Acute Myeloid Leukemia: Focus on Novel Therapeutic Strategies

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Abstract: Acute myeloid leukemia (AML) is a heterogeneous disease with variable clinical outcomes. Cytogenetic analysis reveals which patients may have favorable risk disease, but 5-year survival in this category is only approximately 60%, with intermediate and poor risk groups faring far worse. Advances in our understanding of the biology of leukemia pathogenesis and prognosis have not been matched with clinical improvements. Unsatisfactory outcomes persist for the majority of patients with AML, particularly the elderly. Novel agents and treatment approaches are needed in the induction, post-remission and relapsed settings. The additions of clofarabine for relapsed or refractory disease and the hypomethylating agents represent recent advances. Clinical trials of FLT3 inhibitors have yielded disappointing results to date, with ongoing collaborations attempting to identify the optimal role for these agents. Potential leukemia stem cell targeted therapies and treatments in the setting of minimal residual disease are also under investigation. In this review, we will discuss recent advances in AML treatment and novel therapeutic strategies.

Keywords: acute myeloid leukemia, clofarabine, FLT3, gemtuzumab ozogamicin, cancer stem cells

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Acute Myeloid Leukemia (AML) is a rare malignancy with 13,000 new cases diagnosed in the US each year. The majority patients die from their disease with an estimated 9,000 deaths annually. Despite remarkable progress in therapy for acute promyelocytic leukemia (APL) with long-term cure likely in up to 90% of patients, outcomes for patients with non-APL AML remain unsatisfactory. Induction chemotherapy given at diagnosis for the majority of patients has undergone little change in over 30 years. The most commonly used post-remission therapy, cytarabine, is given in similar fashion as when described in 1994. Elderly AML remains notoriously difficult to manage, with rare cures in patients over age 65 from chemotherapy alone and 5-year survival rates of less than 10%. Novel strategies to maximize remission rates in response to the initial treatment and to prolong remission duration are clearly needed.

Cytogenetics remains the most important prognostic feature of newly diagnosed AML. Three risk categories—favorable, intermediate and poor risk—have been recognized based upon outcomes by chromosomal abnormalities in several large series of patients. The median survivals in each category are as follows: favorable risk, 7.6 years; intermediate risk, 1.3 years; and poor risk, 0.5 years. More recently, emerging data on molecular markers of prognosis within the traditionally defined risk groups had led to additional refinements (see Table 1). Within favorable risk disease, data demonstrate inferior outcomes for patients with an additional c-KIT mutation. Progress in molecular profiling of the intermediate risk cytogenetics normal AML (CN-AML) have led to the identification of mutations conferring improved (mutations of NPM1 or CEBPA) or inferior (FLT3) outcomes. Although these better defined prognostic risk categories suggest which patient will have shorter remission duration, there is no effective therapy specifically targeted to these subtypes, and when more aggressive therapy is indicated for poor prognosis disease, the only curative treatment option remains allogeneic stem cell transplant. In this review, we will discuss recent refinements to the standard induction regimen, new treatment strategies in elderly AML, approved drugs in the setting of relapsed or refractory disease, and novel therapies that are under investigation (Table 2).

### Strategies to Improve Response to Intensive Induction Chemotherapy

**Dose-intensification**

Induction chemotherapy with “7+3” remains the US standard of care for patients less than age 60 with newly diagnosed AML. Cytarabine (Ara-C) is given by continuous infusion for seven days with an anthracycline [DNR (DNR) or idarubicin (IDA)] given daily for 3 days. IDA is given at a dose of 12 mg/m², and DNR was historically given at doses of 45–60 mg/m². A phase III study by the Eastern Cooperative Oncology Group addressed the issue of higher doses of DNR in patients ages 17–60 with newly diagnosed AML. A higher complete remission (CR) rate (71 versus 57%, \( P = 0.003 \)) and longer median survival (24 versus 16 months, \( P = 0.003 \)) was observed in the higher dose DNR patients. The survival advantage was limited to those patients under age 50 and those with favorable or intermediate risk karyotype. Cardiac and hematologic toxicities were similar between the two groups. However, there was concern that the CR rate was lower than previously

