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Acute myeloid leukemia: advancing clinical trials and promising therapeutics

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Abstract

Recent progress in understanding the biology of acute myeloid leukemia (AML) and the identification of targetable driver mutations, leukemia specific antigens and signal transduction pathways has ushered in a new era of therapy. In many circumstances the response rates with such targeted or antibody-based therapies are superior to those achieved with standard therapy and with decreased toxicity. In this review we discuss novel therapies in AML with a focus on two major areas of unmet need: (1) single agent and combination strategies to improve frontline therapy in elderly patients with AML and (2) molecularly targeted therapies in the frontline and salvage setting in all patients with AML.

Keywords

AML; clinical trials; molecular therapy; monoclonal antibodies; multikinase inhibitors

INTRODUCTION

The standard of care treatment for AML has remained relatively unchanged over the past 4 decades. Treatment consists of intensive induction therapy, most commonly with a combination of an anthracycline and cytarabine, followed by post-remission consolidation, with cytarabine-based chemotherapy or stem cell transplantation (1). However, between 50% and 60% of patients relapse, so only 40% to 50% will achieve long-term disease-free survival (2).

Elderly patients (typically 60–65 years in most publications) with AML have a significantly less favorable prognosis that is attributable to having a disease that is inherently more resistant to current standard cytotoxic agents and/or having relatively poor tolerance of these agents (3–5). Importantly, this group constitutes the majority of patients with AML.

Advances in the molecular characterization of pathogenic mechanisms of leukemogenesis have resulted in the identification of mutations in a number of genes that regulate somatic

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and epigenetic pathways with prognostic and therapeutic implications (1, 6–12). A number of clinically active agents targeting the aberrant protein products of these mutated genes are currently in clinical trials(13). These include agents targeting FLT3 kinase (quizartinib, sorafenib, crenolanib, FLX925, E6201), *IDH1/IDH2* (such as AG221, AG120, IDH305 and CB839), MEK (activated in patients with *NRAS/KRAS* mutations) (GSK1120212 and MEK-162), BCL2 (ABT-199), MLL (DOT1-L1 inhibitors), TP53 (bromodomain inhibitors and MDM2 inhibitors), STAT inhibitors, Axl inhibitors, and others which are being investigated as single agents or in combinations (14–17). Another approach to targeted therapy includes naked or conjugated-antibodies to leukemia specific antigens or immunomodulatory pathways. Clinical trials with antibody-drug conjugates to CD33 (SGN33A), CD123 (SL-401), CD56 (IMGN901), bi-specific monoclonal antibodies (AMG330), and immune checkpoint inhibitors including PD1 (nivolumab, pembrolizumab) as single agents or in combination with standard AML therapies during induction, consolidation, and maintenance are ongoing(18). In this review, we outline emerging therapies for newly diagnosed and relapsed patients with AML in the two major areas of unmet need: (1) strategies to improve frontline therapy in elderly AML patients and (2) molecularly targeted therapies in the frontline and salvage setting.

STRATEGIES TO IMPROVE FRONTLINE THERAPY IN ELDERLY AML

AML is primarily a disease of elderly. Over two thirds of patients with newly diagnosed AML in the United States are 65 years and older (19–21). Despite steady progress in the therapy of AML in younger patients, the treatment of elderly AML has not improved significantly over the last four decades (21–23). The 4–8 week mortality with intensive chemotherapy is 15%-50% in these patients, and the median survival is 4–7 months with <10% of elderly patients with AML achieving long-term disease free survival. Hypomethylating agents are the most frequently used agents in the treatment of less fit, older patients with AML in the United States and Europe (24). The DACO-016 study compared the efficacy and safety of decitabine (20 mg/m²/day for 5 days every 4 weeks) versus treatment of choice (TC) [including low dose cytarabine (LDAC) 20 mg/m²/day for 10 days every 4 weeks or best supportive care] in 485 patients with AML (median age 73 years) ineligible for cytotoxic chemotherapy (24, 25). The initial analysis showed a trend toward improved survival with decitabine (7.7 versus 5.0 months; *P* = 0.108) that became significant (*P* = 0.037) with further follow-up. The CR/CRi rate with decitabine was 17.8% as compared to a CR/CRi rate of 7.8% with TC. Azacitidine has shown benefit in elderly patients with AML in two large multicenter trials. In one trial patients with AML with 20–30% blasts were evaluated as a subset analysis of the phase III AZA-001 trial(26). The patients were randomly assigned to receive subcutaneous azacitidine or conventional care regimen CCR (best supportive care [BSC] only, LDAC, or intensive chemotherapy [IC]). Of the 58 patients in the CCR group, 27 (47%), 20 (34%), and 11 (19%) received BSC only, LDAC, and IC, respectively. At a median follow-up of 20.1 months, the median overall survival (OS) for azacitidine-treated patients was 24.5 months compared with 16.0 months for CCR-treated patients (*P* = 0.005), and 2-year OS rates were 50% and 16%, respectively (*P* = 0.001) (26). A relatively large proportion of patients in the CCR group received BSC only (47%) and there were no CR/CRi's among the patients who received BSC. It is possible

that this may have contributed to the observed difference between the two groups. Among the patients who received LDAC a morphologic CR was noted in 15%. This morphologic CR rate is higher than the morphologic CR rate of 8.4% noted in the DACO-016 study. The reasons for these differences are not clear but it is difficult to make a clear comparison across studies. In another trial, 488 elderly patients with AML with >30% blasts and age 65 years were randomized to receive either azacitidine (75 mg/m²/day for 7 days every 4 weeks) or a CCR (standard induction chemotherapy, LDAC, or supportive care only)(27). Median OS was 10.4 months (1-year survival 47%) for patients receiving azacitidine compared to 6.5 months (1-year survival 34%) for patients receiving CCR ($P = 0.0829$). A prespecified analysis censoring patients who received AML treatment after discontinuing study drug showed median OS with azacitidine versus CCR was 12.1 months vs 6.9 months (stratified log-rank $P = .0190$). Although it appears there is a role for hypomethylating agents with a signal for improved response rate and possible improved survival over supportive care and LDAC-based regimens the improvements have been modest with scope for significant improvement in outcomes in elderly AML. The dismal prognosis in elderly AML patients has resulted in increased efforts and clinical trials to improve the frontline therapy in elderly patients (>60 years) with AML including de-novo AML, therapy-related AML and AML arising from preexisting antecedent hematologic disorder (AHD), AML in very old (>75 years) or infirm patients, and AML harboring targetable mutations such as *FLT3* and *IDH1/2*.

