CLINICAL INVESTIGATION

Chemotherapeutic treatment of acute myeloid leukemia: incorporating the results of recent clinical trials

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Better outcomes for patients with acute myeloid leukemia over the last 30 years have been largely achieved by improvements in supportive care measures rather than therapeutic advances. The combination of daunorubicin and cytarabine has remained the standard of care for patients undergoing intensive induction–consolidation treatment. In less fit older patients, low-dose cytarabine is the equivalent, although the hypomethylating agent azacitidine may be challenging current practice. Enhanced understanding of disease pathogenesis and therapy resistance has enabled the entry of novel chemotherapeutic and nonchemotherapeutic agents into clinical development with varied levels of activity. This article examines the evidence behind established chemotherapy practices for intensive and nonintensive acute myeloid leukemia treatments with an emphasis on emerging clinical trial data from novel chemotherapeutic and nonchemotherapeutic agents.

Keywords: acute myeloid leukemia • aminopeptidase inhibitors • farnesyl transferase inhibitors • FLT3 inhibitors • gemtuzumab ozogamicin • histone deacytylase inhibitors • hypomethylating • Polo-like kinase 1 inhibitors

Methodology

An electronic database search (EMBASE, MEDLINE and PubMed) was undertaken to identify and review clinical studies in acute myeloid leukemia (AML) undertaken between 1 January 2009 and 1 June 2014.

Background

The treatment of AML has remained immensely challenging for clinicians due to the disease's heterogeneity and the multiple confounding factors that influence its prognostic landscape. Younger patients, generally defined as those aged less than 60 years, have seen sustained increases in survival as a result of improved supportive care measures, better risk stratification and developments in stem cell transplantation [1–3]. Despite approximately 70–80% of these patients achieving complete remission [4,5], the majority still ultimately relapse and overall survival (OS) remains only 40–45% at 5 years [6]. However, this age group only accounts for a third of all AML cases [7]. Older patients over the age of 60 years thus represent an important population from both demographic and therapeutic perspectives. Older patients frequently have multiple comorbidities, unfavorable cytogenetic and molecular profiles, pre-existing myelodysplasia and poor performance status and, as a result, have poor tolerability and resistance to standard chemotherapy with far inferior outcomes. Only 40–50% of those over 60 years of age with a good performance status achieve a complete remission (CR), and cure rates remain less than 10% and median survival is less than 1 year [8,9].

Improvements in the understanding of the molecular biology of AML using techniques such as gene expression profiling and miRNA platforms has helped to further delineate prognosis. This is particularly important for patients with a normal karyotype who comprise 40% of the total AML patients [11,12]. From such developments, the isolation of novel targets to manipulate and control

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driving factors in the disease has been possible. A simple model that exemplifies this can be found in the APL-RARA fusion protein underlying the pathogenesis of acute promyelocytic leukemia. Knowledge and, importantly, inhibition of this pathway have resulted in superior outcomes [13,14] for acute promyelocytic leukemia (APL) patients. Other AML entities are, however, genetically far more heterogeneous, with complex interactions between numerous cell surface and intracellular pathways; the concept of targeting leukemic cells and sparing normal cells from the unselected attack of chemotherapy, while appealing, remains problematic, as there are multiple potential targets in AML cells that may require simultaneous targeting [6]. Recent genomic analyses have emphasized the genetic complexity of AML with at least one potential driver mutation and a complex interplay of genetic events contributing to AML pathogenesis [15].

Traditional trial design, involving drug safety (Phase I), suggestion of efficacy (Phase II) and true efficacy, survival and quality of life effects (Phase III), is still deep rooted in clinical practice. This approach may hinder efficiency and increase the costs of drug development in AML, especially with the ever-expanding number of targets and molecules being developed. To overcome this, selection 'pick-a-winner' trials, screening trials and randomized discontinuation trials have been developed [16–18]. The MRC trials for older patients with untreated AML now employ the pick-a-winner design [19]. In these trials, the goal is to select therapies with superior early responses for further testing, with early rejection of unpromising agents based on a strict statistical algorithm [17]. Despite this shift in design, other limiting factors remain problematic to the collection of reliable data. Most notably, these include small sample sizes, poor patient recruitment, lack of a control group, patient heterogeneity and the use of surrogate end points that do not predict clinical benefit [19].

