CLINICAL INVESTIGATION

Novel treatment options for acute myelocytic leukemia

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Acute myelocytic leukemia (AML) is an extremely heterogeneous disease with outcomes that vary widely according to subtype of the disease. Nevertheless, the majority of patients are not cured of the disease, and improvements in therapy are required. A number of recent studies challenge the notion that '7 + 3' chemotherapy (cytarabine combined with an anthracycline) is the standard of care for most patients with AML. Targeted therapy with monoclonal antibodies and small-molecule kinase inhibitors are very promising strategies to help improve the cure rate. This review will highlight the results of recent clinical trials in which outcomes have been influenced significantly. Novel approaches to sequencing and combining available therapies will also be covered. Finally, molecularly targeted therapies relevant to AML will be discussed.

Keywords: acute myelocytic leukemia • gemtuzumab ozogamicin • FLT3 inhibitors

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Acute myelocytic leukemia (AML) is a heterogeneous malignancy of the bone marrow predominantly diagnosed in patients older than 60 years of age [101]. Patients tend to present with typical signs and symptoms secondary to inadequate hematopoiesis, namely fatigue, abnormal bruising or bleeding, and infection. While there are subsets of AML that confer a good prognosis, most patients will die from the disease or complications of its treatment. The international gold standard for the majority of newly diagnosed patients is multiagent chemotherapy based on clinical trials published more than two decades ago [1,2]. Several modifications to the '7 + 3' regimen have been attempted, but most have not led to substantial improvements in overall survival (OS). However, intensifying the dose of daunorubicin has recently been shown to improve survival in certain subgroups of patients. In addition, a meta-analysis evaluating high-dose cytarabine compared with standard doses of cytarabine during induction, found improved outcomes for the high-dose cohort [3]. Patients are often stratified by age and/or performance status when selecting the initial treatment approach.

Advances in the genetic and molecular characterization of AML have improved the outlook for the development of targeted approaches to therapy. Cytogenetics at baseline has repeatedly been shown to be one of the most powerful prognostic factors for survival [4]. Patients are typically considered to have favorable, intermediate or unfavorable disease based on these results, which ultimately influences the overall treatment plan. Molecular studies allow the identification of gene mutations that influence cell signaling, proliferation and survival. Most notably, mutations in the *FLT3* gene have been shown to be associated with a very poor prognosis [5]. This is relevant, as several small molecules are able to specifically inhibit FLT3.

The most dramatic example of targeted therapy to date in AML is the treatment of acute promyelocytic leukemia (APL) with all-trans retinoic acid (ATRA) [6]. APL is a form of AML driven by a specific fusion protein, PML-RAR-α, which essentially blocks myeloid differentiation. ATRA binds to RAR-α, leading to normal transcription and terminal differentiation of myeloid cells [7]. The use of ATRA-based

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therapy allows a high number of patients to be cured of this AML subtype without the use of cytotoxic chemotherapy [6,8]. That said, the remainder of this review will focus on treatment options for non-APL AML. Monoclonal antibodies directed against cell surface antigens have represented a major advance in the treatment of acute lymphocytic leukemia (ALL) [9–11]. For example, rituximab, an antibody directed against CD20, has been shown to improve the OS in younger patients with ALL when used in the frontline setting [9]. The development of monoclonal antibodies has not been as successful in AML thus far, with the anti-CD33 agent gemtuzumab ozogamicin (GO) being withdrawn from the US market.

This review will briefly highlight the current management strategy for AML, but will mainly focus on novel and investigational agents under development that may ultimately improve patient outcomes. Most of these agents are being tested in the salvage setting, with some of the more promising agents being evaluated as part of the frontline approach. Recent clinical trials evaluating unique chemotherapy regimens, and also studies using new and 'old' monoclonal antibodies will also be discussed. When reviewing clinical trials, it is important to note the patient population that is being studied to allow proper interpretation of the results. For studies involving anthracyclines, both the agent and dose used are critical to place any results or conclusions in proper context. A summary of agents and regimens discussed in this review can be found in Table 1.

Management of adult AML: frontline approach

The combination of an anthracycline (i.e., daunorubicin or idarubicin) given for 3 days combined with continuously infused cytarabine over 7 days is the currently accepted standard of care for younger and fit elderly patients with AML. Recently, a higher dose of daunorubicin (90 mg/m^2) was found to improve the outcome for certain subsets of patients, and this has now become the standard dose recommended for these groups [12,13]. Idarubicin may be a more powerful anthracycline, and studies have shown it to be at least as good as high-dose daunorubicin [14,15].

Patients who achieve complete remission (CR) require consolidation with chemotherapy, autologous hematopoietic stem cell transplantation or allogeneic hematopoietic stem cell transplantation (alloSCT). The standard chemotherapy for consolidation is repeated courses of high-dose cytarabine (3 g/m^2 intravenously [iv.] every 12 h on days 1, 3 and 5) [16,17]. The need for such a high dose of cytarabine during consolidation was called into question when a large, randomized trial found moderate-dose cytarabine-based therapy equally effective (5-year OS similar between groups) [18]. Patients with high-risk disease are recommended to undergo alloSCT in first CR if there is a matched stem cell donor.