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**Table 1. Prognosis and associated chromosomal and molecular abnormalities in AML.**

| Risk status  | Karyotype                        | Molecular abnormalities                          |
|-------------|----------------------------------|-------------------------------------------------|
| Favorable   | Inversion (16) or t(16;16)       | Normal cytogenetics with NPMI mutation          |
|             | t(8;21)                           | or CEBPA mutation                               |
|             | t(15;17)                          | in absence of FLT3-ITD mutation                 |
| Intermediate| Normal cytogenetics              | t(8;21), inv (16), or t(16;16) with c-KIT        |
|             | Trisomy 8                         | mutation                                        |
| Poor risk   | Complex (≥3 abnormal clones)     | Normal cytogenetics with FLT3-ITD mutation      |
|             | -5, -5q, -7, -7q 11q23            |                                                 |
|             | Inversion 3 or t(3;3)            |                                                 |
|             | t(6;9)                            |                                                 |
|             | t(9;22)                           |                                                 |
reported in studies of DNR at 60 mg/m². There are no studies which have directly compared DNR at 60 mg/m² versus 90 mg/m². In the European ALFA-9801 study, patients ages 50–70 were randomized to induction regimens of standard dose Ara-C and varying anthracycline dose—standard dose IDA (12 mg/m² × 3 days), increased IDA (12 mg/m² × 4 days) or higher dose DNR 80 mg/m² for 3 days. Although a significant difference in CR rate was observed (83% in IDA3, 78% in IDA4 and 70% in DNR, P = 0.04), there was no difference in incidence of relapse, event-free survival or overall survival. A similar study in older adults was conducted by the Leukemia Working Group of the Dutch-Belgian Cooperative Trial Group for Hemato-Oncology (HOVON) and the Swiss Group for Clinical Cancer Research (SAKK) Collaborative Group. Patients age 60 or older were randomized to induction therapy with standard dose Ara-C and DNR at either 45 mg/m² or 90 mg/m². Higher CR rates were seen in the higher dose DNR arm (64% vs. 54%, P = 0.002), and this advantage was more pronounced in those aged 60–65 with a trend towards significance (CR 73% vs. 51%, P = 0.07). There were no increased toxicities seen at the higher dose. Event-free and overall survival was similar between the arms. Exploratory post-hoc analysis suggests a survival advantage with higher dose DNR in patients with favorable risk cytogenetics. Based on these large cooperative studies, NCCN Guidelines advocate the use of escalated dose DNR or IDA as a Category 1 recommendation. The survival benefit of higher dose DNR appears greater in patients with favorable or intermediate cytogenetics; however, this information is generally not available at the time of chemotherapy initiation. Currently, many practitioners use higher dose DNR in nearly all fit patients, and this is our clinical practice. A clinical trial is also underway assessing the toxicity and efficacy of increasing doses of IDA. A novel compound, CPX-351 (Celator), is a liposomal formulation combining Ara-C and DNR in a 5:1 molar ratio. Preclinical data demonstrates that this formulation accumulates and persists in the bone marrow with greater efficacy compared to the two drugs given in combination. Clinical trials are ongoing in relapsed AML (see below) and are expected to open shortly in untreated patients.

### Table 2. Agents currently under investigation for induction of untreated AML or re-induction of relapsed/refractory disease.

| Drug class | Drugs in clinical trials |
|------------|--------------------------|
| Re-formulations of AML drugs | Elacatisine, CPX-351 |
| Nucleoside analogue | Clofarabine, sapacitabine, 5-Fluoro-2′-Deoxyuridine |
| Hypomethylating agents | Azacitidine, decitabine |
| Immunomodulator (IMID) | Lenalidomide |
| CXCR4 antagonist | Plerixafor |
| BCR-ABL tyrosine kinase inhibitors | Dasatinib, imatinib, nilotinib |
| Histone deacetylase inhibitors | Entinostat, MS-275, Panobinostat, vorinostat |
| Proteosome inhibitor | Bortezomib, Everolimus, temsirolimus |
| mTOR pathway inhibitors | AC220, PLX3397, sorafenib |
| FLT3 inhibitors | All-trans retinoic acid, bexarotene |
| Retinoids | Gemtuzumab |
| Antibody-drug conjugate | Bendamustine |
| Alkylating agents | Tipifarnib (PF-0449) |
| Farnesyltransferase inhibitor | ON 01910.Na |
| Hedgehog pathway inhibitor | Elesclomol sodium |
| PI3K/AKT pathway inhibitor | BIBF 1120 |
| HSP-90 inhibitor | Pravastatin, lovastatin |
| Angiokine inhibitor | Tigecycline |
| Statins | Tegafur,obufine |
| Mitochondrial translation inhibitor | Erlotinib |
| EGFR inhibitor | CWP232291 |
| WNT pathway inhibitor | Ribavirin |
| Oncogene eIF4E inhibitor | PK2-391 |
| Src kinase inhibitor | Pazopanib |
| VEGFR inhibitor | Vosaroxin |
| Anticancer quinolone derivative | Flavopiperide |
| CDK inhibitor | AZD1208 |

Other chemotherapy or targeted agents have been studied in combination with conventional “7+3” induction. Gemtuzumab ozogamicin (GO) (Mylotarg, Pfizer) is an antibody-drug conjugate linking an anti-CD33 antibody to the DNA-damaging agent calicheamicin. It received accelerated FDA approval in 2000 based on results in elderly patients with relapsed AML. Several trials have examined the benefits and toxicity of adding GO to conventional induction chemotherapy with encouraging results for subgroups of patients; however, increased toxicity in a US confirmatory trial led to its withdrawal from the US market.