In this article, we aim to present and critically evaluate the data published over the past 10 years using novel chemotherapeutic and targeted agents in AML. To provide context, the historical evidence underpinning established induction–remission approaches will also be summarized. Judicious use of allogeneic bone marrow transplantation (allo-SCT) remains central to the management of selected patients, but in-depth analyses of transplantation or of the management of APL are beyond the scope of this article, and the reader is directed to other reviews of these areas [20–23].

Evidence base for current induction strategies

The mainstay of treatment for AML over the past 40 years has been the combination of daunorubicin and cytarabine (DA). This combination is classically delivered using the '3 + 7' schedule, in which daunorubicin (45–60 mg/m^2) is administered for 3 days with a continuous infusion of cytarabine (AraC; 100–200 mg/m^2) over 7 days. There have been many variations on this theme, with the MRC AML trials employing a '3 + 10' approach. Attempts to augment and/or manipulate this standard 'backbone' combination have met with variable success.

Dose intensification of daunorubicin (45 vs 90 mg/m^2) has been extensively studied. The Eastern Cooperative Onclology Group (ECOG) 1900 trial showed improved CR rates in younger patients (<60 years of age) treated with higher doses (71 vs 57%; $p < 0.001$) with no excess toxicity [24]. A Korean study noted similar outcomes regarding CR rate (82 vs 72%; $p = 0.014$) and toxicity [25]. The HOVON-SAKK study of patients aged 60–83 years also demonstrated benefit with superior CR rates (64 vs 54%; $p = 0.014$), but the survival benefit was only demonstrated in the 60–65 years of age subgroup [26]. Despite these promising data, caution needs to be exercised before daunorubicin 90 mg/m^2 is established as the new standard of care; similar rates of survival have been reported in trials featuring conventional-dose daunorubicin, including the MRC AML12 and 15 studies, in wihch patients received daunorubicin 50 mg/m^2, but for two courses, giving a cumulative dose of 300 mg/m^2 [27]. The National Cancer Research Institute (NCRI) AML17 study of younger patients continues to address the anthracycline dose intensification question by comparing intermediate- with high-dose daunorubicin (60 and 90 mg/m^2) in the first induction followed by a second course of 50 mg/m^2.

'High-dose AraC' (HDAC) generally refers to a AraC dose of 1000–3000 mg/m^2. The use of HDAC in induction achieved no improvement in CR rates and OS with increased toxicity in comparison to standard-dose AraC in a randomized trial of 860 patients (<60 years of age) comparing 200–1000 and 1000–2000 mg/m^2 of AraC over two cycles given, respectively, with idarubicin and amsacrine cycles 1 and 2. CR rates (82 vs 80%; $p = 0.45$), OS (42 vs 40%; $p = 0.87$) and event-free survival (35 vs 34%; $p = 0.79$) were similar between the two groups, but grade 3–4 adverse events were increased in those receiving HDAC (61 vs 51%; $p = 0.005$) [28].

Studies analyzing the addition of agents such as etoposide or 6-thioguanine to the DA backbone have generally been disappointing. The MRC AML15 trial randomized 2673 younger adults between standard DA, DA plus etoposide (ADE) and fludarabine, AraC, G-CSF and idarubicin (FLAG-Ida) [29]. Induction CR rates were similar across the three groups (DA: 72% vs

ADE: 82% vs FLAG-Ida: 84%), with greater toxicity seen in the ADE and FLAG-Ida groups. Although relapse rates in the FLAG-Ida group were lower, no difference in OS was seen.

The Polish Acute Leukemia Study Group demonstrated a potential benefit from the addition of cladribine to DA in two randomized clinical trials [30,31]. In the larger trial, of 652 younger (<60 years of age) patients, DA was compared with DA plus cladribine (DAC; 5 mg/m^2, days 1–5) and DA plus fludarabine (DAF; 25 mg/m^2, days 1–5) [31]. Higher CR rates were noted with the cladribine-containing combination (DA: 56% vs DAC: 67.5% vs DAF: 59%), which translated into improved OS in patients receiving DAC over the other regimens, especially in those patients aged >50 years (40 vs 18 months; p = 0.05) and with leukocyte counts greater than 50 × 10^9/l (47 vs 18 months; p = 0.03). A further randomized assessment of the DAC regimen is currently being made in comparison to DA and FLAG-Ida in patients aged >60 years with high-risk disease features or persisting detectable minimal residual disease (MRD) on recovery from induction chemotherapy (the NCRI AML18 study).

