyond morphologic complete remission (CR) such as progression-free survival and minimal residual disease-negative CR must also be explored [6].

Several new agents are currently in advanced development to help address this need. These agents, which span several mechanisms of action, have undergone years of preclinical and early clinical testing, building on the successes and failures of prior approaches. Here we review some of the most promising new drugs for the treatment of AML, divided into categories based on their mechanisms of action: cytotoxic agents, small-molecule inhibitors, and targeted therapies (Table 1).

Cytotoxic agents

Vosaroxin

Vosaroxin is a first-in-class anticancer quinolone derivative that intercalates into DNA and, similar to anthracyclines, potently inhibits DNA topoisomerase II and induces double-stranded (ds) DNA breaks. It may have several advantages over traditional anthracyclines. First, vosaroxin is not associated with the formation of free radicals, reactive oxygen species, or toxic metabolites, potentially limiting its cardiotoxicity [7–9]. Second, vosaroxin is not a substrate of P-glycoprotein and can induce
p53-independent apoptosis [8, 10]. Based on preclinical activity in leukemia cell lines and evidence of safety in a phase I trial in leukemia, several phase II trials have been completed, demonstrating efficacy as a single agent and in combination with cytarabine [11–14]. In a phase Ib/II study of vosaroxin (10–90 mg/m² on D1 and 4) combined with cytarabine (400–1000 mg/m² on D 1–5) in patients with relapsed or refractory (R/R) AML, the recommended phase II dose was 90 mg/m² on D1–4 combined with cytarabine (400–1000 mg/m² on days 1, 8, 15).