The above strategies lead to long-term survival in approximately 20–40% of adult patients with AML [18]. Patients with favorable risk disease may do better, and those with unfavorable risk disease tend to do worse. There have been several attempts to improve the frontline management of patients with AML. What are considered to be the biggest recent developments in the initial management of patients are highlighted below.

Purine analogs

The purine analogs, in particular, compounds mimicking adenine, have been used in the treatment of AML. The mechanisms of action include being incorporated into DNA as well as through inhibition of ribonucleotide reductase [19]. The latter mechanism may lead to synergistic effects when combined with cytarabine. Fludarabine and cladribine are older examples of purine analogs, while clofarabine was designed as an attempt to optimize the characteristics of these agents. Each of the drugs have been studied alone or in combination approaches and the results have been promising.

Fludarabine combined with high-dose cytarabine and filgrastim was studied in newly diagnosed patients with core binding factor AML [20]. This patient population tends to be highly sensitive to cytarabine, making the addition of a purine analog a rational approach to attempt to improve the outcome. Patients received fludarabine 30 mg/m^2 iv. daily for 5 days, cytarabine 2000 mg/m^2 iv. daily for 5 days, and filgrastim starting 1 day prior to chemotherapy and continuing through day 5 (FLAG). Patients receiving FLAG had superior event-free survival (EFS) when compared with historical controls that did not receive fludarabine-containing chemotherapy.

In a large randomized trial, the British Medical Research Council tested three induction chemotherapy regimens in patients with newly diagnosed AML [21]. FLAG plus idarubicin was compared with daunorubicin and cytarabine (DA), or daunorubicin, cytarabine and etoposide. While there were fewer relapses at 5 years on the FLAG-Ida arm compared with daunorubicin, cytarabine and etoposide (42 vs 54%), there were more myelosuppression-related deaths in CR that negated any potential benefit in OS. Nevertheless, FLAG-Ida is a very promising regimen and strategies should be explored to optimize drug dosing, administration and supportive care to reduce deaths in CR.

Cladribine was approved by the US FDA in 1993 for the management of hairy cell leukemia [22]. The addition of cladribine has been shown to enhance the cellular uptake of cytarabine into leukemic cells [23]. It also exhibits some antileukemic activity as a single agent.

Table 1. Novel strategies for the treatment of adult acute myeloid leukemia.

Agent	Target/Class	Comment
Fludarabine	Nucleoside analog	When used first line in the FLAG-Ida regimen, has been shown to be more effective than standard induction chemotherapy
Cladribine	Nucleoside analog	When added to '7 + 3' during induction, improved survival compared with 7 + 3 alone
Clofarabine	Nucleoside analog	Has been studied in combination with standard chemotherapy, as well as sequenced with hypomethylating agents in elderly patients
Gemtuzumab	Monoclonal antibody	Improves survival in subsets of younger and older patients when added to chemotherapy
Decitabine	Hypomethylating agent	In elderly patients, approved in Europe based on improved survival compared with standard treatment; extending the regimen to 10 days is a promising strategy; used prior to standard chemotherapy as epigenetic 'priming' is an innovative approach
Flavopiridol	Cyclin-dependent kinase inhibitor	High response rates noted when sequenced with chemotherapy in high-risk patients
CPX-351	Liposomal formulation of cytarabine and daunorubicin	High response rates noted in Phase II trials, particularly in patients with secondary AML; also being studied in the salvage setting
Omacetaxine	Protein synthesis inhibitor	Improved outcomes in patients with favorable or intermediate cytogenetics compared with 7 + 3
FLT3 Inhibitors	Tyrosine kinase inhibitors	Several promising oral agents being studied alone or in combination with chemotherapy or hypomethylating agents (midostaurin, sorafenib, quizartinib, crenolanib)
Elacytararbine	Fatty acid derivative of cytarabine	Being evaluated as a reinduction strategy for patients with residual leukemia after initial therapy
Vosaroxin	DNA intercalating agent, topoisomerase II inhibitor	Large, ongoing Phase III study comparing moderate dose cytarabine with or without vosaroxin for relapsed AML

AML: Acute myeloid leukemia.

Therefore, the Polish Adult Leukemia Group conducted a multicenter, Phase III, randomized trial evaluating three induction chemotherapy regimens in younger patients (up to 60 years of age) with AML: DA, DA + fludarabine and DA + cladribine (DAC) [24]. Cladribine was given at a dose of 5 mg/m^2 iv. for 5 days with DA. The daunorubicin dose in all groups was 60 mg/m^2. Patients could receive one or two courses of the induction regimen, then proceeded to standardized consolidation and maintenance therapies per protocol (consolidation and maintenance regimens did not include cladribine or fludarabine). There was a significantly higher CR rate in patients treated with DAC compared with DA (68 vs 56%; p = 0.01). With 2.8 years of follow up, the 3-year probability of survival was significantly higher for the DAC group compared with the DA group (45 vs 33%; p = 0.02). Subgroups that benefited in particular included patients older than 50 years and those with unfavorable cytogenetics. Toxicity and induction-related mortality were similar in all groups. However, an induction related mortality of 10% is quite high for a group of younger patients only receiving daunorubicin 60 mg/m^2 combined with cytarabine, considering a previous study using daunorubicin 90 mg/m^2 reported a rate of 5.5% [12].