Consolidation

Consolidation therapy refers to any therapy delivered following the attainment of CR with the aim of reducing the risk of relapse. Randomized controlled trials (RCTs) have aimed to shed light on the optimal management of patients in this period. Questions for research include the best choice of chemotherapy regimen, the optimal number of consolidation courses, the role of allo-SCT in first complete remission (CR1) and the use of maintenance therapy [27].

HDAC has established itself as the gold standard consolidation therapy largely based on data from Cancer and Leukemia Group B (CALGB). In one RCT, 596 patients were randomized to four cycles of 100 or 400 mg/m^2 AraC for 5 days or 3 g/m^2 on days 1, 3 and 5 [32]. Response and survival benefit were gained upon increasing the dose in younger patients (aged < 60 years). For older patients (aged >60 years), however, survival benefit was lost due to high toxicity rates, with sometimes irreversible neurotoxicity noted. The CALGB group also demonstrated a particular survival advantage with HDAC postremission therapy for patients with core binding factor AML [32]. The NCRI AML15 trial (patients aged < 60 years) compared 1.5 and 3 g/m^2 AraC, with no differences being seen in major outcome end points [33]. The optimum number of cycles of consolidation is still not firmly established. The NCRI AML14 trial in older patients (aged >60 years) concluded no benefit of four courses over three courses using both 200 and 400 mg/m^2 of AraC [27]. Equally, the NCRI AML15 trial demonstrated no survival difference between a total of four and five cycles of therapy in younger patients.

The addition of mitoxantrone and daunorubicin to an attenuated dose of AraC in consolidation has shown no significant overall benefit, although in the NCRI AML15 trial, an amsacrine–etoposide–mitoxantrone-based consolidation approach proved superior to both HDAC dose levels (1.5 and 3 g/m^2) in patients with poor-risk cytogenetics [34].

Therefore, although HDAC in consolidation treatment has proved efficacious in younger patients, concerns over toxicity in older patients are warranted. The optimal dose and number of treatment courses remain unknown in both older and younger patients. The NCRI AML17 trial in younger patients (aged <60 years) is currently comparing three with the standard four courses of HDAC in favorable and intermediate-risk patients, while AML16 has recently compared three versus two cycles of a combination of DA and daunorubicin-clofarabine (DClo) in patients who achieved CR or partial remission after the first induction.

In contrast to the standard management of acute lymphoblastic leukemia, maintenance therapy has never been considered 'standard of care' in AML, with a paucity of data to question this approach. In a recent Phase III trial, 320 adult patients who had attained CR after induction–consolidation therapy were randomized to receive either ten cycles of the immunomodulatory combination histamine dihydrochloride (0.5 mg/12 h) and IL-2 (400 U/kg/12 h) or no treatment [35]. After a 3-year follow-up period, although there was some evidence of improved leukemia-free survival, no difference in OS was demonstrated. This combination may warrant further clinical investigation.

Novel agents

The major cause of therapeutic failure in AML is resistance to treatment, rather than treatment-related mortality [36,37]. The reasons for treatment failure are varied and are linked to the heterogeneous nature of patients' disease on genetic, molecular and cellular grounds. Approaches to translate increasing understanding of the pathogenesis of AML have resulted in the development of both novel cytotoxic and noncytotoxic agents (Table 1 & Figure 1), with varying degrees of success gained in clinical trials (Tables 2 & 3). Prognostic molecular markers (e.g., FLT3 and NPM1) have evolved to further risk stratify patients with normal karyotypes into more meaningful prognostic groups. The careful selection and understanding of prognostic profiles has enabled a more personalized care approach

Table 1. Novel chemotherapeutic groups and proposed mechanisms of action.