### Table 1. New drugs being developed for AML, their proposed mechanism of action, and ongoing clinical trials

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of action</th>
<th>Comments</th>
<th>Clinical trials</th>
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<tbody>
<tr>
<td>Vosaroxin</td>
<td>Cytotoxic; DNA-intercalating agent</td>
<td>Multicenter randomized phase III trial in combination with cytarabine did not meet primary end point, but demonstrated improved outcomes in older patients. Lower intensity trials ongoing.</td>
<td>NCT01893320; NCT01913951; NCT01980056</td>
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<tr>
<td>CPX-351</td>
<td>Cytotoxic; liposomal formulation of cytarabine and daunorubicin in 5:1 molar ratio</td>
<td>Randomized trial of CPX-351 versus 7 + 3 demonstrated improved outcomes in patients with secondary AML.</td>
<td>NCT02286726; NCT01804101</td>
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<tr>
<td>Sapacitabine</td>
<td>Cytotoxic; orally bioavailable novel nucleoside analog</td>
<td>Single-agent sapacitabine had outcomes similar to LDAC, but sequential combination study with decitabine showed promising results. A randomized study of this approach is ongoing.</td>
<td>NCT01303796</td>
</tr>
<tr>
<td>SGI-110</td>
<td>Cytotoxic; longer acting hypomethylating agent</td>
<td>Single-agent activity in AML and myelodysplastic syndrome seems promising. A randomized study versus conventional care is ongoing, as are combination studies.</td>
<td>NCT02096055; NCT02131597; NCT02348489</td>
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<tr>
<td>Volasertib</td>
<td>Small-molecule inhibitor of Polo-like kinase</td>
<td>A phase II study of volasertib combined with LDAC demonstrated improved outcomes over LDAC. A randomized phase III study is underway to confirm these results.</td>
<td>NCT01721876; NCT02003573</td>
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<tr>
<td>AG-221</td>
<td>Small-molecule inhibitor of isocitrate dehydrogenase (IDH)-2 enzyme</td>
<td>Single-agent studies have reported significant activity in patients with IDH2-mutated AML. Combination studies with conventional chemotherapy have been planned.</td>
<td>NCT01915498; NCT02577406</td>
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<tr>
<td>AG-120</td>
<td>Small-molecule inhibitor of IDH-1 enzyme</td>
<td>Single-agent study has demonstrated activity in patients with IDH1-mutated AML. Combination studies are planned.</td>
<td>NCT02074839</td>
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<tr>
<td>ABT-199</td>
<td>Small-molecule BH3 mimetic, inhibitor of BCL-2</td>
<td>Single-agent ABT-199 demonstrated a signal of response in patients with AML, particularly in patients with IDH mutations. Combination studies are now underway.</td>
<td>NCT02287233; NCT02203773</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Small-molecule multikinase inhibitor with activity against mutant FLT3 (FLT3-ITD) in AML</td>
<td>Following several single-arm studies, a phase III randomized study of 7 + 3 with or without sorafenib demonstrated improved outcomes (but not improved OS) with sorafenib in younger patients with AML. A randomized study in older patients did not show a benefit. Lower intensity therapy such as hypomethylating agents are being studied in combination with sorafenib.</td>
<td>NCT01296857; NCT01253070</td>
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<tr>
<td>Midostaurin</td>
<td>Small-molecule multikinase inhibitor with activity against mutant FLT (FLT-ITD) in AML</td>
<td>Results from a large phase III randomized double-blind study of 7 + 3 with or without midostaurin in newly diagnosed patients with FLT3-mutated AML were recently presented. Among younger patients (median age 48 years), the midostaurin arm was associated with a significant improvement in event-free- and overall survival.</td>
<td>NCT00651261</td>
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<tr>
<td>Quizartinib</td>
<td>Small-molecule multikinase inhibitor with potent activity against FLT3-ITD</td>
<td>Single-agent dose-finding studies have demonstrated efficacy, but DLT is QT prolongation. Lower doses of quizartinib have demonstrated similar activity but less toxicity. These are now being studied in combination studies.</td>
<td>NCT01892371; NCT02039726</td>
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<tr>
<td>Crenolanib</td>
<td>Small-molecule kinase inhibitor with activity against mutant FLT3 (both FLT3-ITD and FLT3-D835)</td>
<td>Single-agent studies have demonstrated activity in heavily pretreated patients; combination studies are now being conducted.</td>
<td>NCT01657682; NCT02400281; NCT02283177</td>
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<td>ASP2215</td>
<td>Small molecule both FLT3 and AXL kinases</td>
<td>Single-agent studies are just underway, with preliminary data anticipated.</td>
<td>NCT0214558; NCT02236013; NCT02421939</td>
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<tr>
<td>FLX925</td>
<td>Small-molecule inhibitor of both FLT3 kinase and CDK4/6</td>
<td>Single-agent studies are just underway, with preliminary data anticipated.</td>
<td>NCT02335814</td>
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<tr>
<td>SGN-33a</td>
<td>Monoclonal antibody-drug conjugate directed at CD33, carrying a pyrrolobenzodiazepine dimer (toxin)</td>
<td>Single-agent and combination studies are underway in several clinical settings. Preliminary experience is positive, demonstrating good safety profile and efficacy in clearing bone marrow blasts.</td>
<td>NCT02326584; NCT01902329</td>
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<tr>
<td>AMG-330</td>
<td>Monoclonal bispecific antibody directed at CD33 and CD3</td>
<td>Single-agent studies are just getting underway.</td>
<td>NCT02520427</td>
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with cytarabine 1000 mg/m² on D1–5 [13]. Among 69 patients with primary refractory AML, or those in first relapse, the CR/CRi with incomplete blood count recovery (CRi) rate was 28%. The 30-day all-cause mortality was 2.5% and the dose-limiting toxicity (DLT) was stomatitis [13]. Based on these encouraging results, a phase III randomized, placebo-controlled study of cytarabine with or without vosaroxin in patients with first-relapsed or refractory AML was conducted [15]. A total of 711 patients were randomized to receive either cytarabine (1000 mg/m² i.v. on D1–5) plus placebo or cytarabine plus vosaroxin (90 mg/m² i.v. on D1 and 4 of cycle 1 and 70 mg/m² in subsequent cycles). The vosaroxin arm was associated with a significantly higher rate of CR (30% versus 16%, P < 0.0001) and an improvement in OS (7.5 versus 6.1 months, P = 0.061). Although the study did not achieve its primary end point, in prespecified subgroup analyses, vosaroxin was associated with a significant improvement in OS for patients ≥ 60 years (7.1 versus 5 months, P = 0.003) and when censoring for allogeneic SCT (6.7 versus 5.3 months, P = 0.024). Importantly, the combination was not associated with increased early mortality (30-day mortality with vosaroxin 8% versus 7%; 60-day mortality 20% versus 19%, respectively). There were more adverse events in the vosaroxin arm, including higher rates of febrile neutropenia and stomatitis, but the regimen was tolerable. Vosaroxin is also being studied in a lower intensity combination with decitabine in older patients with newly diagnosed AML (NCT01893320).