The results of the above study are highly encouraging, and while cladribine is not a new drug, this novel combination warrants attention when considering frontline AML therapy, particularly in patients with high-risk disease. It is interesting that one nucleoside analog improved the outcome and another did not in this study. The authors hypothesize that although cladribine and fludarabine are structurally similar, they have distinct mechanistic properties that may explain the results.

Clofarabine is the newest purine nucleoside analog, and is currently approved for third-line treatment of pediatric ALL. It has also recently been incorporated into the frontline management of AML in both younger and older adults. Nazha and colleagues presented a single arm, open label study evaluating clofarabine combined with idarubicin and cytarabine (CIA) [25]. Patients could receive up to two induction courses and six consolidation courses. In total, 59 patients were enrolled and 74% of patients achieved CR, while an additional 5% achieved CR with incomplete recovery of the platelet count. With short follow up, the median

OS had not been reached and the predicted median EFS was 13.5 months. Building on these results, collaborators from the same institution initiated a randomized trial comparing CIA versus fludarabine combined with idarubicin and cytarabine (FIA) [26]. These data were recently updated, and included 28 relatively young patients with newly diagnosed AML (n = 17 on CIA; n = 11 on FIA). Rates of CR were high in both groups (76% for CIA vs 82% for FIA). Patients continue to be enrolled on this study, and longer follow up will be required to determine if one regimen is superior in terms of long-term outcome.

Clofarabine has also been explored as an option for elderly patients with AML. One innovative approach recently published attempted to provide an extended period of consolidation and maintenance, as well as alternate clofarabine with the non-cross resistant agent decitabine [27]. Patients received an induction course of clofarabine 20 mg/m^2 iv. daily for 5 days combined with cytarabine 20 mg subcutaneously (sc.) twice daily for 10 days. Once remission was achieved, patients began consolidation with an abbreviated cycle of the same regimen as above alternating every three cycles with decitabine 20 mg/m^2 iv. daily for 5 days. The response rate was high, with 66% of patients achieving CR or CR with incomplete recovery of the platelets. There was very little induction-related mortality and the median OS was 12.7 months.

■ Gemtuzumab ozogamicin

GO is an antibody–drug conjugate that was previously approved for salvage therapy in elderly patients with AML. The monoclonal antibody portion is directed against CD33, a cell surface marker that is almost exclusively expressed on myeloid cells. Once GO binds to CD33, it is internalized, where it releases a potent cytotoxin, calicheamicin, which subsequently leads to cell death. This offered one of the first targeted approaches commercially available for AML. However, it was withdrawn from the market after preliminary results of a randomized trial evaluating the drug as a component of frontline therapy were presented [28]. In this study, GO did not appear to improve the outcome, and there were also some concerns regarding toxicity, including early death. Nevertheless, several large international studies were already underway, and their results have reopened the debate about the efficacy and toxicity of GO [29].

The Acute Leukemia French Association conducted a randomized trial evaluating the addition of GO to standard chemotherapy in newly diagnosed AML patients aged 50–70 [30]. All patients received the 7 + 3 regimen (daunorubicin 60 mg/m^2) with or without fractionated doses of GO (3 mg/m^2 [capped at 5 mg] iv. on days 1, 4 and 7 with induction). For patients not achieving CR after one course, a second cycle of daunorubicin 60 mg/m^2 combined with moderate doses of cytarabine was given (1000 mg/m^2 over 2 h iv. q12 h for six doses) was given. The second induction course did not contain GO. While the CR rate between the two groups was similar (72% for the control arm vs 73% for the GO arm), patients in the GO group had superior estimated 2-year EFS (41 vs 17%; p = 0.0003) and OS (53 vs 41%; p = 0.0368). Induction-related mortality was similar between the two groups. Grades 3–4 thrombocytopenia was the most pronounced adverse event occurring more frequently in the patients receiving GO. Hepatic veno-occlusive disease has been associated with the use of GO. In this study, there were two fatal cases in the GO group (none reported in the control arm), and this issue will need to be carefully monitored going forward.

The results of the French study are supported by two reports from the British Medical Research Council [31,32]. First, subgroup analysis of a large, randomized trial in younger adults with AML identified patients who significantly benefited from the addition of GO to induction chemotherapy [31]. In the study, patients were randomized to receive one dose of GO (3 mg/m^2) added to one of three chemotherapy regimens. They could also be randomized to receive one additional dose of GO during consolidation. There was a survival benefit detected for patients with favorable-risk cytogenetics and a trend for a benefit in patients with intermediate-risk cytogenetics. There was no benefit for patients in the high-risk group. The same group also studied whether the addition of GO to induction chemotherapy benefited elderly AML patients (the majority of the patients were older than 60 years of age) [32]. Patients received one of two chemotherapy regimens, and were subsequently randomized to one dose of GO (3 mg/m^2) or chemotherapy alone. With nearly 3 years of follow up, there was a clear advantage in terms of relapse and OS for the group who received GO. Unlike the results of the trial in younger adults, patients in all age and cytogenetic categories appeared to benefit in this study.