I. AML in Elderly Patients (Table 1)

(A) SGI-110—SGI-110 is a second-generation hypomethylating agent formulated as a dinucleotide of decitabine and deoxyguanosine. It has a longer half-life and produces an extended decitabine exposure as compared to intravenous decitabine infusion. In a phase I study SGI-110 produced potent hypomethylation and clinical responses in relapsed and refractory MDS and AML patients including patients previously treated with azacitidine or decitabine (28). SGI-110 was well tolerated in a Phase II, open label, multi-center study in elderly treatment-naïve patients with AML who were not suitable for induction cytotoxic chemotherapy. Responses (CR/CRi) were observed in 55% of the patients with an 8-week mortality of 14–16%. Response rate was similar at the two dose levels evaluated: 60 mg/m² Qday × 5 and 90 mg/m² Qday × 5(29). These data compare favorably with previous results reported for first-generation hypomethylating agent therapies and have led to the initiation of a Phase III pivotal study of SGI-110 versus treatment of choice (azacitidine, decitabine or LDAC) in older adults with previously untreated AML who are not considered candidates for intensive remission induction therapy (ClinicalTrials.gov Identifier: NCT02348489).

(B) ABT-199 + Decitabine or Azacitidine—Bcl-2 overexpression has been implicated in maintaining the survival of AML cells and has been associated with resistance to chemotherapy and inferior overall survival (30). ABT-199 is a potent and selective small-molecule inhibitor of Bcl-2 that has demonstrated cell-killing activity against a variety of leukemia cell lines, primary patient samples and leukemia stem/progenitor cells (31, 32). ABT-199 also has been found to synergize with agents known to down-regulate Mcl-1, including azacitidine (33). In a phase II multicenter trial single agent ABT-199 produced an overall response in 5/32 relapsed/refractory AML patients (CR in 1 patient, CRi in 4

patients) (34). Of the 5 patients with CR/CRi, 3 had *IDH* mutations suggesting that patients with *IDH* mutations may be particularly sensitive to ABT-199. Two ongoing trials are evaluating ABT-199 combination regimens in treatment naïve patients with AML who are 65 years of age and who are not eligible for standard induction: (a) to evaluate the efficacy and tolerability of the combination of ABT-199 with a methyltransferase inhibitor (azacytidine or decitabine) (ClinicalTrials.gov Identifier: NCT02203773). Interim results from this study were recently presented and show an encouraging response rate of 75% with a median time to response of only 29.5 days(35), (b) to evaluate ABT-199 in combination with low-dose cytarabine (ClinicalTrials.gov Identifier: NCT02287233).

(C) Vosaroxin + Decitabine—Vosaroxin is a first-in-class anti-cancer quinolone derivative (AQD). The pivotal Phase III, randomized, controlled, double-blind, multinational clinical study to evaluate the efficacy and safety of vosaroxin and cytarabine versus placebo and cytarabine in patients with first relapsed or refractory AML (VALOR) (36). A total of 711 patients with AML aged 18 years of age or older with refractory disease or who were in first relapse after one or two cycles of previous induction chemotherapy, including at least one cycle of anthracycline (or anthracenedione) plus cytarabine were enrolled. The study demonstrated improved overall survival (7.5 months versus 6.1 months, unstratified log-rank $P = 0.061$; stratified $p = 0.024$) and complete remission rates 30% versus 16%, $P < 0.0001$) in the vosaroxin plus cytarabine group compared to the placebo plus cytarabine group.

Vosaroxin and decitabine have non-overlapping mechanisms of action. Interim results of the single arm, open-label study of this combination in previously untreated patients with AML/high-risk MDS who are 60 and older are encouraging with a response rate (CR/CRp) of 74% and an 8-week mortality of 13%. The regimen is moderately intense and is especially attractive among patients aged 60–75 years with >60% of these patients alive one year after initiation of therapy (37). The response rate among patients with complex cytogenetics was 67% and among patients harboring a TP53 mutation was 75%. These response rates compare favorably to response rates with hypomethylator therapy alone in patients with these adverse features. However, the median OS among patients with these adverse features remained similar to that observed with single agent hypomethylator therapy with patients with complex cytogenetics having a median OS of 4.1 months. This suggests that vosaroxin may have increased potency to induce a response due to its p53 independent mechanism of action but the overall survival is still driven by the baseline adverse features.

(D) SGN-CD33A in Patients With CD33-Positive Acute Myeloid Leukemia—

Several CD33 antibody-drug conjugates (ADCs) such as lintuzumab, VE9633 and gemtuzumab ozogamicin (Mylotarg) have been evaluated in the treatment of AML (38–40). Among these, gemtuzumab demonstrated single-agent activity in relapsed AML and improvement in survival for a subset of patients when used as part of frontline therapy (40–42). SGN-CD33A is a next-generation ADC with uniform drug loading, a highly stable linker, and a novel drug payload. In preclinical models, SGN demonstrated targeted cell killing and appeared to be insensitive to common resistance mechanisms (43). A Phase I first-in-human trial of SGN including treatment-naïve patients who have declined or are not suitable for high-dose induction/consolidation therapy is ongoing (ClinicalTrials.gov

Identifier: NCT01902329). Sutherland et al have demonstrated that 5-azacytidine significantly enhanced the ability of an earlier generation anti-CD33 antibody (lintuzumab; SGN33) to promote tumor cell killing through antibody-dependent cellular cytotoxicity (ADCC) and phagocytic (ADCP) activities (44). These results suggest that an anti-CD33 antibody and 5-azacytidine may act in concert to promote tumor cell killing. Daver et al demonstrated that decitabine and gemtuzumab ozogamicin improved the response rate but not overall survival compared with historical outcomes in untreated AML 60 years (45). To further explore the hypothesis of combining a DNA-methyltransferase inhibitor with an anti-CD33 antibody the ongoing study includes a cohort combining SGN-CD33A with a DNA-methyltransferase inhibitor (azacytidine or decitabine) for elderly treatment naïve patients with AML. Initial results from this study were recently presented and revealed a CR/CRi rate of 65% and 8-week mortality of 4%. 85% of treated patients had a 50% reduction in blasts (46).