CPX-351

While the ‘7 + 3’ regimen has been the standard induction regimen for AML for decades, several attempts have been made to modify the treatment program to improve outcomes. Intensifying the dose of cytarabine [16, 17] or daunorubicin [18, 19] or even changing the anthracycline [20, 21] has led to improved response rates but only modest improvements in long-term outcome. One innovation, aimed to reduce extramedullary toxicity and increase exposure of leukemic cells to the doublet, involves packaging the ‘7 + 3’ combination in a liposome. CPX-351 is nano-scale liposome which includes within it, a fixed molar ratio of ara-C and daunorubicin of 5:1 [22]. This molar ratio was studied and found to be an optimal combination, maximizing synergy, and avoiding antagonism. A first-in-man phase I dose-escalation study in patients with relapsed and refractory AML confirmed safety and efficacy and produced a CR/CRi with incomplete platelet recovery (CRp) rate of 23% [22]. A multicenter phase II study randomized (2:1) 125 patients with relapsed AML to CPX-351 versus physician’s choice of intensive chemotherapy for first salvage [23]. CPX-351 was associated with a higher rate of CR/CRi compared with the control arm (49.3% versus 40.9%), but there was no difference in median OS (8.5 versus 6.3 months, P = 0.19). In a prespecified subgroup analysis of poor risk patients, CPX-351 was associated with a significant improvement in median OS (6.6 versus 4.2 months, P = 0.02) and median event-free survival (EFS; 1.9 versus 1.2 months, P = 0.08) [23]. The drug was well tolerated in this population and was associated with a lower 60-day mortality (16.1% versus 24.1%) compared with the control arm. A second randomized phase II trial was conducted in frontline AML patients aged ≥ 60 years [24]. A total of 127 patients were randomized (2:1) to CPX-351 (100 U/m² on D1,3,5) or conventional ‘7 + 3’(cytarabine 100 mg/m² i.v. on D1–7 with daunorubicin 60 mg/m² i.v. on D1–3). Overall response rate (ORR) was the primary end point. CPX-351 produced a higher response rate (66.7% versus 51.2%, P = 0.07) [24]. In a predefined cohort of patients with secondary AML, CPX-351 demonstrated a higher response rate (58% versus 32%, P = 0.06) and prolongation of EFS [hazard ratio (HR) = 0.59, P = 0.08] and OS [HR = 0.46, P = 0.01]. Prolonged myelosuppression was noted with CPX-351 compared with 7 + 3, but this did not translate into increased infection-related deaths. CPX-351 was associated with a lower 60-day mortality compared with ‘7 + 3’ (4.7% versus 14.6%). A phase III registration trial in patients with newly diagnosed secondary AML is ongoing (NCT01696084).

Sapacitabine

Sapacitabine is an N4-palmitoyl derivative of 2‘,C-cyano-2‘-deoxy-β-D-arabinino-pentofuranosylcytosine (CNDAC) which is orally bioavailable and resistant to deamination and inactivation [25–27]. CNDAC, the active component of sapacitabine, is a deoxycytidine analog similar to cytarabine with a unique mechanism of action. Upon phosphorylation to the nucleotide and incorporation into actively synthesized DNA, replication is not immediately inhibited in a cytotoxic manner like cytarabine or clofarabine [25–27]. Instead, the cyano group within the ring triggers rearrangement of the nucleotide while it is incorporated in the DNA, creating a single-strand (ss) DNA break. This is then converted to a ds-DNA break after a round of DNA replication, which leads to cell death. This may explain the effect it has on actively dividing hematopoietic cells and the observation that responses and myelosuppression are more profound with successive courses of therapy.

