REVIEW

Acute Myeloid Leukemia: Focus on Novel Therapeutic Strategies

Tara L. Lin[1] and M. Yair Levy[2]

[1]Division of Hematology/Oncology, Department of Internal Medicine, University of Kansas, Kansas City, KS.
[2]Texas Oncology—Baylor Charles A. Sammons Cancer Center, Dallas, TX. Corresponding author email: tlin@kumc.edu

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

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.[1] Despite remarkable progress in therapy for acute promyelocytic leukemia (APL) with long-term cure likely in up to 90% of patients,[2] 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.[3,4] The most commonly used post-remission therapy, cytarabine, is given in similar fashion as when described in 1994.[5] 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%.[6] 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.[7–9] 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.[9] More recently, emerging data on molecular markers of prognosis within the traditionally defined risk groups had led to additional refinements (see Table 1).[10] Within favorable risk disease, data demonstrate inferior outcomes for patients with an additional *c-KIT* mutation.[11,12] Progress in molecular profiling of the intermediate risk cytogenetics normal AML (CN-AML)[13–16] have led to the identification of mutations conferring improved (mutations of *NPM*1 or *C*EBPA) or inferior (*FLT-*3) outcomes.[17–19] 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 addition to needed therapies in the upfront setting for newly diagnosed AML, relapsed and refractory disease remains a formidable problem. New agents have been approved in recent years for patients with relapsed and refractory AML, and those achieving remission in this setting may be eligible for potentially

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

Risk status	Karyotype	Molecular abnormalities
Favorable risk	Inversion (16) or t(16;16) t(8;21) t(15;17)	Normal cytogenetics with NPMI mutation or CEBPA mutation in absence of FLT3-ITD mutation t(8;21), inv (16), or t(16;16) with c-KIT mutation
Intermediate risk	Normal cytogenetics Trisomy 8 t(9;11)	
Poor risk	Complex (≥3 abnormal clones) -5, -5q, -7, -7q 11q23 Inversion 3 or t(3;3) t(6;9) t(9;22)	Normal cytogenetics with FLT3-ITD mutation

curative 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%, $P < 0.001$) 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.[20] However, there was concern that the CR rate was lower than previously

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	Elacytarabine, CPX-351
Nucleoside analogue	Clofarabine, sapacitabine, 5-Fluoro-2′-Deoxycytidine
Hypomethylating agents	Azacitdine, decitabine
Immunomodulator (IMiD)	Lenalidomide
CXCR4 antagonist	Plerixafor
BCR-ABL tyrosine kinase inhibitors	Dasatinib, imatinib, nilotinib
Histone deactylase inhibitors	Entinostat, MS-275, Panobinostat, vorinostat
Proteosome inhibitor	Bortezomib
mTOR pathway inhibitors	Everolimus, temsirolimus
FLT3 inhibitors	AC220, PLX3397, sorafenib
Retinoids	All-trans retinoic acid, bexarotene
Antibody-drug conjugate	Gemtuzumab
Alkylating agents	Bendamustine
Farnesyltransferase inhibitor	Tipifarnib
Hedgehog pathway inhibitor	PF-0449
PI3K/AKT pathway inhibitor	ON 01910.Na
HSP-90 inhibitor	Elesclomol sodium
Angiokinase inhibitor	BIBF 1120
Statins	Pravastatin, lovastatin
Mitochondrial translation inhibitor	Tigecycline
EGFR inhibitor	Erlotinib
WNT pathway inhibitor	CWP232291
Oncogene eIF4E inhibitor	Ribavirin
Src kinase inhibitor	KX2-391
VEGFR inhibitor	Pazopanib
Anticancer quinolone derivative	Vosaroxin
CDK inhibitor	Flavoperidol
Pan-PIM kinase inhibitor	AZD1208

reported in studies of DNR at 60 mg/m^2. There are no studies which have directly compared DNR at 60 mg/m^2 versus 90 mg/m^2. 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/m^2 × 3 days), increased IDA (12 mg/m^2 × 4 days) or higher dose DNR 80 mg/m^2 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.[21] 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/m^2 or 90 mg/m^2. 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.[22] Based on these large cooperative studies, NCCN Guidelines advocate the use of escalated dose DNR or IDA as a Category 1 recommendation.[10] 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.[23] 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.[24] Clinical trials are ongoing in relapsed AML (see below)[25] and are expected to open shortly in untreated patients.[23]