Monoclonal antibody drug conjugates not only may be beneficial in the initial therapy but may also improve outcomes by eradicating minimal residual disease when administered in maintenance in patients with high-risk AML who are unable to receive allogeneic stem cell transplant. Such strategies are being evaluated in ongoing clinical trials. A concurrent phase Ib dose-escalation study to find the best dose and schedule for SGN-CD33A when given in combination with induction treatment, and in combination with consolidation treatment, and as monotherapy for maintenance is ongoing (ClinicalTrials.gov Identifier: NCT02326584).

(E) Purine analogues (Clofarabine, Cladribine) and LDAC Alternating With

Decitabine—We have previously demonstrated the efficacy and safety of a sequential low-intensive therapy using the combination of clofarabine and LDAC alternating with decitabine. Patients received clofarabine 10 mg/m² daily × 5 plus cytarabine 20 mg subcutaneously twice daily for 10 days every month for three courses alternating with decitabine 20mg/m² daily x 5 every month for three courses, for a total of 18 months. Among 118 patients treated with this regimen the CR rate was 60% (ORR = 68%), median survival was 11.1 months, 4- and 8- week mortality were 3% and 7%, respectively (47). We are now evaluating a similar alternating regimen of cladribine + LDAC alternating with decitabine. Cladribine is a purine analog that modulates deoxycytidine kinase and is thought to possess more specific activity against myeloid blasts than clofarabine. Cladribine has been shown to improve survival when combined with cytarabine. The combination of cladribine and LDAC alternating with decitabine was well tolerated with 1% 4-week mortality and no treatment-related grade 3/4 non-hematologic adverse events. The CR rate is 57%, and the overall response rate (CR+CRp+PR) is 69%. The median OS is 12.1 months and estimated 1-year overall survival is 57%(48).

(F) CPX-351—CPX-351 is a liposomal formulation of a fixed combination of the antineoplastic drugs cytarabine and daunorubicin. CPX-351 markedly prolongs plasma drug levels and maintains the 5:1 molar ratio for optimal leukemic cell killing. A phase II study of CPX randomized 125 patients with AML in first relapse after initial an CR lasting 1 month 2:1 to CPX-351 or investigators' choice of first salvage chemotherapy. The investigators choice was usually based on cytarabine and anthracycline, often with 1 or more additional

agents. Patients were stratified per the European Prognostic Index (EPI) into favorable, intermediate, and poor-risk groups based on duration of first CR, cytogenetics, age, and transplant history. Subset analyses of the EPI-defined poor-risk strata demonstrated higher response rates (39.3% versus 27.6%) and improvements in event-free survival (1.9 months versus 1.2 months, HR=0.63, $P=0.08$) and overall survival (6.6 months versus 4.2 months, HR=0.55, $P=0.02$) and improved outcomes over standard 3+7 in first relapse AML patients with EPI-defined poor-risk disease (49, 50). In another phase II study CPX-351 produced a higher response rates than 7+3 with no significant differences in event free survival (EFS) or OS in patients with newly diagnosed AML(51). The CR/CRi rate was improved with CPX-351 versus 3+7 among patients with adverse cytogenetics (77.3% versus 38.5%; $P=0.03$) and among patients with secondary AML (57.6% versus 31.6%; $P=0.06$). A planned analysis of secondary AML patients ($n=52$) found a statistically significant improvement in OS (median 12.1 versus 6.1 months, HR = 0.46, $P=0.01$) and EFS (median 4.5 versus 1.3 months, HR = 0.59, $P=0.08$) in favor of the CPX-351 cohort. However, this difference was not seen among patients with adverse cytogenetics wherein the median survival among the CPX-351 and 7+3 patients was 10.6 months and 12.2 months, respectively. The secondary AML subset was well balanced with respect to demographic characteristics, type of antecedent hematologic disorder, and proportion of patients with prior hypomethylating (5-azacitidine or decitabine) agent therapy. These data led to a phase III multicenter, randomized study of CPX-351 liposome injection versus cytarabine and daunorubicin in patients 60–75 years old with untreated high risk (secondary) AML that has recently completed accrual (Clinicaltrials.gov Identifier: NCT01696084).

(G) Hedgehog Inhibitor (PF-04449913) + Low-Dose Cytarabine—Hedgehog and Gli signaling (Hh-Gli) are critical pathways in cell cycling and angiogenesis and have been implicated in both hematopoietic and solid malignancies (52, 53). Aberrant Hh signaling has been described in human leukemia (54). PF-04449913 is a novel small molecule inhibitor of the Sonic Hedgehog Pathway being developed for the treatment of hematologic malignancies and solid tumors. A Phase IB/II, open label, international, multi-center, safety and efficacy study of PF-04449913 in combination with intensive chemotherapy (cytarabine and daunorubicin), LDAC, or decitabine in previously untreated patients with AML or high-risk MDS unsuitable for conventional induction regimens is ongoing (ClinicalTrials.gov Identifier: NCT01546038).

(H) Volasertib and LDAC—Published preclinical and clinical data suggest that Polo-like kinase 1 (Plk1) is highly expressed in AML cells as compared to normal cells and plays an important role in centrosome maturation, spindle formation, and cytokinesis during mitosis making it a potentially interesting therapeutic target for the treatment of AML(55). Volasertib (BI 6727) is a first in class, highly selective and potent cell cycle kinase inhibitor targeting Plk1 by competitive binding to the ATP-binding pocket of the kinase. In a phase II trial volasertib in combination with LDAC had a higher remission rate than LDAC monotherapy (30% versus 13%, $P=0.05$) in older patients (median age 75 years) with previously untreated AML considered ineligible for intensive treatment. The combination of volasertib and LDAC resulted in improved event-free survival (5.6 versus 2.3 months, $P=0.02$) and overall survival (8.0 versus 5.2 months, $P=0.047$). An ongoing randomized,

double-blind, phase III trial is evaluating whether patients ≥ 65 years of age with previously untreated AML will have higher response rates with volasertib and LDAC as compared to placebo and LDAC (clinicaltrials.gov: NCT01721876). This trial includes patients who are deemed ineligible for intensive induction chemotherapy or traditional clinical protocols due to poor performance status, concomitant diagnosis, or organ dysfunction.