After demonstrating broad preclinical activity in multiple human tumor cells, including leukemia cell lines, a phase I study was conducted in advanced leukemias [26]. Forty-seven patients were treated with sapacitabine on two different schedules: (A) orally (p.o.) twice daily (b.i.d.) for 7 days every 3–4 weeks, or (B) p.o. b.i.d. on days 1–3 and 8–10 every 3–4 weeks. The DLTs on both schedules were gastrointestinal in nature, including diarrhea, abdominal pain, neutropenic colitis, and small bowel obstruction. The maximum tolerated dose (MTD) for schedule A was 325 mg b.i.d.; the MTD for schedule B was 425 mg b.i.d. Grade 3 or 4 myelosuppression was common and 30% had febrile episodes associated with myelosuppression. Thirteen patients (28%) achieve an objective response, including 4 CR (9%), 2 CRp (4%), and 7 CRi (15%). The estimated 30-day mortality was 4% [26]. The encouraging safety and efficacy profile prompted further development in AML. A randomized phase II study in older patients with newly diagnosed AML studied three schedules of sapacitabine [28]: (A) 200 mg b.i.d. d. × 7 days, (B) 300 mg b.i.d. × 7 days, and (C) 400 mg b.i.d. on D1–3 and 8–10. Among the 60 patients who were randomized to the three schedules, the 1-year OS rate was 35% for cohort A, 10% for B, and 30% for C [28]. The most common adverse events were myelosuppression and the 60-day mortality was 26%. A second randomized phase II trial compared sapacitabine (300 mg b.i.d. on D1–3 and 8–10) in a ‘pick-the-winner’ design to standard low-dose cytarabine (LDAC, 20 mg s.c. b.i.d. × 10
days) in 143 older patients with AML [29]. At the planned interim analysis, there was no difference in response (CR/Cri, 27% versus 16%, P = 0.09), 2-year relapse-free survival (10% versus 14%, P = 0.4), or 2-year OS (12% versus 11%, P = 0.2) for sapacitabine compared with LDAC [29]. The trial was stopped early for insufficient evidence of benefit. It was clear from these earlier studies that, although active, sapacitabine as a single agent was inadequate therapy. In a pooled analysis of patients in a lead-in phase of a randomized phase III trial, 46 patients were treated with sapacitabine alternating with decitabine [30]. The approach was well tolerated, yielded a response rate of 37% (22% CR), 35% 1-year survival, and 13% 60-day mortality [30]. A randomized phase III trial comparing this approach to single-agent decitabine is ongoing (NCT01303796).

**SGI-110**

Hypomethylating agents (HMAs) such as 5-azacytidine and decitabine have improved outcomes in patients with AML and have become important low-intensity options for older patients and those unfit for intensive therapy. In these select subgroups, HMAs produce outcomes similar to those obtained with intensive chemotherapy, with lower toxicity [31]. Both 5-azacytidine and decitabine have also been shown to be effective in older patients with higher blast count AML and associated with a survival benefit when compared with conventional therapy [32, 33]. Resistance to these agents and the development of HMA failure is a major limitation. Although the exact mechanism of resistance is not known, altered metabolism of the nucleoside analog or modification of the drug target have been proposed. SGI-110 or guadecitabine is a second-generation HMA designed to be resistant to degradation by cytidine deaminase, one of the major enzymes responsible for decitabine degradation and shortened half-life [34–36]. SGI-110 (2’-deoxy-5-azacytidylyl-(3’ → 5’)-2’deoxyguanosine sodium salt) is a dinucleotide of decitabine and deoxyguanosine linked by a phosphodiester bond that gives it properties of longer half-life and more prolonged in vivo exposure to decitabine. A first-in-human phase I dose-escalation study evaluated three different schedules of SGI-110 (3–125 mg/m²/day) in patients with RR myelodysplastic syndrome or AML: (A) s.c. daily on D1–5, (B) s.c. on 1, 8, 15 of 28-day cycle, and (C) s.c. twice weekly [37]. The drug was well tolerated, with the most common grade 3 or higher adverse events being myelosuppression and infection. The recommended phase II dose was 60 mg/m² daily × 5. Six of 74 patients with RR had an objective response, with 5 of the 6 being treated at a dose of 60 mg/m² or higher [37]. Preliminary results from a phase II study using a 10-day schedule of SGI-110 in RR AML were recently reported [38]. Among 53 heavily pretreated patients (median age 57 years), the response rate (CR + Cri + CRp) was 30%, with 30- and 60-day mortality rates of 1.9% and 11.3%, respectively [38]. The doses studied, 60 and 90 mg/m²/day, were both tolerable with myelosuppression and febrile neutropenia being the most common grade ≥3 adverse events. This signal of activity has prompted several studies in newly diagnosed AML—both as single agent in a randomized phase III study compared with ‘treatment choice’ (NCT02348489) and in a four-arm study randomizing between the 5-day schedule, 10-day schedule, in combination with idarubicin, or in combination with cladribine (NCT02096055). Both studies are ongoing and preliminary data are awaited.