Antibody-drug conjugate
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

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.[26]

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.[27]

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 in 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.[28] 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.[29]

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.[30] Another strategy involves intensive chemotherapy with flavopiridol, Ara-C and mitoxantrone (FLAM). This regimen has been studied in elderly and relapsed patients[31] or younger patients with poor risk features[32] 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.[33] 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.[23]

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.[34] 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.[35] Recent studies, though, demonstrate that older patients with AML may tolerate intensive chemotherapy with increasing doses of DNR,[22] suggesting that comorbidities and performance status, rather than age per se, determine fitness for therapy.[36] Authors argue that each patient should be considered individually, particularly given that no less intensive induction regimen has proven superior to 7+3.[37] 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.[38] 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.[39]

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.[40] Studies examining the efficacy of sequential azacitidine plus lenalidomide as well as decitabine in combination with other agents are currently ongoing.[23]

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.[41] SWOG conducted a phase II clinical trial for untreated elderly patients with 5q deletion with 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.[42] 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).[42] 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.[23]

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 ≥ 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 × 5 days as induction followed by consolidation cycles at 20 mg/m^2/day × 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.[43] 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 ≥ 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/m^2/ day × 5 days and Ara-C at 1 g/m^2/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 the 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/m^2/day × 5 days) with or without low-dose Ara-C (20 mg/m^2/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.

Decitabine	Dasatinib
Bortezomib	Panobinostat
IL-2	AC220
Imatinib	Lenalidomide
Azacitidine	

In the intervening years, however, there has been no consistent data to recommend any maintenance strategy over another.[54–56] 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;[57,58] and the farnesyltransferase inhibitor tipifarnib.[59] 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.[23]

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.[9] 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.[60–63] 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/m^2 daily for 5 days.[64] A subsequent phase I-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.[65] The CLASSIC I trial was a phase III prospective randomized trial comparing clofarabine/Ara-C (clofarabine at 40 mg/m^2/day × 5 days, Ara-C 1 g/m^2/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.[66] 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/m^2/day × 5 days, Ara-C at 2 g/m^2/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.[23]

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.[67,68] 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%.[69] The WHO revised its AML classification schema in 2008 to include FLT3 mutant AML as a distinct entity with poor prognosis.[70] 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.[71] 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,[72] and patients with unfavorable risk disease who received CPX-351 had a significant improvement in OS (6.6 months versus 4.2 months, $P = 0.02$).[25]

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,[75] acute lymphocytic leukemia,[76] multiple myeloma[77] and lymphoma[78,79] 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.[80] 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/m^2/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.[23]

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
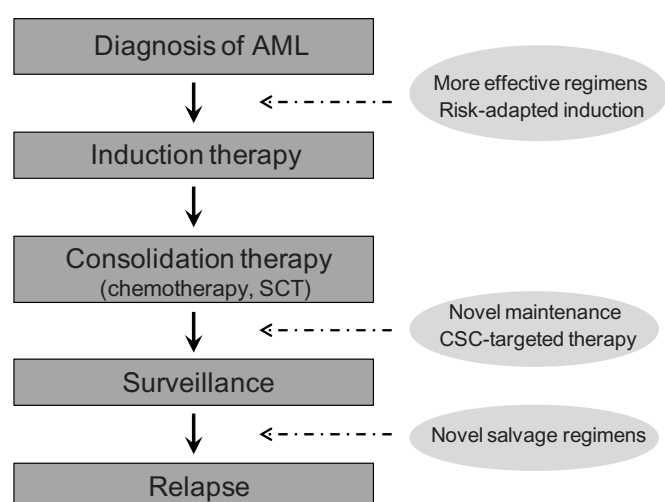
Figure 1. Treatment paradigm of AML and potential therapeutic interventions.

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).[84,85] Other attempted interventions with FLT-3 inhibitors have thus far led to disappointing clinical results.[67,68] 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.