Taken together, there is currently a call for reappraisal of the role of GO in AML [33]. It may not be appropriate to derive conclusions about a drug's utility based on the results of large groups of AML patients, who are without doubt comprised of many distinct disease subsets biologically. Preplanned subgroup analysis may be a valuable tool for identifying the ideal population of AML patients for a given therapy, though this will always be hindered by a smaller number of patients. Optimization of the dose and schedule of a drug is vital also.

Hypomethylating agents

DNA methyltransferase inhibitors (or hypomethylating drugs), such as azacitidine and decitabine, have been shown to be highly effective in the management of myelodysplastic syndromes [34,35]. Given the hypothesis that aberrant methylation is present in most hematologic malignancies, these drugs have been investigated both as monotherapy and in combination approaches for AML [36]. Novel strategies for the use of azacitidine and decitabine have been reported recently. These include both extending the duration of each cycle and sequencing/alternating azacitidine or decitabine with conventional chemotherapy [27,37–39].

Elderly patients with AML have particularly poor outcomes. For patients who cannot tolerate intensive chemotherapy, the current standard of care is low-dose cytarabine sc. or supportive care alone with or without hydroxyurea [17]. These strategies, however, do not lead to long-term survival or cure. In a large, Phase III, randomized trial, Kantarjian and colleagues tested whether decitabine could improve the outcome for this patient group [36]. Patients were randomized to receive decitabine at 20 mg/m^2 iv. daily for 5 days or one of the standard strategies mentioned above (cytarabine sc. or supportive care). Cytarabine sc. was administered at a dose of 20 mg/m^2 once daily for 10 days each month. The median age of all patients enrolled (n = 485) was 73 years and 36% had poor risk AML. At the time of analysis prespecified by the protocol, median OS for the decitabine group was 7.7 months versus 5.5 months in the cytarabine or supportive care group, which was not statistically significant. However, when more mature data were analyzed at an unplanned time point, the median OS for each group remained the same, but the result was now statistically significant indicating a survival benefit for decitabine. The results of this trial subsequently led to approval of decitabine by the European Medicines Agency [40].

Blum and colleagues used a 10-day administration schedule of decitabine as opposed to the 5-day schedule normally used in MDS [37]. This study focused on newly diagnosed, older AML patients; the youngest patient enrolled to the study was 60, and median age overall was 74 years. Patients received decitabine at 20 mg/m^2 for 10 days until morphologic remission and then the number of days was adjusted down according to tolerability. Of the 53 patients enrolled, 47% achieved CR, and an additional 17% achieved an 'incomplete CR', for an overall response rate of 64%. The median OS for the entire cohort was 55 weeks, which is similar to the survival reported for older AML patients who received standard chemotherapy [13].

Hypomethylating drugs have been shown to sensitize cancer cells to chemotherapy, as well as induce re-expression of tumor suppressor genes [41]. Based on this, a group from Cornell University designed a Phase I study to test the concept of 'epigenetic priming', or administering a DNA methyltransferase inhibitor followed by cytotoxic chemotherapy [38]. Patients who were less than 60 years old with newly diagnosed AML were eligible for this trial. Multiple dosing cohorts were explored, but all involved decitabine at 20 mg/m^2 given for 3, 5 or 7 days. They also evaluated whether bolus dosing or a 24-h continuous infusion altered the response. Following decitabine, all patients received 7 + 3 chemotherapy with daunorubicin dosed at 60 mg/m^2. In total, 30 patients were enrolled and 83% of the patients achieved CR within two cycles of chemotherapy. The toxicity appeared similar to what would be expected from chemotherapy alone, and a maximum-tolerated dose of decitabine was not reached. The authors concluded that the strategy was safe and could be studied further to determine if it might improve outcomes.

A group from Germany evaluated the addition of azacitidine to standard chemotherapy, with a particular focus on determining the optimal timing of administration [39]. The study was focused on AML patients lacking the well-known genetic aberrations that are targetable or associated with better outcome. Patients were randomized to one of four arms: idarubicin 12 mg/m^2 iv. on days 1, 3 and 5, cytarabine 100 mg/m^2 iv. continuous infusion on days 1–7, etoposide 100 mg/m^2 iv. on days 1–3 (ICE, control arm) and azacitidine 100 mg/m^2 sc. before, during, or after idarubicin and etoposide (at similar doses in ICE). It is interesting that cytarabine was omitted from the experimental arms. The primary objective of this study was to determine the CR rate produced in each arm, allowing the investigators to eliminate the regimens performing sub-optimally. Eventually, the arms with azacitidine given before and during chemotherapy were closed. Patients continued to be enrolled on the ICE arm and the arm calling for azacitidine after chemotherapy, and the CR rates after treating 100 patients in each group were similar (59 and 52%, respectively). This study also plans to evaluate azacitidine maintenance for 2 years after chemotherapy, though longer follow up is necessary. A large study using decitabine as maintenance after chemotherapy found no benefit [42].