II. MOLECULAR AND MULTIKINASE INHIBITOR THERAPIES IN THE FRONTLINE AND SALVAGE SETTING

A number of mutated or deregulated genes conferring unfavorable, indeterminate or favorable prognosis have been identified (7, 56). There are three predominant ways by which molecular mutations impact therapy in leukemia(57). Firstly, mutated or aberrantly expressed genes are potential targets for small-molecule inhibitors or monoclonal antibodies. A number of clinically active agents targeting *FLT3 ITD* and/or *D835* (such as quizartinib, crenolanib and sorafenib), MEK (activated in patients with *NRAS/KRAS* mutations) (such as GSK1120212 and MEK-162) and *IDH1/IDH2* (such as AG221) are being investigated in AML (Table 2). Secondly, specific molecular abnormalities stratify patients to risk-adapted strategies and aid in the selection of ideal post-remission therapy (e.g. allogeneic stem cell transplant for molecular poor-risk patients)(6). Thirdly, the presence of mutations that regulate DNA methylation and chromatin structure may define epigenetically distinct forms of leukemia, thus potentially assisting in the identification patients with a higher likelihood of responding to epigenetic therapy (58). Novel therapies that may actively target specific cytogenetic aberrations/pathways such as EPZ-5676 that inhibit DOT1L are also attractive targeted approaches. The prelim results with this compound show modest activity(59). Studies with this and other inhibitors of specific pathways/molecular aberrations are ongoing.

I. FLT3-MUTATED AML

FLT3 plays a crucial role in normal hematopoiesis and cellular growth in primitive hematopoietic stem and progenitor cells (60). Signaling via receptor tyrosine kinases is frequently deregulated in hematological malignancies. FLT3 (FMS-like tyrosine kinase III) is a transmembrane tyrosine kinase that belongs to the Class III family of RTKs. FLT3 is activated following binding of FLT3 ligand, leading to activation of downstream signaling pathways including Stat5, MAPK/ERK, and PI3K/AKT. FLT3 stimulates survival and proliferation of leukemic blasts. FLT3 is expressed on the leukemic cells of 70% to 100% of patients with AML. Additionally, activating mutations in *FLT3* are observed in approximately 30% of adult patients with AML. The leading types of mutations found in AML include internal tandem duplication mutations in the juxtamembrane domain (ITD, ~30%) and mutations in the activation loop (approximately ~7–10%)(6). Patients with mutations in *FLT3* have a worse prognosis when treated with conventional chemotherapy compared to patients with wild-type FLT(6).

Several small-molecule FLT3 inhibitors currently undergoing evaluation in phase I, II, and III trials have shown promising activity as single agents and in combination with hypomethylating agents or chemotherapy. These include quizartinib, sorafenib, midostaurin,

and crenolanib (61–68). These TKIs act as direct inhibitors of *FLT3* via competitive inhibition of ATP-binding sites in the *FLT3* receptor kinase domain (KD) (69, 70). The variations in conformational states (inactive versus active) of the *FLT3* KDs have led to the development of different *FLT3* inhibitors. Most *FLT3* inhibitors including midostaurin, quizartinib, sorafenib, and lestaurtinib target the inactive conformation (type II inhibitors). However, next generation *FLT3* inhibitors, such as crenolanib target both the inactive and active conformational states (type I inhibitor) (71). Initial studies using small molecule *FLT3* inhibitors as single agents or in combination with standard chemotherapy have demonstrated clinical benefit in patients with AML expressing mutated *FLT3*. A number of phase III studies of *FLT3* inhibitors (including midostaurin, quizartinib, ASP2215) in different settings in AML are ongoing or have recently completed accrual and will hopefully continue to show positive results in the randomized setting.

(A) Midostaurin (PKC)—Midostaurin is a multi-target *FLT3* inhibitor with efficacy and good tolerability in phase 1–2 studies. Midostaurin was evaluated in the largest *FLT3*-targeted study to date, the RATIFY study, a CALGB-led intergroup, international, randomized, double-blinded, placebo controlled trial of young adult (age 18–59) patients with *FLT3*-mutated AML(72). 717 patients were randomized to receive induction chemotherapy and 4 post-remission cycles of high dose cytarabine with placebo (n=360) or midostaurin 50 mg BID (n=357) on days 8–22 of each cycle, followed by 1 year of maintenance with midostaurin 50 mg twice a day or placebo continuously. The addition of midostaurin did not improve the CR rate (59% versus 54%; $P = 0.18$) but improved the 5-year overall survival (51% versus 43%, HR 0.77, $P = 0.007$) and 5-year EFS (27% versus 19%, HR 0.80, $P = 0.004$). After censoring for transplant the addition of midostaurin continued to show improvement in the 5-year OS (63% versus 55%, HR 0.77, $P = 0.047$) and 5-year EFS (24% versus 22%, HR 0.84, $P = 0.03$). No significant differences were noted in the overall rate of grade 3/4 hematologic or non-hematologic adverse events. This study is the first large randomized to study to show the benefit of *FLT3*-inhibitors and will hopefully result in the approval of this agent in combination with standard induction therapy in newly diagnosed young patients with *FLT3*-mutated AML. Furthermore, we hope that this study will pave the way for further evaluation of *FLT3*-inhibitors in different settings of AML include salvage, maintenance, combinations with high-dose chemotherapy, combinations with hypomethylating agents, and combinations of targeted agents.

(B) Quizartinib

Quizartinib (AC220) single agent therapy: AC220 is a novel second-generation Class III RTK inhibitor with potent *FLT3* activity in vitro and in vivo(73). In addition to *FLT3*, AC220 inhibits c-KIT, PDGFR and RET. In a phase 1 trial in 76-relapsed/refractory or untreated, elderly AML patients single-agent AC220 had significant clinical activity, inducing complete remissions(74). The maximum tolerated dose was 200 mg per day of continuous dosing, with asymptomatic prolongation of the QT interval as the dose-limiting toxicity. Responses were noted in 23/76 (30%) of patients including 10 (13%) CRs (2 CR, 3 CR with incomplete platelet recovery, and 5 CR with incomplete blood count recovery) and 13 (17%) PRs. The median duration of response was 14 weeks, with some responses lasting 67+ weeks. Higher overall response rates and CR rates were observed in *FLT3*-ITD mutated

patients (56% and 28%, respectively) compared with those lacking the mutation (20% and 7%, respectively). AC220 was well tolerated. The most common possibly drug-related adverse events were grade 2 in severity per CTCAE grading, and included peripheral edema, dysgeusia, and nausea.