### Antibody-drug conjugate
in June 2010. It continues to be used in clinical trials and outside of the US, and here we will review the emerging data for GO in induction therapy.

Two studies from the UK NCRI (AML15 and AML16) addressed the question of adding GO to induction chemotherapy. In AML15, over 1100 patients with newly diagnosed AML were randomized to one of three induction chemotherapy regimens with or without the addition of GO. A second randomization was performed for patients in CR to one of three consolidation regimens with or without GO. There were no differences in CR rate or 30-day all cause mortality between patients receiving and not receiving GO with induction chemotherapy. There were no differences in rates of relapse, relapse-free or overall survival. A pre-specified subset analysis by cytogenetic risk category did show a highly significant benefit of induction GO in patients with favorable risk cytogenetics (79% versus 51% overall survival, P = 0.001). Patients with poor risk cytogenetics appeared to have no benefit, and there was a non-significant trend for benefit in patients with intermediate risk cytogenetics. There were no excess toxicities seen in the GO treated patients. An internally validated prognostic index demonstrated a predicted benefit of 10% in 5 year survival attributable to the addition of GO in approximately 70% of patients.

In AML16, over 1100 older patients (median age 67, range 51–84) were randomized to intensive chemotherapy with either DNR/Ara-C or DNR/Clofarabine with or without a single dose GO on day 1, followed, or not, by a third cycle of therapy (DNR/Ara-C) followed by azacitidine maintenance. Preliminary results presented at the American Society of Hematology (ASH) Annual Meeting in 2011 showed no significant differences in CR rate or toxicities. There was a significant decrease in the rate of relapse (61% in patients receiving GO versus 70% in control arms, P = 0.004) and significant improvement in all patients overall survival at 2 years (35% with GO versus 29% in control, P = 0.04). The benefit was lower in patients with secondary AML or poor risk cytogenetics.

The plenary session at the 2011 ASH Annual Meeting featured preliminary results from the ALFA (Acute Leukemia French Association) 0701 trial. Castaigne, et al. presented data from 271 patients with newly diagnosed AML, aged 50–70. Patients were randomized to induction chemotherapy with 7+3 (with DNR dosed at 60 mg/m²) with or without GO at 3 mg/m² on days 1, 4 and 7. Patients in CR could proceed on to an additional 2 courses of consolidation therapy with or without GO as per initial randomization. There was no significant difference in rates of CR, induction death or primary refractory disease. Significant improvements were seen in the 2 year event-free survival (15.6% versus 41.4%, P < 0.002) and disease-free survival (18.1% versus 48.5%, P < 0.001) between the control group and the group receiving GO. Subgroup analysis showed that the EFS benefit persisted in all age groups (> or <65), but not in those with poor risk cytogenetics. In the entire cohort, overall survival was longer in the GO arm than control (25.4 versus 15.3 months, P = 0.037), although this benefit was non-significant when cytogenetics were considered. Prolonged thrombocytopenia (19 patients) and veno-occlusive disease (3 patients, 2 fatal events) were seen in the GO arm. Also presented at the meeting were preliminary results from the GOELAMS AML 2006 IR study. This Phase III trial randomized 238 patients ages 18 to 60 (median age 50) with intermediate cytogenetics to induction chemotherapy with or without GO, followed by consolidation chemotherapy and/or autologous or allogeneic stem cell transplant. There were no significant differences in CR rate or early death. An increased incidence of veno-occlusive disease (4 cases versus 0) and grade 3/4 hepatic toxicities (23% versus 13%) was seen in those receiving GO. Event-free and overall survival at 3 years were not statistically different between those receiving GO or not. In the subset of patients who did receive an allogeneic transplant, EFS was significantly higher in those patients receiving GO (53.7% versus 27%, P = 0.03), although there was no difference in OS at 3 years.

In the US, SWOG conducted a multicenter, randomized Phase III trial of 7+3 with or without the addition of GO (S0106) in adults ages 18–60 with untreated AML. Preliminary results presented in 2009, after a planned interim analysis, showed no clinical benefit and, in fact, excess deaths in the treatment arm versus standard therapy. There has been concern that the standard treatment patients had clinical results better than expected/historical controls, and that this may have obscured the true clinical benefit of GO. Also, preliminary results from the European studies suggest that the clinical benefit to GO in induction...
therapy seems restricted to subsets of AML patients (favorable or intermediate risk cytogenetics), which may also, in part, explain the negative preliminary results of the SWOG trial. However, since S0106 was designed as the confirmatory trial for FDA approval of the medication, it was withdrawn from the US market in 2010 in light of these results. Clinical trials of GO are ongoing, and the drug’s ultimate future in the US is unknown.