Other strategies

New strategies are necessary for patients with high risk AML, as conventional chemotherapy regimens only rarely cure this group. CDKs are important proteins that serve as regulators of the normal cell cycle [43]. Inhibition of CDKs leads to cell cycle arrest, which is advantageous in a disease such as AML. Flavopiridol is a potent CDK inhibitor that has previously been

studied in patients with CLL [44]. Recently, it was added to chemotherapy as a strategy for treating newly diagnosed patients with high-risk AML [45]. Patients were considered high risk if they were older than 50 years, had secondary disease or had poor-risk cytogenetics. Patients were randomized to one of two dosing strategies of flavopiridol based on previous studies indicating that duration of administration may impact the response. Patients either received 50 mg/m^2 iv. over 60 min (bolus regimen) daily for 3 days, or 30 mg/m^2 iv. over 30 min followed by 40 mg/m^2 given as an iv. infusion over 4 h (hybrid regimen) daily for 3 days. On day 6, all patients received cytarabine 667 mg/m^2 iv. continuous infusion over 24 h daily for 3 days, followed by mitoxantrone 40 mg/m^2 iv. for one dose on day 9. There were 39 patients in each arm, and remission was achieved in 62 and 74% of the bolus and hybrid arms, respectively (including patients that did not have complete hematologic recovery). Despite a high rate of response, median OS was only between 11 and 13 months, though patients were older at baseline (median age was 60 years). There was a clear association between OS and age, with younger patients doing better. The toxicity profile was manageable, though there was one death due to tumor lysis syndrome, which is concerning given the supportive care available. The authors concluded that the administration strategies for flavopiridol were equivalent and planned to move forward with the bolus dosing due to convenience. A randomized trial of this regimen compared with 7 + 3 is underway.

The combination of anthracycline and cytarabine has been the accepted standard in AML therapy for decades. The drugs may be synergistic at the optimal molar ratio and research has been conducted in an effort to exploit this relationship [46]. CPX-351 is a liposomal formulation containing a 5:1 molar ratio of cytarabine and daunorubicin. A randomized study was conducted in elderly patients with newly diagnosed AML evaluating CPX-351 compared with standard 7 + 3 [47]. CPX-351 was given at a dose of 100 units/m^2 iv. on days 1, 3 and 5, while patients in the control arm received 7 + 3 with daunorubicin 60 mg/m^2. Patients receiving CPX-351 had a higher response rate, mostly driven by patients achieving CR with incomplete platelet recovery. Notably, there appeared to be less mortality at 60 days in the CPX-351 group (4.7 vs 14.6%) despite a significant increase in infections. The investigators also observed a larger difference in responses favoring CPX-351 in patients with secondary AML.

Homoharringtonine (HHT) is a naturally occurring compound that has been used to treat AML for nearly 40 years [48]. HHT and its derivatives exert their antileukemic effects via protein synthesis inhibition and induction of apoptosis. Over the decades, semi-synthetic compounds have been formulated and HHT has continued to be actively investigated as a treatment for multiple types of leukemia. One compound in particular, omacetaxine, has been approved for use in the USA. Recently, results from a multicenter, Phase III study in China were presented [49]. Patients with newly diagnosed AML were randomized to one of three induction chemotherapy regimens:

- Omacetaxine at 2 mg/m^2 on days 1–7, cytarabine 100 mg/m^2/day iv. continuous infusion on days 1–7, aclarubicin 20 mg/day iv. on days 1–7 (OAA);
- Omacetaxine and cytarabine as in regimen 1 with daunorubicin 40 mg/m^2 iv. on days 1–3;
- 7 + 3 with daunorubicin dosed at 40–45 mg/m^2 iv. on days 1–3. There were slightly more than 200 patients enrolled on each arm of the study.

Both omacetaxine-containing arms led to a significantly higher CR rate compared with the 7 + 3 group. EFS at 3 years was improved for the patients receiving OAA compared with 7 + 3. Furthermore, patients with favorable or intermediate risk cytogenetics had improved OS compared with the group treated with 7 + 3. These results are intriguing, but some will argue that the 7 + 3 arm was destined to be inferior due to a suboptimal dose of anthracycline. The OAA regimen included aclarubicin, an anthracycline not available in the USA and it was given for 7 days. The authors did not provide the relative potency of this anthracycline to daunorubicin. It is also interesting that one omacetaxine-containing arm improved EFS and OS, but not the other. The authors await the publication of this study in a peer-reviewed journal where the authors may elaborate on some of these issues.

The Cancer and Leukemia Group B recently tested the efficacy and safety of the addition of a proteasome inhibitor, bortezomib, to standard chemotherapy in patients aged 60–75 [50]. Standard induction chemotherapy included daunorubicin dosed at 60 mg/m^2 iv. on days 1–3 and cytarabine given via continuous infusion at 100 mg/m^2/day on days 1–7. During induction, bortezomib was administered iv. on days 1, 4, 8 and 11 at a dose of 1.3 mg/m^2. Responding patients were eligible to receive consolidation chemotherapy with cytarabine 2000 mg/m^2 iv. over 3 h on days 1–5 combined with bortezomib. A separate goal in this trial was to establish the maximum-tolerated dose of bortezomib in the consolidation portion. Overall, 95 patients were treated, and the CR rate was 65%, establishing the regimen as active. The investigators also found that tolerability was reasonable, and concluded that the regimen may warrant further study.