References

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*. Jul–Aug 2011;61(4):212–36.
2. Sanz MA, Lo-Coco F. Modern approaches to treating acute promyelocytic leukemia. *J Clin Oncol*. Feb 2011;29(5):495–503.
3. Rai KR, Holland JF, Glidewell OJ, et al. Treatment of acute myelocytic leukemia: a study by cancer and leukemia group B. *Blood*. Dec 1981;58(6):1203–12.
4. Yates JW, Wallace HJ, Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep*. Nov–Dec 1973;57(4):485–8.
5. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. *N Engl J Med*. Oct 1994;331(14):896–903.
6. Farag SS, Archer KJ, Mrózek K, et al. Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. *Blood*. Jul 2006;108(1):63–73.
7. Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood*. Oct 1998;92(7):2322–33.
8. Slovak M, Kopecky K, Cassileth P, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood*. Dec 2000;96(13):4075–83.
9. Byrd J, Mrózek K, Dodge R, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. Dec 2002;100(13):4325–36.
10. Members NAMLP. NCCN Practice Guidelines in Oncology v.2.2010. Acute Myeloid Leukemia. 2010; http://www.nccn.org/professionals/physician_gls/PDF/aml.pdf. Accessed Jul 5, 2010.
11. Care RS, Valk PJ, Goodeve AC, et al. Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. *Br J Haematol*. Jun 2003;121(5):775–7.
12. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. *J Clin Oncol*. Aug 2006;24(24):3904–11.
13. Bienz M, Ludwig M, Leibundgut E, et al. Risk assessment in patients with acute myeloid leukemia and a normal karyotype. *Clin Cancer Res*. Feb 2005;11(4):1416–24.
14. Mrózek K, Marcucci G, Paschka P, Whitman S, Bloomfield C. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? *Blood*. Jan 2007;109(2):431–48.
15. Schlenk R, Döhner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med*. May 2008;358(18):1909–18.
16. Döhner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. Jan 2010;115(3):453–474.
17. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. Jan 2005;352(3):254–66.
18. Fröhling S, Schlenk R, Breitruck J, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood*. Dec 2002;100(13):4372–80.
19. Fröhling S, Schlenk R, Stolze I, et al. CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. *J Clin Oncol*. Feb 2004;22(4):624–33.
20. Fernandez HF, Sun Z, Yao X, et al. Anthracycline dose intensification in acute myeloid leukemia. *N Engl J Med*. Sep 2009;361(13):1249–59.
21. Pautas C, Merabet F, Thomas X, et al. Randomized study of intensified anthracycline doses for induction and recombinant interleukin-2 for maintenance in patients with acute myeloid leukemia age 50 to 70 years: results of the ALFA-9801 study. *J Clin Oncol*. Feb 2010;28(5):808–14.
22. Löwenberg B, Ossenkoppele GJ, van Putten W, et al. High-dose daunorubicin in older patients with acute myeloid leukemia. *N Engl J Med*. Sep 2009;361(13):1235–48.
23. http://www.clinicaltrials.gov. Accessed Mar 1, 2012.
24. Lim WS, Tardi PG, Dos Santos N, et al. Leukemia-selective uptake and cytotoxicity of CPX-351, a synergistic fixed-ratio cytarabine:daunorubicin formulation, in bone marrow xenografts. *Leuk Res*. Sep 2010;34(9):1214–23.
25. Cortes J, Feldman E, Goldberg S, et al. CPX-351: A Randomized Phase 2b Study of CPX-351 v. Intensive Salvage Therapy in ≤65 Yo First Relapse AML Patients: Initial Efficacy and Safety Report. *Blood ASH Annual Meeting Abstracts*. 2011;118:254.
26. Burnett AK, Hills RK, Milligan D, et al. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. *J Clin Oncol*. Feb 2011;29(4):369–77.
27. Burnett A, Hills R, Hunter A, et al. The addition of gemtuzumab ozogamicin to intensive chemotherapy in older patients with AML produces a significant improvement in overall survival: results of UK NCRI AML16 randomized trial. . *Blood ASH Annual Meeting Abstracts*. 2011;118:582.
28. Castaigne S, Pautas C, Terre C, Raffoux E, Bordessoule D. Fractionated Doses of Gemtuzumab Ozogamicin (GO) Combined to Standard Chemotherapy (CT) Improve Event-Free and Overall Survival in Newly-Diagnosed De Novo AML Patients Aged 50–70 Years Old: A Prospective Randomized Phase 3 Trial From the Acute Leukemia French Association (ALFA). *Blood ASH Annual Meeting Abstracts*. 2011;118:6.
29. Delauney J, Recher C, Pigneux A, et al. Addition of gemtuzumab ozogamicin to chemotherapy improves event-free survival but not overall survival of AML patients wtih intermediate cytogenetics not eligible for allogeneic transplantation. Resiults of the GOELAMS AML 2006 IR study. *Blood ASH Annual Meeting Abstracts*. 2011;118:615.
30. Scandura JM, Roboz GJ, Moh M, et al. Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML. *Blood*. Aug 2011;118(6):1472–80.
31. Karp JE, Passaniti A, Gojo I, et al. Phase I and pharmacokinetic study of flavopiridol followed by 1-beta-D-arabinofuranosylcytosine and mitoxantrone in relapsed and refractory adult acute leukemias. *Clin Cancer Res*. Dec 2005;11(23):8403–12.