Novel induction regimens
Clinical trials are ongoing with novel agents added to induction regimens in AML. The hypomethylating agent decitabine, commonly used in myelodysplastic syndrome (MDS), is also under investigation in combination with intensive chemotherapy in fit patients. This concept is termed “epigenetic priming,” using decitabine prior to initiation of chemotherapy. Another strategy involves intensive chemotherapy with flavopiridol, Ara-C and mitoxantrone (FLAM). This regimen has been studied in elderly and relapsed patients or younger patients with poor risk features with encouraging results. The regimen is now in a multicenter randomized trial evaluating the efficacy of FLAM versus 7+3 in patients aged 18–70 with non-core binding factor AML. An induction regimen consisting of the histone deacetylase inhibitor vorinostat in combination with IDA and Ara-C were presented at the 2011 ASH Annual Meeting. Untreated adults received 3 days of vorinostat with IDA/Ara-C induction, along with consolidation cycles of vorinostat, IDA and Ara-C (5 cycles) followed by vorinostat maintenance. CR rates were higher than historical controls across the entire cohort (85% versus 72%, P = 0.01), and subset analyses showed a trend toward improvements in CR rate for patients with abnormalities of chromosomes 5 or 7 or FLT3 mutations. Efforts to capitalize on known molecular aberrations in specific subtypes of AML include trials of imatinib in c-KIT mutated AML and FLT3 inhibitors in FLT3-mutant AML.

Strategies to Develop Less Toxic Induction Regimens
Intensive induction chemotherapy is recommended for all patients who are fit to tolerate it. However, for many elderly patients with AML, physicians are reluctant to prescribe intensive chemotherapy due to comorbidities and poor performance status. Rates of complete remission and overall survival decline with advancing age, due in part to more aggressive disease biology, preponderance of poor risk cytogenetics as well as limited tolerance to therapy. Recent studies, though, demonstrate that older patients with AML may tolerate intensive chemotherapy with increasing doses of DNR, suggesting that comorbidities and performance status, rather than age per se, determine fitness for therapy. Authors argue that each patient should be considered individually, particularly given that no less intensive induction regimen has proven superior to 7+3. Alternate induction strategies of less toxic and/or more effective agents are under investigation for older or unfit patients with AML. These include the hypomethylating agents, azacitidine and decitabine, and the immunomodulatory derivative (IMiD) lenalidomide which are already approved and in use for myelodysplastic syndromes, as well as novel therapies.

Hypomethylating agents
Azacitidine was studied in a Phase III international trial comparing azacitidine (75 mg/m² subcutaneously for 7 days of each 28 day cycle) to “conventional care regimens” (CCR) including best supportive care, low-dose chemotherapy and intensive chemotherapy in patients with high-risk MDS or AML (30% with AML). The majority of patients were considered unfit for intensive chemotherapy. At a median follow-up of 20 months, patients receiving azacitidine had significantly prolonged overall survival (24.5 months versus 16 months for CCR patients, P = 0.005) with OS rates of 50% versus 16%, favoring azacitidine (P = 0.001). This randomized trial showed a benefit for azacitidine and suggests that hypomethylating agents are an effective strategy in patients unfit for intensive chemotherapy. In a non-randomized Phase II trial of untreated elderly patients with AML, decitabine monotherapy (20 mg/m² intravenously for 5 consecutive days of each 28 day cycle) resulted in a CR rate of 25% consistently across all cytogenetic subgroups. The median OS was 7.7 months with the majority of toxicities related to bone marrow suppression.

Researchers at M.D. Anderson conducted a study of 81 patients with high risk MDS or AML (46% with AML) with abnormalities of chromosomes 5 or 7, with or without additional cytogenetic
abnormalities. These patients were treated with one of the hypomethylating agents, either decitabine or azacitidine, as initial therapy. An additional 151 patients (83% with AML) were treated with intensive induction chemotherapy. Retrospective analysis compared the outcomes of these two groups (median ages 66 and 61 years, respectively) and found no significant difference in CR rate or median duration of CR. However, overall survival favored the hypomethylating agents (median OS of 9 months versus 5 months, \( P = 0.019 \)) demonstrating a benefit to the use of these agents particularly in patients with chromosome 5 or 7 abnormalities. Studies examining the efficacy of sequential azacitidine plus lenalidomide as well as decitabine in combination with other agents are currently ongoing.