Management of adult AML: salvage approach

Designing a treatment plan for a patient with relapsed AML generally depends on the prior therapy received and the duration of the previous remission. It is also important to revisit the cytogenetic and molecular studies in the event the patient harbors a mutation that might be targetable. All patients should be considered for available clinical trials, as most salvage chemotherapy regimens are inadequate. If the patient did not undergo alloHSCT previously, an attempt should be made to find an appropriate stem cell donor. For those unable to participate in clinical trials, several chemotherapy regimens have been tested and endorsed by expert panels [16,17]. Regimens are selected based on what the patient has received previously and according the patient's current organ function and performance status. Below some of the more significant progress that is being made in the management of relapsed or refractory AML, particularly in patients with FLT3 mutated disease, will be discussed. It is important to note, that using these agents as a salvage therapy is only a start, and the goal is to take the most promising agents into the frontline setting.

FLT3 inhibitors

FLT3 is a receptor tyrosine kinase that normally plays a role in hematopoiesis. Nevertheless, the receptor is expressed on the surface of AML cells, and is thought to be involved in cell signaling and proliferation [51]. The discovery that the *FLT3* gene is often mutated in AML blasts led investigators to explore FLT3's influence on the natural history and prognosis of the disease. Two distinct types of activating mutations have been found, and include internal tandem duplication (ITD) of the intracellular juxtamembrane region, and point mutations in the tyrosine kinase domain (TKD). FLT3 ITDs have clearly been associated with a dismal prognosis, while TKDs do not appear to significantly impact prognosis [52]. Evidence suggests a possible benefit from early alloHSCT for FLT3 ITD patients in first CR [53]. Furthermore, recent basic and clinical research has established that TKDs may confer resistance to small-molecule FLT3 inhibitors [54]. This area is rapidly evolving, and this article will review what the authors feel are the most significant findings to date.

Lestaurtinib (formerly CEP-701), one of the first FLT3 inhibitors under development, was evaluated in a randomized, multicenter study comparing the drug combined with chemotherapy versus chemotherapy alone [55]. Patients were enrolled if they had FLT3-mutated (ITD or point mutation) AML in first relapse. Chemotherapy was assigned according to the duration of first CR. Patients randomized to lestaurtinib received the drug starting 2 days after the completion of chemotherapy (day 7) at a dose of 80 mg orally every 12 h. In total, 224 patients were randomized, and the groups were well balanced for baseline characteristics. The majority of patients in each group had a FLT3 ITD as opposed to a TKD. Unfortunately, lestaurtinib failed to improve either the CR rate or OS, and was more toxic when compared with the control group. The 30-day mortality rate was twice as high in the lestaurtinib group (12 vs 6%), though this was not statistically significant.

The investigators performed several correlative studies that offered possible explanations for the negative results. First, there was substantial variability in the steady state plasma levels of the drug. Furthermore, remission rate was correlated with *in vivo* FLT3 inhibition, which was only achieved in approximately 50% of patients. The authors also uncovered a mechanism of resistance to multiple FLT3 inhibitors. Plasma levels of FLT3 ligand (FL) were noted to be drastically increased in response to chemotherapy, and such high concentrations of FL were shown to impair FLT3 inhibition [56]. It was concluded that future studies may have to consider FL levels when deciding what to combine FLT3 inhibitors with and the optimal way to sequence these combinations.

Another nonselective FLT3 inhibitor, midostaurin, has also been evaluated relapsed in AML. The largest study published to date was a randomized trial looking at two doses of midostaurin administered as a single agent [57]. Importantly, this trial also enrolled patients with wild-type FLT3. Overall, 95 patients were treated with either 50 or 100 mg orally twice daily on a continuous basis. Most patients had relapsed or refractory disease, though some untreated patients deemed unable to tolerate standard chemotherapy were included. There were no CRs documented on this trial, though a substantial reduction in blast percentage was noted for mutated and wild-type patients at both doses that were explored. While a reduction in blast percentage and hematologic improvement are encouraging and establish the drug as being active, the median OS for the entire cohort was only slightly longer than 2 months. Therefore, combination therapy, or adding the drug to a frontline regimen may improve on these results.

Sorafenib is a multikinase inhibitor that is FDA approved for renal cell and hepatocellular carcinomas [58]. While known to be a potent FLT3 inhibitor, other important kinase targets of sorafenib include NRAS and c-KIT, which may also be important in AML. The drug has been shown to be active in refractory AML in small studies [59,60]. In view of the data indicating that chemotherapy can induce a rise in FL and that FL confers resistance to FLT3 inhibitors, Ravandi and colleagues designed a trial evaluating sorafenib in combination with azacitidine [61].