**small-molecule targeted therapies**

**Volasertib**

Plk-1 belongs to the family of serine/threonine kinases and plays an important role in centrosome maturation, spindle formation, and cytokinesis during mitosis. Plk-1 is aberrantly highly expressed in leukemia cell lines and in freshly isolated leukemia cells from patients with AML relative to normal bone marrow cells from healthy donors [39]. Inhibition of Plk-1 using small-interfering RNA (siRNA) in leukemia cells has been shown to inhibit their proliferation, suggesting important therapeutic potential [39].

Volasertib is a small-molecule serine threonine kinase inhibitor of the cell cycle kinase Plk1. It binds competitively to the adenosine triphosphate binding pocket of this kinase and inhibits its enzymatic activity at low nanomolar concentrations. Volasertib is also an inhibitor of two related Polo-like kinase family members, Plk2 (Polo-like kinase 2) and Plk3 (Polo-like kinase 3), at low nanomolar concentrations. Treatment of cells with volasertib leads to cell cycle arrest in early prometaphase due to impaired spindle formation (leading to formation of a monopolar spindle instead of a bipolar spindle), which is followed by apoptotic cell death [40]. A phase I dose-finding study of volasertib in combination with low-dose araC (LDAC, 20 mg s.c. b.i.d. × on D1–10) in AML provided evidence of clinical efficacy and determined the MTD to be 350 mg i.v. on D1 and 15 [41]. This led in to a randomized phase II study of LDAC with or without volasertib in patients deemed unsuitable for intensive induction therapy [42]. Eighty-seven patients with a median age of 75 years were randomized to either LDAC or LDAC + volasertib at the above established doses. The response rate (CR/Cri) was higher for the combination arm (30% versus 13.3%, P = 0.052) and associated with a significant improvement in median EFS (5.6 versus 2.3 months, P = 0.021) and median OS (8 versus 5.2 months, P = 0.047) [42]. While there was an increased frequency of adverse events associated with the combination arm (myelosuppression, infections, gastrointestinal), there was no difference in 30-day (9.5% versus 8.9%, respectively) or 60-day (21.4% versus 17.8%, respectively) mortality between the two arms. These data earned volasertib the FDA’s Breakthrough Therapy Designation for AML to help expedite further development of this drug. An ongoing multicenter phase III randomized control trial of LDAC with or without volasertib in patients with newly diagnosed AML ineligible for intensive chemotherapy may help confirm these data (NCT01721876). Other studies combining volasertib with intensive chemotherapy and with HMAs are being planned.

**Isocitrate dehydrogenase inhibitors**

Newer techniques in whole-genome deep-sequencing have uncovered novel mutations that may be involved the pathogenesis of AML. Better understanding the biological significance of these mutations can provide opportunities for biologically targeted therapies. Mutations in isocitrate dehydrogenase (IDH) 1/2 have been identified in up to 20% of patients with normal
karyotype AML and have been shown to induce a neomorphic enzyme activity in the IDH protein. This leads to the aberrant production of the onco-metabolite 2-hydroxylutarate (2-HG). 2-HG has been shown to dysregulate enzymes involved in epigenetic function, promote hypermethylation, and may be sufficient in causing leukemia [43–45]. Strategies to target IDH mutant AML are being investigated with promising early results. AG221, an orally bioavailable small-molecule inhibitor of IDH2, has demonstrated inhibition of 2-HG production and objective clinical responses [46]. In a phase I study, 158 patients with IDH2-mutated AML have been treated once or twice daily with doses ranging from 30 to 200 mg. The ORR was 40%, including 16.5% CR (updated data from presentation) [46]. The drug was very well tolerated, with the most common adverse events being nausea, fever, diarrhea, and fatigue. Similarly, IDH1 inhibitors are being developed. Preliminary results from a phase I trial of the IDH1 inhibitor AG-120 in R/R IDH1-mutated AML were recently presented including 52 treated patients [47]. Investigators reported responses in 16 of 52 (31%) patients, including 8 (16%) CR (updated data from presentation) [47]. Unlike traditional cytotoxic chemotherapy, responses with these agents have occurred progressively over several cycles, were durable and were associated with differentiation or maturation of the leukemic blasts. Both studies are ongoing with dose escalation and no MTD has been reached. These signals of clinical activity with IDH inhibitors in a molecularly defined subset of AML have generated excitement about individualizing the treatment of AML.