Novel chemotherapeutic group	Mechanism of action	Examples	Ref.
FLT3 inhibitors	Inhibit proliferation of leukemic blasts	Lestaurtinib Midostaurin Quizartinib Sorafenib Sunitinib Pacritinib Ponatinib	
HDAC inhibitors	Multiple: induce apoptosis, mitotic failure, autophagic cell death and reactive oxygen species-facilitated cell death	Vorinostat Mocetinostat Entinostat Panobinostat	[38,39,40]
Farnesyl transferase inhibitors	Multiple: inhibit microtubule function via downstream pathways (e.g., Ras, Rho-B and Rac among others)	Tipifarnib Lonafarnib	[41]
Polo-like kinase 1 inhibitors	Multiple: inhibit mitotic spindle formation and play key roles in the regulation of cell division, DNA damage repair pathways and apoptosis	Volasertib	[42,43]
Hypomethylating agents	Correction of epigenetic deregulation of gene expression	Decitabine Azacitidine	
Heat shock proteins inhibitors	Multiple: molecular chaperones that regulate the folding/stability of labile 'client proteins' required for tumor development	Ganetespib	
Aminopeptidase inhibitors	Exact mechanism unknown, suggestion of antiproliferative effect via depletion of tumor cells of amino acids by the inhibition of protein recycling	Tosedostat	[10]

to develop. For younger patients this is vital, especially in deciding which patients proceed to allo-SCT. The wider availability of therapeutic agents in practice is also important in the setting of relapsed and refractory disease, in which previously only palliative measures were available. There may be a role for using novel agents as a bridge to allo-SCT or, more ambitiously, eliminating the need for allo-SCT altogether.

In addition to genetic risk stratification and assessing the ability of patients to tolerate treatment, drug resistance mechanisms need to be considered in delivering optimal anti-AML therapy. A number of novel agents have been designed with the aim of circumventing these mechanisms.

Novel chemotherapy agents

A number of 'novel cytotoxic agents' have been introduced over the last decade. All have potentially novel mechanisms of action and have continued through preclinical characterization and early-phase studies, with some reaching Phase III evaluation, where the majority have unfortunately failed to make an impact.

Laromustine (VNP-40101M)

Laromustine is a sulfonylhydrazine compound that is metabolized to two active agents – VNP-4090-CE and methyl isocyanate – which, respectively, induce DNA alkylation and the inhibition of DNA repair [78]. Synergy between the two mechanisms of action is explained by the relative selective inhibition by chloroethylating species responsible for DNA alkylation at the O6 guanine position [79] and inhibition of the nucleotidyl transferase activity of purified human DNA polymerase-β, a principal enzyme of DNA base excision repair [80]. Modest clinical activity was recorded in 85 treatment-naive older patients (>60 years of age) with high-risk features when laromustine was combined with AraC consolidation (overall response rate [ORR]: 32%; OS: 3.2 months) [45]. A similar but larger Phase III placebo-controlled trial confirmed the

superior initial response rate in comparison to AraC monotherapy (CR: 35 vs 19%; p = 0.005), but only a modest survival improvement was noted (median OS: 177 vs 128 days; p = 0.087) [46]. In addition, pulmonary toxicity was problematic in the laromustine/HDAC compared with the HDAC/placebo arm (34 vs 17%; p = 0.006). The development of this agent has been discontinued.

Elacytarabine (CP-4055)

AraC is dependent on the transmembrane hENT1 for effective intracellular uptake. Reduced hENT1 expression and activity is associated with adverse therapeutic outcomes and reduced cytotoxicity for patients treated with AraC [81,82]. Elacytarabine, the lipophilic 5′-elaidic acid ester of AraC, potentially circumvents this resistance mechanism by entering cells independently of hENT1 [51], as well as exhibiting prolonged intracellular distribution and inhibiting DNA synthesis for twice the duration of AraC [83]. Modest responses were seen (ORR: 34%) in relapsed–refractory patients in two Phase II single-agent nonrandomized studies of elacytarabine [51,52], although correlative hENT1 expression analysis was not undertaken to substratify those patients most likely benefit in either study. In a similar study using elacytarabine in combination with idarubicin, however, hENT1 expression did not predict elacytarabine activity [53]. The 30-day mortality with elacytarabine was deemed significantly better than that of a historical control population (13 vs 25%) [51]. Recent Phase III data from 381 relapsed–refractory AML patients randomized between single-agent elacytarabine and an investigator's choice (ranging from supportive care to HDAC) disappointingly showed no difference in OS (3.5 vs 3.3 months), ORR (23 vs 21%) or relapse free survival (RFS) (5.1 vs 3.7 months) [50], and following these results, the clinical development of elacytarabine has been discontinued.