32. Karp JE, Blackford A, Smith BD, et al. Clinical activity of sequential flavopiridol, cytosine arabinoside, and mitoxantrone for adults with newly diagnosed, poor-risk acute myelogenous leukemia. *Leuk Res*. Jul 2010; 34(7):877–82.

33. Garcia-Manero G, Tambaro F, Bekele N, Yang H. Final report of a phase II trial of vorinostat with idarubicin and cytarabine for patients with newly diagnosed acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS). *Blood ASH Annual Meeting Abstracts*. 2011;118:763.

34. Menzin J, Lang K, Earle CC, Kerney D, Mallick R. The outcomes and costs of acute myeloid leukemia among the elderly. *Arch Intern Med*. Jul 2002;162(14):1597–603.

35. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood*. May 2006;107(9):3481–5.

36. Walter RB, Othus M, Borthakur G, et al. Prediction of early death after induction therapy for newly diagnosed acute myeloid leukemia with pretreatment risk scores: a novel paradigm for treatment assignment. *J Clin Oncol*. Nov 2011;29(33):4417–23.

37. Schiffer CA. "I am older, not elderly," said the patient with acute myeloid leukemia. *J Clin Oncol*. Feb 2010;28(4):521–3.

38. Fenaux P, Mufti GJ, Hellström-Lindberg E, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol*. Feb 2010;28(4):562–9.

39. Cashen AF, Schiller GJ, O'Donnell MR, DiPersio JF. Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. *J Clin Oncol*. Feb 2010;28(4):556–61.

40. Ravandi F, Issa JP, Garcia-Manero G, et al. Superior outcome with hypomethylating therapy in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome and chromosome 5 and 7 abnormalities. *Cancer*. Dec 2009;115(24):5746–51.

41. Blum W, Klisovic RB, Becker H, et al. Dose escalation of lenalidomide in relapsed or refractory acute leukemias. *J Clin Oncol*. Nov 2010;28(33):4919–25.

42. Sekeres MA, Gundacker H, Lancet J, et al. A phase 2 study of lenalidomide monotherapy in patients with deletion 5q acute myeloid leukemia: Southwest Oncology Group Study S0605. *Blood*. Jul 2011;118(3):523–8.

43. Kantarjian HM, Erba HP, Claxton D, et al. Phase II study of clofarabine monotherapy in previously untreated older adults with acute myeloid leukemia and unfavorable prognostic factors. *J Clin Oncol*. Feb 2010;28(4):549–55.

44. Burnett AK, Russell NH, Kell J, et al. European development of clofarabine as treatment for older patients with acute myeloid leukemia considered unsuitable for intensive chemotherapy. *J Clin Oncol*. May 2010;28(14):2389–95.

45. Faderl S, Verstovsek S, Cortes J, et al. Clofarabine and cytarabine combination as induction therapy for acute myeloid leukemia (AML) in patients 50 years of age or older. *Blood*. Jul 2006;108(1):45–51.