**ABT-199**

Another approach to targeting AML involves activating the intrinsic or mitochondrial pathway of apoptosis. This pathway of apoptosis is regulated by the BCL-2 family of proteins, involving a dynamic balance of proapoptotic effectors (Bak, Bax) and antiapoptotic proteins (BCL-2, BCL-XL, and MCL-1). In the balanced state, the antiapoptotic proteins bind to and sequester the proapoptotic proteins, preventing them from triggering mitochondrial outer membrane permeabilization, release of cytochrome c, and apoptosis. Small-molecule ‘BH3-mimetics’ have been developed that bind to the antiapoptotic proteins in the BH3 domain and liberate the proapoptotic proteins to trigger apoptosis. Earlier investigational BH3 mimetics were found to bind efficiently to multiple antiapoptotic proteins, including BCL-2, BCL-XL, and MCL-1 and were consequently associated with on-target toxicity, including thrombocytopenia. ABT-199, a more advanced clinical candidate BH3 mimetic, was specifically designed to retain specificity for BCL-2, but lack affinity for BCL-XL. ABT-199 has demonstrated significant activity in lymphoid neoplasms including chronic lymphocytic leukemia. Since AML blasts and AML stem cells are dependent on BCL-2 for survival, while normal hematopoietic cells are dependent on MCL-1, ABT-199 was tested in AML. In preclinical studies, Pan et al. [48] tested the activity of ABT-199 in leukemia cell lines, murine primary xenografts, and primary samples of patients with AML. Their studies demonstrated significant activity of ABT-199 in all three AML model systems, correlating with dependence on mitochondrial apoptosis and predicted by BH3 profiling. Interestingly, they also demonstrated activity against AML stem and progenitor cells in the primary samples, immunophenotypically defined as CD34(+), CD38(−), and CD123(+) cells [48]. These data prompted a clinical trial of single-agent ABT199 in AML [49]. Among 32 patients with R/R AML, ABT-199 (20 mg escalated to 800 mg daily) therapy demonstrated a CR/CRI rate of 15.5%. Importantly, 19% of patients had a reduction of blasts by at least 50% from baseline, suggesting antileukemic activity [49]. The responses were particularly enriched in patients with IDH mutations, with three of the four CR/CRI occurring in patients with IDH mutations. Several other patients with IDH mutations had significant blast count reduction that did not meet IWG response criteria. These clinical observations confirm laboratory observations that suggest BCL-2 as a synthetic lethal partner for IDH2-mutated AML [50]. If these observations are confirmed, IDH mutations could be utilized as a predictive biomarker for response. Promising clinical activity as a single agent has led to ongoing studies of combination of ABT-199 with HMAs (NCT02203773) and LDAC (NCT02287233).

**FLT3 Inhibitors**

Activating mutations in FMS-like tyrosine kinase 3 (FLT3) receptor tyrosine kinase are present in about a third of patients with AML and are associated with a high risk of relapse and an overall adverse prognosis. Recognized activating mutations are in the form of internal tandem duplications (ITD) of the juxtamembrane domain or point mutations in the tyrosine kinase domain (TKD) of FLT3, with the ITD being most closely associated with an adverse prognosis. These activating mutations lead to constitutive signaling that may promote growth and survival of AML blasts. A high allelic burden of mutant FLT3 in an AML sample may suggest dependence on FLT3 signaling and a greater sensitivity to FLT3-inhibitor signaling [51]. Several small-molecule FLT3-ITD inhibitors have been developed and demonstrated benefit as single agents and in combination with chemotherapy.

sorafenib. Sorafenib is a multikinase inhibitor that potently inhibits FLT3-ITD and yields significant single-agent activity in FLT3-ITD-mutated AML [52]. Combination studies with higher and lower intensity chemotherapy have also demonstrated improvement in outcomes for patients with FLT3-ITD-mutated AML. In a phase I/II study of sorafenib (median age 53 years) combined with idarubicin and HiDAC, the overall CR rate was 75% [53]. In patients with FLT3-ITD mutation, the CR rate was 93%, with a 1-year OS of 74%. The SORAML trial randomized 276 patients (aged 18–60 years) to daunorubicin and cytarabine (7 + 3) with or without sorafenib [54]. The CR rates between the two arms were similar. However, there was a significant prolongation of 1-year EFS in the sorafenib arm (64% versus 50%, P = 0.023). This result was in all patients, with a trend for more significant improvement in patients with FLT3-ITD mutations [54]. A second randomized study in older patients (61–80 years) evaluated the same approach in 201 patients [55]. In contrast to the younger patient study, there was a trend toward lower CR rate (48% versus 60%, P = 0.12), higher early death (17% versus 7%, P = 0.052), and no improvement in EFS or OS with sorafenib [55]. These results suggest an important role for sorafenib combined
with intensive chemotherapy in AML, particularly patients with FLT3-ITD mutations. However, this combination may be too toxic for older patients. A lower intensity approach using sorafenib in combination with 5-azacytidine (5-AZA) has generated promising results [56]. Thirty-seven assessable patients (median age 64 years) with R/R FLT3-mutated AML received sorafenib (400 mg b.i.d.) combined with 5-AZA (75 mg/m² i.v. on D1–5). The ORR was 46% (16% CR; 27% CRi; 3% PR), with 3% 4- and 8-week mortality [56]. Combination sorafenib studies are ongoing to better define the efficacy and toxicity of this FLT3 inhibitor and determine its place in AML treatment.