Clofarabine

Clinical applications of the purine analogs fludarabine and chlorodeoxyadenosine have been partly hindered by dose-limiting extramedullary toxicities, notably renal and neurological toxicities [84]. Clofarabine was developed in an attempt to reduce toxicity and combine the contrasting mechanisms by which fludarabine and chlorodeoxyadenosine inhibit DNA synthesis [85]. Predominantly in older patients, clofarabine demonstrated some additive but little single-use benefit. The UK NCRI AML14 trial randomized 404 elderly patients to receive either low-dose AraC (LDAC) or clofarabine resulting in superior response rates (ORR: 38 vs 19%) that did not equate to improved OS after two years of follow-up (12 vs 13%) [49]. In 57 treatment-naive younger patients (aged < 60 years) treated with clofarabine in combination with idarubicin and AraC as induction–consolidation treatment, an ORR of 79% was achieved [48], but with a median follow-up of only 10.9 months, meaningful survival conclusions are limited. The published results of randomized trials of clofarabine used in combination with daunorubicin in newly diagnosed older patients (MRC AML16) and in high-risk or treatment-refractory younger patients (AML17) are currently awaited.

Vosaroxin (SNS-595)

Vosaroxin is a first-in-class cytotoxic quinolone derivative that intercalates in DNA and inhibits topoisomerase II [86]. Favorable pharmacological properties and the potential to overcome resistance mechanisms make it a promising agent when compared with other topoisomerase II inhibitors (e.g., anthracyclines, epipodophyllotoxins [etoposide and teniposide] and the anthracenediones [mitoxantrone]). Phase II data [54] from 113 older patients (aged >60 years) deemed unsuitable for intensive chemotherapy established that a dosing schedule of 72 mg/m^2 administered on days one and four of the first week of each three-weekly cycle had the best safety profile and outcomes (CR/CR with incomplete platelet count recovery [CRp]: 35%; median survival: 7.7 months; 1-year survival: 38%; 30-day mortality: 7%) [87]. The results of a multinational Phase III RCT (VALOR) of vosaroxin in combination with AraC or placebo, which closed to recruitment in September 2013, are anticipated soon and are likely to determine the future prospects of this agent.

CPX-351

CPX-351 aims to optimize the combined delivery of DA using a 5:1 molar ratio of AraC to daunorubicin within a liposomal carrier [88]. An initial study proved the tolerability and safety of CPX-351 with no significant prolongation of cytopenia despite its extended half-life [89]. A Phase II trial randomized 126 elderly treatment-naive patients between standard DA chemotherapy and an equivalent dose of CPX-351 [44]. Although only trends favoring CPX-351 in both ORR (p = 0.07) and OS (p = 0.61) were observed overall, patients with secondary AML (n = 52) showed a significant improvement in survival (12.1 vs 6.1 months; p = 0.01). Caution should be exercised in interpreting these results, as high-risk patients did not see an overall significant improvement in survival. CPX-351 does appear to show genuine promise, however, and a larger ongoing Phase III trial (NCT01696084) comparing CPX-351 with DA chemotherapy in *de novo* AML patients (aged 60–75 years) may help establish it as a competitor to DA, the current gold standard.