46. Faderl S, Ravandi F, Huang X, et al. A randomized study of clofarabine versus clofarabine plus low-dose cytarabine as front-line therapy for patients aged 60 years and older with acute myeloid leukemia and high-risk myelodysplastic syndrome. *Blood*. Sep 2008;112(5):1638–45.

47. Martin MG, Uy GL, Procknow E, et al. Allo-SCT conditioning for myelodysplastic syndrome and acute myeloid leukemia with clofarabine, cytarabine and ATG. *Bone Marrow Transplant*. Jul 2009;44(1):13–7.

48. Magenau J, Tobai H, Pawarode A, et al. Clofarabine and busulfan conditioning facilitates engraftment and provides significant antitumor activity in nonremission hematologic malignancies. *Blood*. Oct 2011;118(15):4258–64.

49. Andersson BS, Valdez BC, de Lima M, et al. Clofarabine ± fludarabine with once daily i.v. busulfan as pretransplant conditioning therapy for advanced myeloid leukemia and MDS. *Biol Blood Marrow Transplant*. Jun 2011;17(6):893–900.

50. Buchholz S, Dammann E, Stadler M, et al. Cytoreductive treatment with clofarabine/ara-C combined with reduced-intensity conditioning and allogeneic stem cell transplantation in patients with high-risk, relapsed, or refractory acute myeloid leukemia and advanced myelodysplastic syndrome. *Eur J Haematol*. Jan 2012;88(1):52–60.

51. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*. Feb 1994;367(6464):645–8.

52. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. Jul 1997;3(7):730–7.

53. Büchner T, Krug U, Berdel WE, et al. Maintenance for acute myeloid leukemia revisited. *Curr Treat Options Oncol*. Aug 2007;8(4):296–304.

54. Cassileth PA, Lynch E, Hines JD, et al. Varying intensity of postremission therapy in acute myeloid leukemia. *Blood*. Apr 1992;79(8):1924–30.

55. Goldstone AH, Burnett AK, Wheatley K, et al. Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial. *Blood*. Sep 2001;98(5):1302–11.

56. Büchner T, Hiddemann W, Berdel WE, et al. 6-Thioguanine, cytarabine, and daunorubicin (TAD) and high-dose cytarabine and mitoxantrone (HAM) for induction, TAD for consolidation, and either prolonged maintenance by reduced monthly TAD or TAD-HAM-TAD and one course of intensive consolidation by sequential HAM in adult patients at all ages with de novo acute myeloid leukemia (AML): a randomized trial of the German AML Cooperative Group. *J Clin Oncol*. Dec 2003;21(24):4496–504.

57. Buyse M, Squifflet P, Lange BJ, et al. Individual patient data meta-analysis of randomized trials evaluating IL-2 monotherapy as remission maintenance therapy in acute myeloid leukemia. *Blood*. Jun 2011;117(26):7007–13.

58. Baer MR, George SL, Caligiuri MA, et al. Low-dose interleukin-2 immunotherapy does not improve outcome of patients age 60 years and older with acute myeloid leukemia in first complete remission: Cancer and Leukemia Group B Study 9720. *J Clin Oncol*. Oct 2008;26(30):4934–9.

59. Karp JE, Smith BD, Gojo I, et al. Phase II trial of tipifarnib as maintenance therapy in first complete remission in adults with acute myelogenous leukemia and poor-risk features. *Clin Cancer Res*. May 2008;14(10):3077–82.

60. Chowdhury S, Seropian S, Marks PW. Decitabine combined with fractionated gemtuzumab ozogamicin therapy in patients with relapsed or refractory acute myeloid leukemia. *Am J Hematol*. Sep 2009;84(9):599–600.

61. Graef T, Kuendgen A, Fenk R, Zohren F, Haas R, Kobbe G. Successful treatment of relapsed AML after allogeneic stem cell transplantation with azacitidine. *Leuk Res*. Feb 2007;31(2):257–9.

62. Platzbecker U, Wermke M, Radke J, et al. Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial. *Leukemia*. Sep 2011.

63. Wei A, Tan P, Catalano J, Walker P. Azacitidine in combination with the mTOR inhibitor everolimus in relapsed and refractory AML. *Blood ASH Annual Meeting Abstracts*. 2011;118:2599.