midostaurin. Midostaurin is an orally bioavailable multikinase inhibitor with nanomolar inhibitory activity against FLT3-ITD. After observing single-agent activity in FLT3-mutated patients [57], midostaurin was studied in combination with chemotherapy. A phase Ib trial of midostaurin combined with 7 + 3 in newly diagnosed AML demonstrated safety and efficacy at a dose of 50 mg daily [58]. The combination produced a higher CR rate in FLT3-mutated patients (92% versus 74%). This led to a randomized, placebo-controlled phase III trial of 7 + 3 with or without midostaurin in patients with newly diagnosed FLT3-mutated AML whose results were recently released [59]. Among 717 patients, with a median age of 48 years, there was no significant difference in toxicity or CR rate between the two arms. However, the study demonstrated a significant improvement in EFS and OS favoring the combination with midostaurin [59]. This benefit persisted after censoring for transplant and remained across FLT3 subgroups. The clear survival benefit with midostaurin in this subset of AML could translate into a new standard of care for a molecular defined patient population. Lower intensity combinations with midostaurin are also being studied [60, 61].

quizartinib. Quizartinib is a highly potent small-molecule TKI designed specifically as FLT3 inhibitor [62]. In a phase I dose-escalation study in patients with R/R AML, the MTD was determined to be 200 mg/day and the DLT was grade 3 QTc prolongation [62]. Among patients with FLT3-ITD mutations, the ORR was 53%, compared with 14% in FLT3-ITD-negative patients. Due to the higher incidence of QTc prolongation associated with higher doses and preserved FLT3-inhibitory activity at lower doses, subsequent phase II trials studied lower dose levels. A phase II trial of quizartinib in patients with R/R AML used doses of 90 mg/day in females and 135 mg/day in males. In patients ≥60 years of age, the composite CR rate was 54% in FLT3-ITD-positive patients and 32% in FLT3-negative patients [63]. These were associated with remission durations of 12.7 and 22 weeks, respectively [63]. Among younger patients, the composite CR rates were 44% and 34% in the FLT3-ITD-positive and -negative groups, respectively [64]. These were associated with remission durations of 11.3 and 25.6 weeks, respectively [64]. Recently, the preliminary results of a randomized trial studying even lower doses of quizartinib were reported [65]. Seventy-six patients with R/R FLT3-ITD-positive AML were randomized to receive either 30 or 60 mg of quizartinib per day. The composite CR rate was 50% in both arms, and the median OS was 146 versus 197 days, respectively [65]. Notably, the rate of grade 2 QTc prolongation was 11% versus 17%, respectively, and the rate of grade 3 QTc prolongation was only 3% in both arms. There was no grade 4 QTc prolongation [65]. The development of this drug demonstrates the importance of targeting a biologically effective dose, rather than the clinical MTD, when studying a targeted agent, to minimize toxicity. Combination studies investigating quizartinib in relapsed and frontline FLT3-ITD-positive AML are ongoing [66, 67] (NCT01892371).