AC220 was subsequently evaluated in two-phase II trials. In the first study 133 patients (92 FLT3-ITD mutated, 41 FLT3-ITD wild type) age 60 years and over who were either refractory to primary therapy or in first relapse received AC220 (75). The composite CR rate was 54% (0 CR; 3% CRp; and 51% CRi) among FLT3-ITD mutated patients and 32% among FLT3-ITD wild type patients. In the second phase II study primarily younger AML patients, 99 FLT3/ITD positive and 38 FLT3/ITD negative, who were refractory to or had relapsed after a second line of therapy, were included. The response rate was similar to that seen in older patients, a composite CR rate of 44% (4% CR; 0 CRp; and 40% CRi) in *FLT3/ITD* positive patients, a composite CR rate of 34% (3% CR; 3% CRp; and 29% CRi) in *FLT3/ITD* negative patients. The predominant response observed was CRi, defined by the elimination of blasts but without count recovery, and with persistent platelet and red cell transfusion dependence thought to be mediated by off target c-kit inhibition. Furthermore, QT prolongation was seen in approximately 25% of the patients. A subsequent Phase II study enrolled 76 FLT3-ITD mutated patients who were relapsed or refractory to second-line salvage chemotherapy or relapsed after hematopoietic stem cell transplantation at two lower doses of quizartinib monotherapy (30mg and 60mg)(76). The composite CR rate at both dose levels was 50% with a significant decrease in the frequency of QTc prolongation (11% at 30mg, 17% at 60mg). Phase II/III studies combining AC220 with standard induction treatments in elderly and young patients are currently ongoing.

Quizartinib (AC220) combination therapies: In an attempt to improve the response rate and more importantly the duration of response AC220 has been combined with intensive chemotherapy (anthracycline and cytarabine in the “3+7” regimen) in patients ages 18–60 years(77). Initial data from this study demonstrated that quizartinib could be safely administered with induction or consolidation chemotherapy in younger AML patients. Quizartinib has also been successfully combined with low intensity therapy (azacitidine or low-dose cytarabine) in AML patients > 60 years of age who have newly diagnosed or first-relapsed FLT3-ITD mutated AML. Patients received either AC220+azacitidine or AC220+LDAC. The initial reports are encouraging with an overall response rate (CR/CRp/CRi/PR/MLFS) of approximately 65%. A Phase 3 open-label randomized study to determine whether quizartinib monotherapy prolongs OS compared to salvage chemotherapy in FLT3-mutated patients who are refractory or have relapsed within 6 months after first-line AML therapy is ongoing (ClinicalTrials.gov Identifier: NCT02039726).

(C) Sorafenib

Sorafenib single agent therapy: Sorafenib is a multikinase inhibitor approved in the United States and Europe for the treatment of hepatocellular, renal cell, and most recently thyroid cancer. Sorafenib is an orally active multikinase inhibitor with potent activity against FLT3 as well as *VEGF*, *c-kit*, platelet-derived growth factor receptor (*PDGFR*), and *BRAF kinases* (78, 79). Sorafenib is 1000 fold more active against ITD mutant FLT3 than wild-type FLT3

in cell based assays (80). Sorafenib was shown to be safe and clinically effective in phase 1 trials that examined the effects of both dose and schedule of sorafenib in relapsed or refractory AML patients. The most common toxicities were fatigue (16%) and hypokalemia (13%). A randomized phase 1 trial examining 2 schedules of sorafenib, either continuous or intermittent, was conducted in patients with relapsed or refractory AML (n = 38) or untreated MDS (n = 4) (81). One CR was noted in an FLT3-ITD-positive AML patient. Sorafenib administered either before or after allogeneic stem cell transplantation (allo-SCT) in AML patients has also been explored (82). Sorafenib induced clinically meaningful and very rapid responses in all 6 patients treated either before (n = 2), after (n = 3), or both before and after (n = 1) allogeneic stem cell transplantation (allo-SCT). Sorafenib-induced remission allowed for allo-SCT in 2 of the 3 refractory AML patients. Two of the 4 patients treated after allo-SCT survived 216 and 221 days, respectively, while the other 2 remained in ongoing complete molecular remission. Borthakur et al treated 50 patients with relapsed hematologic malignancies with 2 different schedules of sorafenib (62). Dose limiting toxicities were grade 3/4-hypertension, hyperbilirubinemia, and amylase elevation. The recommended phase II dose in hematologic malignancies was 400 mg twice daily. Complete remissions or complete remissions with incomplete recovery of platelets were achieved in 5 (10%) patients (all with FLT3-ITD mutations). Significant reduction in bone marrow and/or peripheral blood blasts was seen in an additional 17 (34%) patients (again all with FLT3-ITD mutations). Melzelder et al reported clinical activity as a single agent in relapsed FLT3-ITD AML (82).

Sorafenib combination therapies: A phase I/II study combining idarubicin, high-dose cytarabine and sorafenib in patients with newly diagnosed AML < 65 years of age (median age, 53 years) has been conducted showing the feasibility of this strategy (83). Of 51 evaluable patients, 38 (75%) achieved a CR, including 12 (92%) of 13 FLT3-ITD, 2 (100%) of 2 patients with FLT3-TKD, and 24 (66%) of 36 FLT3-wildtype patients. The difference in CR rate between the FLT3-mutated and FLT3-wild-type patients was statistically significant ($P = 0.033$). Ravandi et al recently reported the feasibility and efficacy of combining hypomethylator therapy (5-azacytidine) with sorafenib in patients with relapsed/refractory AML (63). A total of 43 patients (93% were FLT3-ITD mutated) met the eligibility criteria and 37 were evaluable. Patients had a median of 2 prior therapies. The response rate was 46% including CR, CRp and CRi in the FLT3-ITD mutated patients. This response rate compares favorably with expected response rates in this population. 64% of the patients achieved >85% FLT3 inhibition during their first cycle of therapy. The combination was reasonably well tolerated. The majority (53%) of patients experienced grade <3 adverse effects attributable to sorafenib. Only 1 patient discontinued treatment because of a grade 4 non-ischemic (proven by cardiac catheterization) cardiomyopathy. These data resulted in a frontline study of 5-azacytidine in combination with sorafenib in older patients with newly diagnosed FLT3-ITD mutated AML that is currently accruing (NCT02196857).