64. Kantarjian H, Gandhi V, Cortes J, et al. Phase 2 clinical and pharmacologic study of clofarabine in patients with refractory or relapsed acute leukemia. *Blood*. Oct 2003;102(7):2379–86.

65. Faderl S, Gandhi V, O'Brien S, et al. Results of a phase 1–2 study of clofarabine in combination with cytarabine (ara-C) in relapsed and refractory acute leukemias. *Blood*. Feb 2005;105(3):940–7.

66. Faderl S, Wetzler M, Rizzieri D, Schiller G, Jagasia M. Clofarabine plus cytarabine compared to cytarabine alone in older patients with relapsed or refractory (R/R) acute myelogenous leukemia (AML): Results from the phase III CLASSIC 1 trial. *J Clin Oncol*. 2011;29(Suppl);Abstr 6503.

67. Fathi AT, Chabner BA. FLT3 inhibition as therapy in acute myeloid leukemia: a record of trials and tribulations. *Oncologist*. 2011;16(8):1162–74.

68. Kindler T, Lipka DB, Fischer T. FLT3 as a therapeutic target in AML: still challenging after all these years. *Blood*. Dec 2010;116(24):5089–102.

69. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood*. Sep 2001;98(6):1752–9.

70. Vardiman J, Thiele J, Arber D, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. Jul 2009;114(5):937–51.

71. Levis M, Ravandi F, Wang ES, et al. Results from a randomized trial of salvage chemotherapy followed by lestaurtinib for patients with FLT3 mutant AML in first relapse. *Blood*. Mar 2011;117(12):3294–301.

72. Breems DA, Van Putten WL, Huijgens PC, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. *J Clin Oncol*. Mar 2005; 23(9):1969–78.

73. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature*. Nov 2004;432(7015):324–31.

74. Taipale J, Beachy P. The Hedgehog and Wnt signalling pathways in cancer. *Nature*. May 2001;411(6835):349–54.

75. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*. Apr 2009;458(7239):776–9.

76. Lin TL, Wang QH, Brown P, et al. Self-renewal of acute lymphocytic leukemia cells is limited by the Hedgehog pathway inhibitors cyclopamine and IPI-926. *PLoS One*. 2010;5(12):e15262.

77. Peacock CD, Wang Q, Gesell GS, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. *Proc Natl Acad Sci U S A*. Mar 2007;104(10):4048–53.

78. Dierks C, Grbic J, Zirlik K, et al. Essential role of stromally induced hedgehog signaling in B-cell malignancies. *Nature Medicine*. 2007;13(8): 944–51.

79. Singh R, Kim J, Davuluri Y, et al. Hedgehog signaling pathway is activated in diffuse large B-cell lymphoma and contributes to tumor cell survival and proliferation. *Leukemia*. Mar 2010.

80. Jamieson C, Cortes JE, Oehler V, et al. Phase 1 Dose-Escalation Study of PF-04449913, An Oral Hedgehog (Hh) Inhibitor, in Patients with Select Hematologic Malignancies. *Blood*. 2011;118:424.

81. Amadori S, Stasi R, Martelli AM, et al. Temsirolimus, an mTOR inhibitor, in combination with lower-dose clofarabine as salvage therapy for older patients with acute myeloid leukaemia: results of a phase II GIMEMA study (AML-1107). *Br J Haematol*. Jan 2012;156(2):205–12.

82. Uy GL, Rettig MP, Motabi IH, et al. A phase I/II study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. *Blood*. Feb 2012.

83. Skrtić M, Sriskanthadevan S, Jhas B, et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. *Cancer Cell*. Nov 2011;20(5):674–88.

84. Bornhäuser M, Illmer T, Schaich M, et al. Improved outcome after stem-cell transplantation in FLT3/ITD-positive AML. *Blood*. Mar 2007; 109(5):2264–5; Author reply 2265.

85. DeZern AE, Sung A, Kim S, et al. Role of allogeneic transplantation for FLT3/ITD acute myeloid leukemia: outcomes from 133 consecutive newly diagnosed patients from a single institution. *Biol Blood Marrow Transplant*. Sep 2011;17(9):1404–9.