### Lenalidomide

The immunomodulatory agent, lenalidomide, appears to influence the bone marrow microenvironment through mechanisms which are not well-described. It is approved and effective for MDS with 5q deletion as well as multiple myeloma, and emerging data suggests a potential role in AML regardless of 5q deletion status. In a phase I study in relapsed and refractory leukemia (31 patients with AML, 4 with acute lymphocytic leukemia), patients were given escalating doses of lenalidomide. The maximum tolerated dose was 50 mg daily. Sixteen percent of AML patients achieved CR with response duration from 5 to 14 months. No patients with 5q deletion were among the responders, but all responders had low blast counts at diagnosis. Interestingly, 2 of 4 patients who had relapsed after an allogeneic stem cell transplant developed acute graft versus host disease of the skin and durable CR. Toxicities included fatigue and infection, but high dose lenalidomide was relatively well-tolerated. SWOG conducted a phase II clinical trial for untreated elderly patients with 5q deletion or without additional cytogenetic abnormalities. Thirty-seven patients were enrolled. Treatment consisted of one cycle of lenalidomide induction at 50 mg daily for 28 days, followed by maintenance lenalidomide at 10 mg daily for 21 days of a 28 day cycle. Only 14 patients completed induction and 8 proceeded to maintenance therapy. Results were disappointing with progression on treatment, deaths during induction and other adverse events precluding completion of planned therapy. Fourteen percent of patients achieved PR or CR and overall survival was 2 months for all patients. A second phase II trial in 33 untreated patients with AML by Fehniger, et al enrolled patients over age 60 and similarly used lenalidomide at 50 mg daily for 28 days as induction therapy. In this trial, patients were able to receive a second 28-day induction cycle at 50 mg. Those with CR or CRi (CR with incomplete blood count recovery) or those not progressing after 2 cycles of induction could proceed on to low-dose lenalidomide at 10 mg daily for a maximum of 12 cycles. In this study, the CR/CRi rate was 53% for patients completing induction therapy, with higher rates of CR seen in patients with lower blast counts at presentation (\( P = 0.01 \)). Median duration of CR was 10 months (range 1–17+ months). These disparate clinical outcomes from two very small phase II studies suggest the need for larger trials to determine the efficacy of high dose lenalidomide in patients with AML. Ongoing trials include lenalidomide in combination with hypomethylating agents and other chemotherapy drugs at varying doses.

### Clofarabine

Clofarabine is a novel nucleoside analogue first studied in relapsed and refractory leukemia (see below). Recent studies have showed responses to single agent clofarabine, as well as in combination with chemotherapy, in untreated elderly patients or those unfit for conventional induction. In the CLASSIC II study, adults \( \geq \)age 60 with untreated AML and at least one additional unfavorable prognostic feature were enrolled. Clofarabine was given as a single agent at 30 mg/m\(^2\)/day \( \times \) 5 days as induction followed by consolidation cycles at 20 mg/m\(^2\)/day \( \times \) 5 days for a maximum of 6 cycles. The CR/CRi rate was 46% and those with best responses had the longest survival with median OS for the entire cohort of 41 weeks, 59 weeks for those with CR/CRi and 72 weeks for those achieving CR. Responses were seen in all cytogenetic risk groups. The toxicity profile was acceptable with the most common non-laboratory side effects being nausea, vomiting, febrile neutropenia, diarrhea, rash and fatigue. Two consecutive European studies of 106 patients similarly examined clofarabine as single agent induction therapy for patients over age 70 or ages 60–69 with ECOG Performance Status >2 (UWCM-001 trial) or patients \( \geq \) 65 years unfit for
intensive chemotherapy (BIOV-121 trial). The rate of CR/CRi was 48% and, similar to CLASSIC II results, responses rates did not differ by cytogenetic risk group. However, survival in these two trials was shorter, with median OS for the entire cohort of 19 weeks. Those in CRi and CR had longer survival, 30 weeks and 47 weeks respectively.44

Clofarabine has also been studied in combination with Ara-C in untreated older patients. A phase II study in untreated AML patients aged 50 and older used a regimen of clofarabine given at 40 mg/m²/day × 5 days and Ara-C at 1 g/m²/day × 5 days followed by additional cycles depending on response. Rate of CR/CRi was 60% with rare grade 3/4 toxicities. Comparison to historical controls, however, showed no survival advantage despite the higher CR rate. Median survival for all patients was 10.3 months, and for those achieving CR was 23.5 months.45