Furthermore, the multikinase inhibition produced by sorafenib led Rollig et al to evaluate the efficacy and tolerability of adding sorafenib to standard induction-consolidation therapy in 267 newly diagnosed younger (18 – 60 years) patients with AML in a randomized placebo control trial (SORAML)(84). On an intent-to treat analysis the addition of sorafenib

significantly improved the median EFS (21 months versus 9 months, $P=0.013$) and 3-year EFS (40% versus 22%) but did not improve the overall survival (OS) in these patients. The 60-day mortality was 4% in both groups, which is comparable to published induction mortality rates in young AML patients treated on non-sorafenib containing standard induction regimens. This is the first randomized study to show that sorafenib produces a clinically meaningful improvement in the therapy of de novo younger AML patients. The wide prevalence of FLT3 expression and inhibition of AML driver kinases beyond FLT3, including *VEGF*, *c-kit*, platelet-derived growth factor receptor (*PDGFR*), and *BRAF* by sorafenib may explain the benefit observed in this study in both FLT3-mutated and non-mutated AML patients. The findings in the SORAML study are in contrast to the findings by Serve et al (SAL study group) who noted that the addition of sorafenib versus placebo to standard chemotherapy in elderly patients resulted in increased toxicity and early mortality without improved antileukemic efficacy(85). It remains to be determined whether sorafenib should routinely be added to frontline induction regimens in younger or older AML patients.

(D) Crenolanib—Crenolanib besylate is an orally bioavailable, selective tyrosine kinase inhibitor (TKI) FLT3 (86, 87). Most FLT3 inhibitors including quizartinib, sorafenib and midostaurin target the inactive conformation (type II inhibitors). A unique feature of crenolanib is that it possesses activity against both TKD1 and TKD2 mutants, including the D835H and D835Y mutants (68, 71, 88). Collins et al reported on the efficacy of crenolanib on the initial 18 evaluable patients (89). Commonly observed side effects included nausea and vomiting and transaminase elevations (primarily grade 1, 2). No patient had to go off study due to toxicity. No QT prolongations on EKG were observed. 11/18 patients had received prior FLT3-inhibitor therapy. The overall response rate was 50% including 1 CR, 2 CRi, 4 PRs, and 2 blast responses. Clinical activity was seen in patients refractory to other FLT3 TKIs via the major clinical resistance mechanism (D835 mutations). In a phase II single center study crenolanib was evaluated in AML patients with FLT3 activation mutations including patients whose leukemia has recurred after prior chemotherapy: (FLT3 TKI-naïve (n=13) and progressed on prior FLT3 TKI (n=21)(90). The composite CR rate was 23% in FLT3-TKI naïve patients and 5% in patients who had received prior FLT3 therapy. Combination therapy (e.g., with chemotherapy or hypomethylating agents) could potentially provide additive efficacy in both treatment-naïve and relapsed/refractory patients and these trials are ongoing (NCT02298166, NCT02400281, NCT02283177).

(E) Lestaurtinib—Lestaurtinib (previously known as CEP-701) is an indolocarbozole derivative that was found to be well tolerated and to reduce peripheral blood and bone marrow blasts in patients with FLT3-mutated AML but the overall clinical activity was modest(91). Lestaurtinib frontline monotherapy in older AML patients unfit for intensive therapy showed modest clinical activity manifested by transient reductions in bone marrow or peripheral blasts or longer periods of transfusion independence in 3 of 5 (60%) FLT3-mutated and 5 of 22 (23%) of FLT3 wild-type patients(67). The Cephalon 204 trial randomized 24 FLT3-mutated AML patients in first relapse with a first remission duration of 1–24 months. Patients received salvage chemotherapy with mitoxantrone, etoposide, cytarabine (if first remission duration 1 to 6 months) or high-dose cytarabine (if first remission duration 6 to 24 months) with or without lestaurtinib. The lestaurtinib was

administered continuously at a dose of 80 mg twice a day beginning 2 days after the completion of chemotherapy (day 7). The addition of lestaurtinib did no significantly improve the CR/CR rate (26% versus 21%, $P=0.35$) or the overall survival. The frequency of serious adverse events (32% versus 21%) and early deaths (12% versus 6%) were higher among the patients receiving lestaurtinib(92). Lestaurtinib is no longer being evaluated in any major FLT3 inhibitor based trials in AML.

(F) ASP2215—ASP2215 hemifumarate is a new chemical entity with an inhibitory effect on tyrosine kinases, mainly FLT3, Axl and ALK. ASP2215 demonstrated favorable efficacy in non-clinical AML models, including mouse xenografts and human AML cell lines. ASP2215 inhibits the growth of FLT3 wild type, FLT3-ITD and FLT3-D835 cells. Axl overexpression in AML is associated with drug-resistance and adverse prognosis (93). Axl inhibition suppresses the growth of human FLT3-positive AML in vivo (94). A phase I/II trial of ASP2215 enrolled 166 patients with relapsed/refractory AML(95). 120 patients were evaluable. The MTD was 300 mg per day and recommended phase 2 dose was 200 mg per day. Overall response rate (ORR) was 57% in 82 patients with FLT3 mutations, and 63% in 68 FLT3 mutated patients in the 80 mg and higher dose levels. Randomized phase III trials of ASP 2215 at 200 mg per day in newly diagnosed and R/R AML are planned (NCT02014558).