Activating point mutations in the FLT3 TKD also lead to constitutive downstream signaling in AML and are associated with resistance to FLT3 inhibitors. Newer FLT3 inhibitors are currently in development that may help circumvent this and other mechanisms of resistance. Crenolanib is a potent small molecular inhibitor that has activity against both the FLT3-ITD and the FLT3-D835 TKD mutation. Preliminary results of crenolanib monotherapy in 19 patients with FLT3-mutated AML demonstrate an ORR of 50% [68]. The dual FLT3/AXL inhibitor ASP2215 and the FLT3/CDK4/6 inhibitor FLX925 are also being studied in R/R AML [69, 70]. Recent data also suggest an important role for FLT3 inhibitors as post-SCT maintenance in patients with FLT3-mutated AML [71–73]. As we gain more insight into the efficacy and toxicity of these newer agents, their role in the treatment of R/R AML will become clearer.

monoclonal antibodies

Monoclonal antibodies have improved outcomes in patients with lymphoid hematologic malignancies and may also have an important role in the treatment of AML. Clinical experience with the anti-CD33 monoclonal antibody-drug conjugate GO underscores the importance of this approach in AML. Although withdrawn from the US market, recent studies of GO [74–76] in combination with chemotherapy have demonstrated improvements in relapse-free and OS in select subgroups of patients, validating CD33 as an important target [77].

SGN-33A

SGN-33A is a humanized anti-CD33 monoclonal antibody conjugated to a potent DNA-crosslinking toxin [78]. Rather than the calicheamicin toxin in GO which is a substrate of drug-efflux enzymes, SGN-33A carries a pyrrolobenzodiazepine dimer [78]. Preclinical evaluation demonstrated more potent antileukemia activity than GO in cell lines, primary patient samples, and xenograft models [78]. SGN-33A is currently undergoing clinical investigation as a single agent and combination in several clinical settings (NCT02326584). Preliminary data from the phase I dose-escalation trial of SGN-33A were recently reported [79]. Among 38 assessable patients with relapsed AML treated with escalating doses of SGN-33A (5–60 µg/kg i.v. every 3 weeks), 16 (42%) had clearance of marrow blasts, with acceptable tolerability [79]. Studies with this new compound are ongoing and more data are expected soon. Careful development of this compound at the optimal dose schedule and in defined subgroups that had benefit with GO may lead to an important candidate for regulatory approval in AML.

AMG-330

Another approach to targeting the CD33 antigen on AML blasts involves recruiting the host immune system to recognize and eradicate the leukemia blasts by cell-mediated cytotoxicity. A new antibody construct, the bispecific antibody, has two motifs

null

null
of specificity—one that recognizes a tumor-specific antigen, and the second one, CD3, which is present on T cells. This bispecific T-cell engaging antibody or 'BITE' can recruit CD3+ effector T cells to the targeting cancer cell and trigger immune cytotoxicity. This approach, using the CD19/CD3 BITE blinatumumab, has shown significant clinical activity in B-cell ALL and is now approved in the salvage setting [80]. A similar approach is now being investigated in AML. AMG-330 is a CD33/CD3 BITE that demonstrated significant antileukemia activity in preclinical experiments [81]. In vivo primary patient samples, AMG-330 led to T-cell recruitment and expansion, followed by significant antibody-mediated cytotoxicity, lysing autologous blasts in the majority of samples [81]. Clinical trials of AMG-330 are underway and may represent an important new tool in the therapy of AML (NCT02520427).

**Conclusion**

The current standard of care for the treatment of adults with AML remains suboptimal. Scientific advances are helping better understand the biological differences among different patients' leukemias and identifying potential targets for novel therapies. It will therefore become mandatory to routinely molecularly characterize cases of newly diagnosed AML to identify these targets before therapy. As we gain more experience with these novel agents on clinical trials and compare them to existing therapies, we hope to raise the bar of efficacy and improve longer term outcomes. Carefully designed studies in selected subsets of patients as well as increased clinical trial enrollment will help advance the field.

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**References**


Breakthrough therapies in B-cell non-Hodgkin lymphoma

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The last 5 years have seen significant advances in our understanding of the molecular pathogenesis of B-cell lymphomas. This has led to the emergence of a large number of new therapeutic agents exploiting precise aspects of the tumor cell’s signaling pathways, surface antigens or microenvironment. The purpose of this comprehensive review is to provide a detailed analysis of the breakthrough agents in the field, with a focus on recent clinical data. We describe agents targeting the B-cell receptor pathway, Bcl-2 inhibitors, emerging epigenetic therapies, new monoclonal antibodies and antibody drug conjugates, selective inhibitors of nuclear export, agents targeting the programmed cell death axis and chimeric antigen receptor T cells.

Key words: lymphomas, treatment, novel agents, ibrutinib, idelalisib, venetoclax

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