A study of lower-dose therapy compared treatment with clofarabine (30 mg/m²/day × 5 days) with or without low-dose Ara-C (20 mg/m²/day subcutaneously × 14 days) using an adaptive randomization strategy. Most patients (54/70) received the combination regimen. Significantly higher CR rates were seen with the combination (63% versus 31%, \( P = 0.025 \)). There was no difference in overall survival.46

The results of the above studies suggest a role for clofarabine in AML induction and ongoing studies will examine the efficacy of clofarabine in combination with various chemotherapy and novel agents.23 However, to date there are no published results showing a survival advantage for clofarabine induction (either single agent or in combination) versus 7+3. Clofarabine is also being tested as part of conditioning regimens for AML prior to allogeneic stem cell transplant.47-50

**Strategies to Improve Remission Duration**

Despite morphologic and cytogenetic CR following induction and consolidation therapy, patients who do not receive additional chemotherapy following induction will relapse, usually within 6 to 9 months. Chemotherapy-based consolidation may prolong remission duration; however, the majority of patients with AML will relapse within 2-3 years. A minority of patients are cured with chemotherapy alone, and others are cured with stem cell transplantation. Long-term survival for elderly patients and those with poor risk cytogenetics is dismal, and various strategies have been studied in the post-remission setting in an attempt to prolong remission duration. Although there is a proven role for post-remission therapy for other hematologic malignancies including acute lymphocytic leukemia, acute promyelocytic leukemia and multiple myeloma, maintenance therapy for AML remains an area of active investigation (Table 3).

It is widely accepted that leukemia relapse results from persistence of chemotherapy-resistant, minimal residual disease, undetectable by morphology or conventional flow cytometry. John Dick and colleagues first described a “leukemia stem cell” (LSC) with properties of self-renewal and differentiation, capable of regenerating the entire spectrum of leukemic cells.51,52 Controversy remains regarding the exact definitions of leukemia or cancer stem cells and whether there is heterogeneity in their phenotype across different leukemia subtypes. Regardless of definition, though, the clinical observation that leukemia relapse is common suggests the existence of these chemotherapy-resistant cells. Various treatments have been tested in the post-remission setting but there is no standard therapy to prolong remission duration in AML beyond a limited number of cycles of consolidation chemotherapy. A complete review of this topic is beyond the scope of this review, and the reader is referred to reference 53 for further details.53 Here, we will summarize the data for post-remission maintenance therapy and review agents under investigation in this setting.

Even early in AML drug development, there was recognition of the need for post-remission therapy. In the landmark 1981 publication establishing 7+3 as the standard induction regimen, there was also provision for maintenance therapy with cycles including Ara-C in alternating combination with thioguanine, CCNU, cyclophosphamide or DNR.3

**Table 3.** Agents currently under investigation in the post-remission (maintenance) setting.

| Drug          | Decitabine | Bortezomib | IL-2 | Imatinib | Azacitidine | Dasatinib | Panobinostat | AC220 | Lenalidomide |
|---------------|------------|------------|------|----------|-------------|-----------|--------------|-------|-------------|

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In the intervening years, however, there has been no consistent data to recommend any maintenance strategy over another. Drugs which have been tested in this setting include common AML chemotherapeutics such as Ara-C, DNR, etoposide and mitoxantrone; IL-2 alone or in combination with histamine, and the farnesyltransferase inhibitor tipifarnib. Ongoing clinical trials will examine the role of varied agents in the post-remission setting including lenalidomide, azacitidine, decitabine, bortezomib, imatinib, dasatinib and sorafenib. Additional trials in the post-stem cell transplant remission setting are also underway with sorafenib, decitabine, azacitidine, panobinostat and the FLT3 inhibitor AC220.

Strategies in Relapsed/Refractory AML

Approximately 25%–30% of patients with AML will have disease that is resistant to standard induction chemotherapy. In addition, the majority of patients who achieve remission will ultimately relapse, including 40%–50% of patients with favorable risk disease. The only option for long-term survival in patients with relapsed or refractory AML is allogeneic stem cell transplant, and transplantation is most successful when the patient is in CR. Therefore, strategies to achieve a sufficiently durable CR in order to identify an appropriate donor are critical as a bridge to transplantation. Early phase clinical trials are examining the safety and efficacy of various drugs either as single agents or in combination with standard therapy for patients with AML. For example, the hypomethylating agents azacitidine and decitabine have been used in the setting of relapsed or refractory leukemia with limited data to support this approach. Here, we will briefly review some of the emerging data.