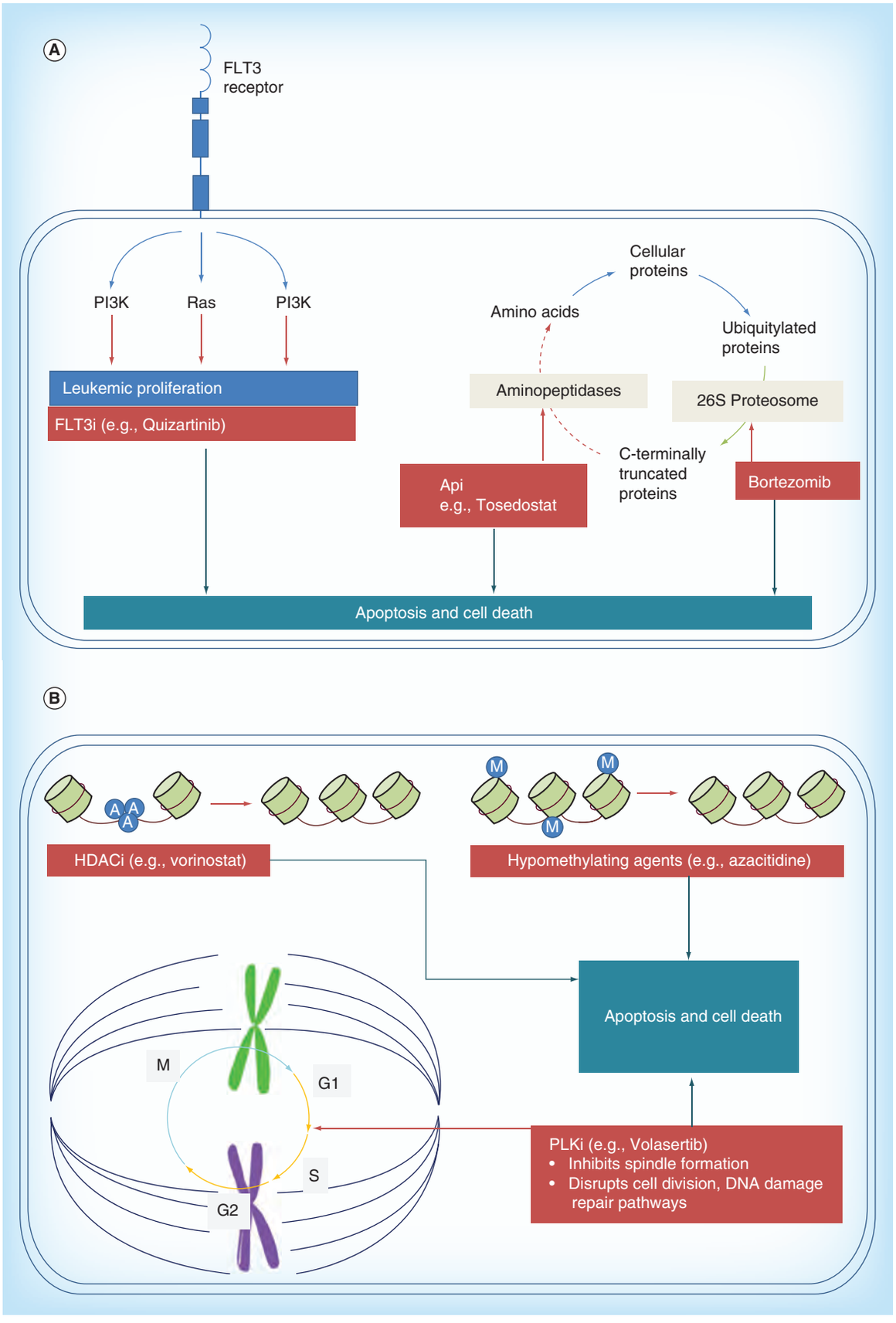
A
FLT3 receptor
PI3K
Ras
PI3K
Leukemic proliferation
FLT3i (e.g., Quizartinib)
Cellular proteins
Amino acids
Ubiquitylated proteins
Aminopeptidases
26S Proteosome
C-terminally truncated proteins
Api e.g., Tosedostat
Bortezomib
Apoptosis and cell death
B
HDACi (e.g., vorinostat)
Hypomethylating agents (e.g., azacitidine)
Apoptosis and cell death
M
G1
S
G2
PLKi (e.g., Volasertib)
• Inhibits spindle formation
• Disrupts cell division, DNA damage repair pathways

Figure 1. Mechanism of action of selected novel agents in acute myeloid leukemia (facing page). (A) Cellular and **(B)** nuclear mechanisms of action of selected novel agents in acute myeloid leukemia.
Api: Aminopeptidase inhibor; FLT3i: FLT3 inhibitor; HDACi: Histone deacetylase inhibitor; PLKi: Polo-like kinase inhibitor.
Adapted partly from [10] © American Society of Clinical Oncology (2010).

Novel molecular agents

Gemtuzumab ozogamicin & other immunotherapies

CD33 is expressed on 85–90% of AML blasts as well as on multipotent myeloid precursors, monocytes and neutrophils, but is absent from pluripotent hematopoietic stem cells [90]. Gemtuzumab ozogamicin (GO), a monocloncal anti-CD33 antibody conjugated to the cytotoxic agent calicheamicin [91], aims to exploit this novel target. GO was withdrawn from the US market by the US FDA in June 2010 based on results from a Phase III Southwest Oncology Group (SWOG) RCT [92] in which the addition of GO to DA in the induction–consolidation setting (treatment-naive) resulted in no difference in efficacy but suggested an increase in early mortality.