Azacitidine has single agent activity in AML, and is far less intense than traditional AML chemotherapy, potentially avoiding a substantial increase in FL. Patients received azacitidine 75 mg/m^2 daily for 7 days per 28-day cycle combined with concurrent sorafenib 400 mg orally twice daily given continuously. While the study permitted the enrollment of patients with wild-type FLT3, 40 out of 43 were positive for a FLT3 ITD. The majority of patients had relapsed or refractory disease and had received a median of two previous therapies. Several patients had failed previous FLT3 inhibitors, including sorafenib. At the time the data were presented, 34 patients were evaluable and the overall-response rate was 44% (29% CR with incomplete hematologic recovery, 12% CR and 3% with partial response). The toxicity profile was manageable, with rash and fatigue occurring most frequently. The regimen was able to bridge several patients to alloHSCT. FLT3 inhibition was measured, and the target was attained in 64% among patients who had samples available. Notably, FL levels were also monitored, and there did not appear to be as drastic a rise as noted in the previous trial with chemotherapy.

More selective inhibitors of FLT3 may be able to optimize responses in AML patients with FLT3 ITD. Quizartinib (formerly AC220) has been shown to be more potent and selective for FLT3 than most other kinase inhibitors under development [62]. Results from a Phase I, open-label, multicenter study evaluating quizartinib as a single agent were recently presented [63,64]. The study enrolled two distinct groups of patients and data were presented separately. The first cohort included elderly patients with primary refractory AML or a short duration of first CR [63]. There were 134 patients in this portion of the study and they could have had either a FLT3 ITD (69%) or a point mutation (31%). Quizartinib was given continuously at a dose of 135 mg/day to male patients and 90 mg/day to female patients. The investigators used an end point known as composite remission (CRc), which was defined as CR, CR without hematologic recovery or CR without platelet recovery. Patients with FLT3 ITD achieved a CRc of 54%, which was almost exclusively due to CR without hematologic recovery (51%). No patient achieved actual CR and the median OS in this group was 25.3 weeks. The most concerning adverse effect was grades 3/4 QT prolongation, which occurred in 13% of the patients. There was one episode of torsade de pointes, which was not fatal.

The second component of the above study evaluated quizartinib in patients with relapsed AML beyond the first salvage chemotherapy regimen or those who were status post alloHSCT [64]. There were 137 patients enrolled and they were given the same doses of quizartinib as described above. When looking only at the FLT ITD patients, the CRc was 44%, with nine patients meeting the definition of CR. Of note, patients with wild-type FLT3 also responded to quizartinib (CRc rate of 34%). Once again, prolongation of the QT interval was a frequently reported adverse event. However, in this group of patients, there were no grade 4 episodes and there were also no occurrences of torsade de pointes. The data thus far for quizartinib are encouraging, though responses do not appear to be durable. That said, approximately a third of the patients were able to be bridged to an alloSCT, which is the goal of therapy in relapsed patients with AML.

As the use of FLT3 inhibitors expands, identifying mechanisms and patterns of resistance will be crucial. One observation that has been made involves the emergence of point mutations in the kinase domain at the time of relapse or progression on FLT3 directed therapy [65]. This phenomenon was predictable, as point mutations play a major role in resistance to tyrosine-kinase inhibitors in chronic myeloid leukemia [66]. Crenolanib is a potent FLT3 inhibitor that was molecularly designed to retain activity in the presence of most known mutations. Investigators from the University of California San Francisco (CA, USA) and the University of Pennsylvania (PA, USA) have presented data indicating that crenolanib maintains potency in cases of quizartinib resistance [65].

Strategies to optimize the use of FLT3 inhibitors will continue to be an active area of research. The most attractive strategy is to use these agents as a component of the frontline treatment approach. This may lessen the impact had on the response to treatment by FL. Trials are underway evaluating quizartinib in this regard. In addition, since the goal is to take all patients with the FLT3 ITD to alloHSCT, post-transplant maintenance therapy with FLT3 inhibitors may be a rational approach.

Other strategies

Since cytarabine remains one of the cornerstone drugs in AML treatment, alternative formulations and drug delivery systems have been explored. Elacytarabine is a fatty-acid derivative of cytarabine, which is able to enter cells in a manner independent of nucleoside transporters [67]. A Phase II study evaluated elacytarabine as a single agent in relapsed or refractory patients [68]. Patients received 2000 mg/m^2 iv. continuous infusion for 5 days every 3 weeks and were compared with matched historical controls to measure efficacy. The CR rate in the elacytarabine group was low at 18%; however, it was significantly higher than what was achieved in the control group (4%). This study essentially established that elacytarabine has activity in a group of patients that likely exhibited cytarabine-resistant disease. Another Phase II, multinational study evaluated elacytarabine combined with idarubicin in patients refractory to

standard induction [69]. Patients with persistent disease on day 12 of therapy received idarubicin 12 mg/m^2 iv. on days 1–3 with elacytarabine 1000 mg/m^2 iv. continuous infusion on days 1–5. A total of 40 patients were evaluable, and the CR/CR with incomplete recovery of blood counts rate was approximately 45%. While this is encouraging, there was no comparator group, so we do not know what percentage of patients may have responded to another cycle of standard induction or high doses of conventional cytarabine. Future studies will likely evaluate elacytarabine in a randomized fashion.

CPX-351 (as discussed in the frontline section) has also been evaluated as salvage therapy for AML [70]. Patients suffering first relapse were randomized to CPX-351 100 units/m^2 iv. on days 1, 3 and 5 or investigator's choice of salvage therapy. More patients in the CPX-351 group had undergone alloHSCT. The CR/CR with incomplete recovery of blood counts rate was slightly higher in the CPX-351 group (51 vs 42%). The improvement in response came at a price of more grades 3–5 sepsis.