Clofarabine

Clofarabine is a second-generation nucleoside analogue recently shown to have efficacy in relapsed and refractory AML. In a phase II trial in patients with relapsed or refractory leukemias, 48% response rate (including 30% CR rate) was observed to single agent clofarabine given at a dose of 40 mg/m² daily for 5 days. A subsequent phase II study examined the efficacy of a combination of clofarabine in combination with Ara-C similarly found a response rate of 38% with the most toxicities limited to grade 2 including nausea/vomiting, rash and mucositis. The CLASSIC I trial was a phase III prospective randomized trial comparing clofarabine/Ara-C (clofarabine at 40 mg/m²/day × 5 days, Ara-C 1 g/m²/day × 5 days) versus Ara-C alone in 320 patients ages 55 and older with relapsed/refractory AML. Results were presented in abstract form at the meeting of the American Society of Clinical Oncology (ASCO). The primary endpoint was overall survival, and overall survival was not different between the two arms (6.6 months for the combination versus 6.4 months in the Ara-C arm, \( P = 0.973 \)). Statistically significant differences favoring the combination were seen in CR rate (41% for the combination versus 16% for Ara-C alone, \( P = 0.001 \)) for relapsed patients. These results have led to the use of clofarabine/Ara-C for relapsed patients with AML as a bridge to transplantation. In addition, clofarabine was studied in combination with Ara-C and granulocyte colony-stimulating factor (G-CSF) in a phase I/II study. Clofarabine was given at 25 mg/m²/day × 5 days, Ara-C at 2 g/m²/day × 5 days, and G-CSF at 5 µg/kg starting the day prior to chemotherapy and continuing until neutrophil recovery. The CR/CRi rate was 61% and responses were seen across all cytogenetic risk categories. Ongoing clinical trials are looking at clofarabine in combination with various agents including gemtuzumab and sorafenib, among others.

FLT3 inhibitors

The recognition of the FLT3-ITD mutation as a marker of poor prognosis in AML was soon matched with the expectation that inhibitors of mutant FLT3 would result in improved outcomes for patients. A comprehensive review of all of the FLT3 inhibitors tested in clinical trials thus far is beyond the scope of this review, and the reader is referred to references 67 and 68 for further details. Here we will briefly summarize the clinical development and challenges of incorporating FLT3 inhibitors into AML therapy.

FLT3-ITD mutations are found in up to 25% of patients with AML and are associated with a 5-year survival rate of 15%. The WHO revised its AML classification schema in 2008 to include FLT3 mutant AML as a distinct entity with poor prognosis. Given its prevalence among patients with AML and high rates of relapse, there is an unmet need to
specifically target this subset of AML. Inhibitors of FLT3, including midostaurin, lestaurtinib, sorafenib, and the second-generation FLT3-TKI AC220, have been tested as single agents. Clinical responses have been variable and transient, and it appears that in vivo inhibition of FLT3 highly correlates with response to therapy. Trials of FLT3 inhibitors in combination with chemotherapy in the upfront and relapsed settings suggest that there is no added toxicity, but long-term data on survival is not yet available.

**CPX-351**

CPX-351 is a liposomal formulation of Ara-C and DNR with increased in vitro and in vivo efficacy as compared to conventional formulations of Ara-C and DNR given in combination. Preliminary data from a randomized trial of CPX-351 re-induction versus standard re-induction therapy (investigator’s choice) was presented at the 2011 ASH Annual Meeting. Results from 126 patients showed non-significant differences in rates of CR/CRi (51% for CPX-351 versus 41% for other salvage). Patients were stratified using the European Prognostic Index, and patients with unfavorable risk disease who received CPX-351 had a significant improvement in OS (6.6 months versus 4.2 months, \( P = 0.02 \)).

**Other drugs in development**

The Hedgehog signalling pathway has been implicated in the pathogenesis and chemotherapy resistance of a variety of human malignancies.73,74 A role for Hedgehog signalling in the self-renewal of leukemia stem cells in chronic myeloid leukemia, acute lymphocytic leukemia, multiple myeloma and lymphoma has been described. Preliminary data was presented at the 2011 ASH Annual Meeting with the Hedgehog inhibitor, PF-04449913 (Pfizer). The Phase I trial enrolled patients with relapsed or refractory hematologic malignancies. One patient with AML arising from CMML achieved a CRi and five other patients with AML had a significant decrease in circulating leukemia cells. Clinical trials of this drug as well as other Hedgehog pathway inhibitors are planned in the relapsed and upfront settings in AML.