II. IDH-MUTATED AML

The isocitrate dehydrogenase enzymes convert isocitrate to α -ketoglutarate (α -KG). α -KG is an important intermediate in the Krebs's cycle and is also an essential co-substrate for several dioxygenases that play a role in epigenetic programming of the cell, including TET2 and histone demethylase (96). Somatic mutations at critical arginine residues in the active site of the metabolic enzymes (R132 in *IDH1* and R140 and R172 in *IDH2*) confer gain-of-function activity in cancer cells (97, 98), resulting in accumulation of the oncometabolite, 2-hydroxyglutarate (2-HG)(99). 2-HG is a competitive inhibitor of α -KG-dependent dioxygenases (100). High levels of 2-HG promote leukemogenesis by inducing epigenetic reprogramming, blocking differentiation and contributing to a transformed phenotype (101). *IDH1/2* mutations are noted in ~20% in AML, including 10–15% for *IDH1* and 8–19% for *IDH2* mutations (102). IDH-mutations are associated with older age, lower frequency of therapy-related disease, and increased incidence among patients with intermediate-risk cytogenetics, and FLT3-ITD and NPM1 mutations (103). The response rate and OS do not appear to differ between patients with *IDH*-mutated and *IDH* wild-type AML, both in the frontline and the salvage setting (104). However, IDH mutations presented novel targets for targeted therapy in AML. Chaturvedi et al demonstrated that a small molecule inhibitor of mutant IDH1 successfully reversed the myeloproliferative effects induced by the oncometabolite 2-hydroxyglutarate (105). Two first-in-class oral, selective, and reversible IDH inhibitors, namely, AG221 (inhibitor of IDH2 mutant enzyme) and AG120 (inhibitor of IDH1 mutant enzyme) are currently in clinical trials.

(A) AG221—AG221 is currently being evaluated in a Phase 1 trial to confirm the safety and clinical activity of AG-221 in relapsed/refractory AML (NCT01915498). The study includes a dose-escalation phase, four expansion cohorts of 25 patients each evaluating (a) patients

with relapsed or refractory AML who are 60 years of age and older and transplant ineligible; (b) relapsed or refractory AML patients under age 60; (c) untreated AML patients who decline standard of care chemotherapy; and (d) patients with other IDH2-mutant positive hematologic malignancies. A fifth expansion cohort of 125 patients with IDH2 mutant-positive AML who are in second or later relapse, refractory to second-line induction or re-induction treatment, or have relapsed after allogeneic transplantation has recently been added and is currently enrolling (expansion cohort e). Data reported as of May 2015 are from 177 patients receiving AG-221 administered from 60 mg to 450 mg total daily doses in the dose escalation arm and 100 mg once daily in the four expansion arms(106). A maximum tolerated dose has not been reached. The data from 177 patients (104 in dose escalation and 73 from the four expansion cohorts) with advanced hematologic malignancies treated with single agent AG-221 showed encouraging clinical activity and a favorable safety profile. Sixty-three out of 158 response-evaluable patients achieved investigator-assessed objective responses for an overall response rate of 40% including CR in 26 of 158 patients (16%). Of the 111 patients with relapsed or refractory AML (dose-escalation phase and cohort a, b), 46 (41%) achieved an objective response, including 20 (18%) CRs. Of the 22 patients with AML that had not been treated (cohort c), seven achieved an objective response, including three CRs. Of the 14 patients with MDS (cohort d), seven achieved an objective response, including two CRs.

As of the analysis date, an estimated 88% of responses lasted three months or longer and 76% of responses lasted six months or longer. More than half of the 177 patients remain on treatment. The majority of adverse events were grade 1 or 2, with the most common being nausea, fatigue, increased blood bilirubin and diarrhea. A multicenter, open-label, randomized, Phase 3 study comparing the efficacy and safety of AG-221 versus conventional care regimens (CCRs) in IDH2-mutated subjects 60 years or older with AML refractory to or relapsed after second- or third-line AML therapy is currently enrolling patients (NCT02577406). Future directions include combination trials to evaluate AG-221 as a potential frontline treatment for patients with AML. These trials are scheduled to open in late 2015.

(B) AG120—AG-120 is being evaluated in an ongoing Phase 1 trial in patients with AML and other IDH1-mutant positive advanced hematologic malignancies (NCT02074839). Data from 57 patients with advanced hematologic malignancies showed an overall response rate of 31% (16 of 52 response-evaluable patients) and a complete remission rate of 15% (8 of 52 response-evaluable patients)(107). Responses were durable estimated 79% of responders were on treatment for three months or longer, and 50% of responders were on treatment for six months or longer. The majority of adverse events reported by investigators were mild to moderate, with the most common being fatigue, diarrhea, pyrexia and nausea. Treatment with AG120 showed substantial reduction in the plasma levels of the oncometabolite 2HG to the level observed in healthy volunteers. Three expansion cohorts to evaluate AG120 in 175 patients with IDH1-mutated advanced hematologic malignancies (125 in relapsed/refractory AML, 25 in untreated AML and 25 in basket IDH1-mutant positive cancers) are currently enrolling. Future directions include combination trials to evaluate AG120 as a potential

frontline treatment for patients with AML and a global phase 3 study in AML patients that harbor an IDH1 mutation in 2016.

A second-generation pan IDH inhibitor AG881 has recently become available in clinical trials for patients with AML (NCT02492737). IDH-305 is another IDH1 inhibitor in phase I trials in patients with advanced malignancies that harbor IDH1R132 mutations.

CONCLUSION

Despite the recent advances in genomic technologies, single agent therapy with targeted agents has not been as successful as previously anticipated (7, 98). A number of potential hurdles have emerged including discriminating driver from passenger lesions, understanding the biological impact of downstream pathways, defining the prognostic and predictive value of individual mutations in a patient with multiple molecular mutations, the emergence of resistant clones and failure to control proliferation of resistant clones (either preferentially selected or spontaneously mutated) on exposure to targeted agents(13). These resistant clones are able to adapt to the therapeutic pressure of targeted agents demonstrating the plasticity of cancer cells. Studies in AML(108), pediatric ALL(109), and in CLL(110) have demonstrated selection and expansion of resistant ancestral leukemia sub-clones at the time of relapse. The emergent resistant clones are often refractory to subsequent targeted and cytotoxic therapies and portend a dismal outcome. Potential strategies to prevent the selection and expansion of such sub-clones include continuous indefinite administration of the targeted agent and/or monoclonal antibody with interruptions only for emergent toxicities; alternating or combining targeted therapies or combined targeted and antibody based as well as cytotoxic therapies. Furthermore, selection of patients for specific targeted agents or combination strategies based on their pretreatment molecular profiles and development of novel maintenance strategies including the use of immune modulating/activating agents may help achieve effective, long term responses.