Nucleoside analogs (as discussed in the frontline section) can also be used for AML in the salvage setting. They may be particularly useful in relapsed patients who have not been exposed to one of these agents previously. Elderly patients are less likely to be exposed to moderate or high doses of cytarabine during initial induction and consolidation, so this strategy may be a standard option for fit patients who relapse and who are ineligible for clinical trials.

Faderl and colleagues conducted a randomized, Phase III, double-blind, placebo-controlled trial comparing moderate dose cytarabine with moderate dose cytarabine plus clofarabine [71]. Cytarabine was given at a dose of 1 g/m^2 iv. daily in both arms, and clofarabine was given at a dose of 40 mg/m^2 iv. daily in the experimental arm. There were 163 patients in each arm, and baseline characteristics were well balanced except for the fact that a higher proportion of patients in the clofarabine group had unfavorable cytogenetics (49 vs 39%). Patients in both groups received a median of one cycle of therapy. The primary end point, median OS, was not statistically different between the groups, though EFS was improved in the clofarabine group. That said, median OS for both groups was greater than 6 months, which compared favorably to previous studies evaluating similar types of patients.

Other ongoing and completed studies may further clarify the role of nucleoside analogs for the management of relapsed AML. A randomized study comparing clofarabine- with fludarabine-containing chemotherapy (both combined with idarubicin and moderate-dose cytarabine) in younger patients (up to 60 years of age) was recently presented [26]. With limited follow up, response rates were similar between the two arms (44% fludarabine and 38% clofarabine). Importantly, 20–30% in each group were able to move to an alloHSCT after receiving the chemotherapy.

In a single-arm study, Jabbour and colleagues evaluated a regimen containing twice daily dosing of fludarabine and cytarabine (BIDFA) for patients with relapsed or refractory disease [72]. Initially this regimen also contained GO, though it was withdrawn from the market during the study. This study was designed to be able to enroll patients of all ages, so inherent dose modifications were included for patients older than 65 years of age to minimize toxicity. Fludarabine was given at 15 mg/m^2 iv. every 12 h for ten doses during induction, and cytarabine 0.5 g/m^2 followed 4 h after each fludarabine dose (total of ten doses). Doses were also adjusted for renal function. Patients could receive up to two induction courses and responding patients could go on to receive six consolidation courses given according to an attenuated dosing schedule. There were 107 patients enrolled and the overall response rate was 26%. The regimen was very well tolerated with a 4-week mortality of 9%. BIDFA could be considered for patients not eligible for clinical trials and for those patients in relapse who may benefit from a moderate dose cytarabine-based strategy.

Vosaroxin is a novel, fluoroquinolone derivative that has demonstrated cytotoxic activity in leukemia and other malignancies [73]. Its mechanism of action is similar to that of the anthracyclines (DNA intercalation and topoisomerase II inhibition), although it is resistant to p-glycoprotein-mediated drug efflux and may be less likely to contribute to free radical-related cardiotoxicity. A Phase III, placebo controlled, randomized trial (VALOR) is currently underway in patients with relapsed or refractory AML [102]. In this trial, patients receive several days of moderate dose cytarabine combined with either vosaroxin or placebo.

Future perspective

While there have been considerable efforts involved in elucidating the genetic and molecular abnormalities that influence outcomes in AML, there is more work to be done. This is especially true from the therapeutic standpoint. It seems clear that we can do better than 7 + 3 for newly diagnosed patients, as there have been several reports showing improvement in OS and other outcomes. The development of monoclonal antibody therapy for AML seems to be lagging behind other malignancies, mainly ALL and a number of the lymphomas. It seems warranted for regulatory bodies to consider 'reapproving' GO, as mounting evidence suggests many AML patients benefit from this antibody–drug conjugate. Important research is ongoing to clarify

the optimal use of FLT3 inhibitors in terms of both when they should be given and with what combination. A large number of mutations have been identified in AML and it will be important to establish which of these are 'druggable' or amenable to disruption of the pathway they influence. Agents that are most promising in salvage studies should be rapidly moved to the frontline approach, when presumably leukemia cells are most susceptible to therapy.

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Executive summary

- Better treatment options are necessary for adults diagnosed with acute myeloid leukemia (AML).
- Chemotherapy regimens including purine nucleoside analogs (fludarabine, cladribine or clofarabine) may improve outcome when compared with the traditional '7 + 3' regimen.
- The development of monoclonal antibody therapy for AML has been slow and this represents an area where large enhancements to therapy may be realized.
- Gemtuzumab ozogamicin, a monoclonal antibody that was withdrawn from the market, has seen a renewal in interest due to emerging data indicating it improves survival in certain subsets of AML patients.
- Optimizing the incorporation of epigenetic therapies, such as azacitidine and decitabine, into AML regimens is a promising strategy.
- Molecularly targeted approaches to disrupt aberrant signaling pathways continue to be actively investigated.
- The ideal strategy for utilizing small-molecule FLT3 inhibitors continues to be refined.

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