In addition to Hedgehog signalling, other pathways have been implicated in AML including mTOR/PI3K, MEK and WNT/β-catenin. Several mTOR inhibitors have been studied as single agents in relapsed/refractory AML as well as in combinations with other chemotherapy. For example, results of a Phase II study of the mTOR inhibitor temsirolimus plus clofarabine in relapsed elderly patients with AML were recently reported. Fifty-three patients received a salvage re-induction with clofarabine 20 mg/m²/day × 5 days and temsirolimus 25 mg on days 1, 8 and 15. Patients attaining CR/CRi could continue on monthly temsirolimus maintenance. Although the rate of CR/CRi was 21%, laboratory correlative studies demonstrated that target inhibition was associated with higher rates of clinical response.81

Trials with histone deactylase inhibitors such as vorinostat, panobinostat and romidepsin, are ongoing in AML and MDS.23 The CXCR4 antagonist plerixafor disrupts the leukemia microenvironment and it is hypothesized that this inhibition of the CXCR4/CXCL12 axis may enhance sensitivity to chemotherapy. A recent publication reports the results of a Phase II study of plerixafor in combination with salvage chemotherapy (mitoxantrone, etoposide and Ara-C) in relapsed or refractory AML. There was no increased toxicity with the addition of plerixafor, and the CR/CRi rate was 46% in this resistant population with a two-fold mobilization in leukemic blasts into the peripheral blood.82 Tigecycline, an antibiotic effective in multidrug resistant soft tissue infections, was identified as an inhibitor of mitochondrial translation with in vitro efficacy against leukemia stem and progenitor cells.83 A phase I study of this agent in relapsed AML is ongoing.

**Discussion**

There is no question that more effective therapy is needed for the majority of patients with AML. In addition, AML incidence is expected to increase with the aging population, underscoring the need for less toxic regimens in patients with co-morbid conditions precluding intensive chemotherapy. Potential opportunities for intervention within the traditional AML treatment paradigm exist in the induction, post-remission and relapsed settings (Fig. 1). Trials of alternate induction regimens are ongoing in both younger and older patients, as are trials of new agents added to the existing “7+3” backbone of AML therapy. Enhanced molecular profiling of the heterogeneous diseases traditionally considered “AML” has provided clinicians with an additional prognostic tool and researchers
with targets to pursue in defined populations of patients. Practically speaking, this refined prognostication has only resulted in practice changes regarding the use of stem cell transplant for patients predicted to have inferior outcomes (increased transplantation for patients with CN-AML with FLT-3 mutation). Other attempted interventions with FLT-3 inhibitors have thus far led to disappointing clinical results. However, it is likely that meaningful advances will require the design of combinations of personalized therapies based on the genetic mutations underlying an individual leukemia.

The heterogeneity and further sub-classification of AML presents both opportunities and challenges for the development and evaluation of novel treatment strategies. It is difficult to accrue large numbers of patients with less common subtypes to clinical trials, and often detailed molecular analysis is not available before the initiation of therapy. Post-hoc subset analyses by age or molecular abnormalities may not be powered to provide robust data demonstrating benefit for particular subtypes. For example, GO has shown improved overall survival in those with favorable risk cytogenetics. However, these benefits were not realized in larger randomized trials of all cytogenetic categories, leading to its withdrawal from the US market. The fate of GO in the US remains unclear, despite growing evidence of efficacy in certain AML patients from maturing European data.

The use of “maintenance” or post-remission therapy has been a mainstay of treatment regimens for Acute Lymphocytic Leukemia and APL, and now is commonly used in the post-transplant setting in Multiple Myeloma. Previous studies have examined the utility of maintenance therapy in AML but are not routinely used in clinical practice. The development of maintenance chemotherapy in AML has been hindered by a lack of uniformity in induction and consolidation chemotherapy regimens as well as the lack of specific targeted maintenance therapy in particular AML subtypes. Maintenance strategies in AML targeting the LSC or specific mutations of the leukemia are ongoing. For example, imatinib is being studied in the post-remission setting in c-KIT mutated AML. Perhaps in the setting of a biologically-targeted agent in AML with a specific molecular derangement, maintenance therapy may prove useful. LSC-targeted agents represent a rational therapeutic strategy to eliminate the chemotherapy-resistant persistent clone in the post-remission setting, and clinical trials with several agents are currently underway.

The biological heterogeneity of AML has been recognized, and there is continued need for adequately powered prospective clinical trials to evaluate new treatments and strategies in these subsets of AML. Molecular profiling of AML, particularly those abnormalities within cytogenetics normal AML, have suggested additional therapeutic targets for development. Laboratory analyses of clinical samples, coupled with outcomes data, have refined the prognosis of AML. Further advances in AML therapy are anticipated with exploration of these newly defined targets.

**Author Contributions**

Wrote the first draft of the manuscript: M.Y.L and T.L.L. Jointly developed the structure and arguments for the paper: M.Y.L and T.L.L. Made critical revisions and approved final version: M.Y.L and T.L.L. All authors reviewed and approved of the final manuscript.

**Competing Interests**

Authors declare no competing interests.

**Disclosures and Ethics**

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including
but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest. Provenance: the authors were invited to submit this paper.

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