EXPERT COMMENTARY

There is an urgent need for development and approval of novel therapies in AML. With the exception of decitabine and gemtuzumab ozogamicin (which was shortly thereafter taken off the market) no drugs have been approved for the therapy of AML in the United States in the last 40 years. The current preclinical and clinical development including encouraging clinical responses and safety of molecular targeted therapies (FLT3-inhibitors and IDH-inhibitors), monoclonal antibodies to CD33 and CD123, novel cytotoxic agents including CPX-351 and vosaroxin, and agents targeting pathways that play a major role in AML emergence and progression such as bcl2-inhibitors, hedgehog inhibitors suggests that we are finally making in-roads in this difficult disease. Rational combinations of a number of these agents are in clinical trials and seem to improve the response rate and time to response. The outcomes have been especially dismal in elderly AML. Clinical trials of novel hypomethylating agents (SGI-110) and hypomethylator-based combinations (decitabine + vosaroxin, decitabine/azacytidine + ABT199, azacytidine + SGN33a) have nearly doubled the response rate seen with prior single agent hypomethylators and seem to improve survival. Identification of biomarkers to select patients best suited to specific therapeutic

modalities and identification of ideal combinations will further improve outcomes and these studies have been incorporated into a number of the ongoing clinical trials.

FIVE-YEAR VIEW

Identification and clinical evaluation of molecular targeted therapies have shown promising results with FLT3 and DH-inhibitors. The FLT3-inhibitor trials are most advanced with midostaurin, quizartinib and ASP2215 in phase III trials as single-agents or in combination regimens. We anticipate successful completion and positive results from the ongoing phase III studies hopefully resulting in the approval of FLT3-inhibitors in the next few years. Combining FLT3-inhibitors with cytotoxic therapies such as 3+7, high dose cytarabine, idarubicin and cytarabine based therapies and with hypomethylating agents will improve the response rate and the durability of the response. IDH1, IDH2, and dual IDH inhibitors are showing promising results and will enter phase III trials as single agents in the next year. Combination trials of IDH inhibitors with 3+7 and hypomethylators are anticipated to open shortly. We anticipate the IDH-inhibitor phase III trials will confirm the efficacy of these agents. Drugs targeting other AML specific molecular mutations including EZH, DNMT3A, ASXL1, TET2 are in preclinical development and are expected to enter clinical trials in the next year. Novel monoclonal antibodies to CD33, CD123, CD56 and other AML specific antigen will enter clinical trials in the next few years. The monoclonal antibodies in combination with hypomethylating agents are a combination to look out for. Immune therapies including checkpoint based combinations, checkpoint + molecular combinations, AML specific vaccines, and CART-cells to AML antigens will be evaluated in clinical trials in the next 1–2 years. Immunotherapy has revolutionized the therapy of solid tumors and we anticipate it will be a significant addition to our battle against AML. We anticipate that better understanding, identification of prognostic and predictive biomarkers, and rational combinations of molecular, antibody based, and immune therapies will produce improved response rates and “cures” with reduced toxicities.

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KEY ISSUES

- In contrast to other hematologic malignancies the treatment of AML has not changes significantly over the last 4 decades.
- In the last 5 years the emergence of molecular targeted therapies, novel monoclonal antibodies, and potent small molecule inhibitors suggests that we may finally have the tools to improve outcomes in AML.
- The FLT3-inhibitors are the most advanced among the molecular targeted therapies and sorafenib, quizartinib, ASP2215, and midostaurin have a single agent CR/Cri rate of 25–55%. Combinations of FLT3-inhibitors with hypomethylating agents or cytotoxic therapies improve the response rate and more importantly the duration of response.
- IDH 1 and 2 inhibitors have a CR/Cri rate of 30–40% and are well tolerated. The responses appear to be durable in some patients. Combination studies with the IDH-inhibitors will begin shortly.
- Novel monoclonal antibodies including SGN33A (CD33) and SL401 (CD123) are producing encouraging results as single agents and in combination regimens.
- Small molecule inhibitors are showing encouraging responses. ABT199 (bcl2-inhibitor) has a single agent response rate of 18% and is showing encouraging response in combination with hypomethylating agents in newly diagnosed AML.
- Novel hypomethylating agents (SGI110) and combinations of hypomethylating agents (decitabine + vosaroxin, decitabine/azacytidine + ABT199, azacytidine + SGN33A) have approximately doubled the response rates seen with single agent traditional hypomethylating agents and are important breakthroughs for elderly AML.
- Immune therapies including checkpoint inhibitors (starting with PD1, PDL1), AML specific vaccines, AML CART cells are entering clinical trials and are a very exciting addition to this field.

Table 1**AML Elderly Induction Regimens**

Regimen	Eligible patients	CR/CRi	Med OS	8-week mortality	Notes
SGI-110(29)	N=51	55%	8.7 mo	15.7%	No major difference in response, survival, toxicity with 60 and 90 mg/m2 daily × 5
DAC/AZA+ABT199(35)	N=34	71%	Not available	Not available	Median time to CR/CRi was 29.5 days (range: 24–112 days)
DAC + vosaroxin(111)	N=61	74%	8.8 mo	13%	Med OS 10.9 mo (vosaroxin 70/m2) vs 5.4 mo (vosaroxin 90/m2)
AZA + SGN33A(46)	N=23	65%	Not Reached	4%	87% of patients had a reduction of bone marrow blasts 50%
Cladribine +LDAC alternating with DAC(48)	N=74	69%	12.1 mo	1%	The 1-year OS estimate is 57%
CPX351 vs 3+7 (Age 60–75)(51)	N=126	67% vs 51%	14.7 mo vs 12.9 mo	4.7% vs 14.6%	CPX significantly improved CR/CRi, OS and EFS as compared to 3+7 in secondary AML

OS: Overall Survival, mo: months, DAC: decitabine, AZA: azacytidine, LDAC: low dose cytarabine, EFS: event free survival

Table 2

Targeted therapeutic agents in single-agent and combination clinical trials

Modality	Target	Agent
Molecular targeted therapy		
FLT3 inhibitors	FLT3-ITD	AC220
		Sorafenib
		PKC412
		Lestaurtinib
	FLT3 ITD and D835	Crenolanib
		ASP2215
		FLX925
		E6201
		Ponatinib
IDH inhibitors	IDH2	AG221
	IDH1	AG120
		IDH305
	IDH 1 and 2	AG881
MLL rearrangement	DOT1L	EPZ5676
Monoclonal antibody		
Conjugated antibodies	CD33	SGN33A
	CD123	SL401
	CD56	IMGN901
Bi-specific T-cell Engaging Antibodies	CD3CD33	AMG330
Checkpoint receptors	PD1	Nivolumab