Results from a recently published meta-analysis [93] of five RCTs [94–98] question the conclusions drawn from the SWOG study. Early mortality from patients seen in the GO arm (6%) of the SWOG study was consistent with that seen in the other studies [95], but there was unexpectedly low mortality in the SWOG control group (1%). In addition, patients in the SWOG study GO group received a 25% daunorubicin dose reduction. The meta-analysis analyzed a total of 3325 patients with unpublished data supplemented separately from three RCTs [94–95,99]. Significant benefit was seen in disease relapse risk (odds ratio [OR]: 0.81; 95% CI: 0.73–0.90; p = 0.0001) with the addition of GO to induction chemotherapy, which was postulated to be as a result of the greater depth of response gained with GO [100]. Importantly, OS was very significantly improved (OR: 0.90; 95% CI: 0.82–0.98; p = 0.01) in those patients who received GO with induction chemotherapy with favorable-risk (OR: 0.47; 95% CI: 0.31–0.73; p = 0.0006) and intermediate-risk (OR: 0.84; 95% CI: 0.75–0.95; p = 0.005) cytogenetic features. Where a fractionated GO dose of up to 5 mg [98] or single doses of <6 mg/m^2 were used [94,99], no differences in early death were observed, suggesting acceptable safety. These pooled data suggest that the abandonment of GO in AML was premature, with demonstration of benefit being most clearly seen in patients with favorable cytogenetic risk profiles. Further studies are now needed in order to better define the optimal dosing strategy. The planned NCRI AML18 trial will compare fractionated and single-dose strategies in older patients.

Despite evidence of benefit with GO, its target, CD33, is often poorly expressed on less differentiated leukemic cells. An increasing body of evidence suggests a hierarchical cellular organization in AML, initiated and maintained by self-renewing leukemic stem cells [101]. Data from animal models have demonstrated promising efficacy using leukemic stem cell monoclonal antibody targets that include CD44 [102] and CD47 [103]. Data featuring the anti-CD123 agent CSL360 in animal models [104] and human subjects in a Phase I study have also been published [105].

Mobilization of AML blasts from the bone marrow stromal environment may also be important in increasing sensitivity to chemotherapy. Approximately 50% of AML blasts express CXCR4, a chemokine that binds to CXCL12 produced by bone marrow stromal cells [106]. Plerixafor, which inhibits the CXCR4–CXCL12 interaction, has been developed principally as a hemopoeitic stem mobilization agent, with Phase I data indicating therapeutic promise in AML, with a twofold mobilization of leukemic blasts into the peripheral blood being demonstrated, along with an ORR (CR/CR with incomplete blood count recovery [CRi]) of 46% [107].

A number of other promising novel immunomodulatory-based therapeutic approaches are currently in relatively early stages of preclinical and clinical development and an exhaustive review is beyond the scope of this article.

FLT3 inhibitors

The possibilities of tyrosine kinase (TK) inhibition were highlighted by the huge success of imatininb in the treatment of chronic myeloid leukemia. This breakthrough emphasized the importance of TK dysregulation in cancer pathogenesis, providing a potentially important therapeutic angle for other hematological malignancies. The example of chronic myeloid leukemia is an extreme one in which the Philidelphia chromosome represents a single and consistent pathogenetic abnormality. The same is not true of most other malignancies and especially of AML, in which there is an especially high level of molecular heterogeneity between individual cases.

Along with other growth factors, the FLT3 plays a central role in normal hematopoiesis and cellular growth in primitive hematopoietic stem and progenitor cells [108,109]. Expression of FLT3 is subsequently lost with cellular maturity [109]. Constitutively activating FLT3 internal tandem mutations (FLT3-ITDs) are seen in approximately 25% of newly diagnosed AML cases and confer a poor prognosis in terms

Table 2. Selected studies of novel chemotherapeutic agents in acute myeloid leukemia.

Agent	Study design	Patients (n)	Patient population	Treatment groups	Results	Ref.
CPX-351	Phase II, randomized	127	Age 60–75 years; treatment-naive	A: CPX-351 (days 1/3/5)	A: ORR: 66.7%; OS: 14.7 months	[44]
				B: AraC 100 mg/m^2 (days 1–7) plus DAU 40–60 mg/m^2 (days 1–3)	B: ORR: 51.2%; OS: 12.9 months	
Larmoustine	Phase II, nonrandomized	85	Age >60 years; treatment-naive; high-risk	LAR 600 mg/m^2 (day 1) for one or two cycles, then AraC 400 mg/m^2/day (day1–5)	ORR: 32%; OS: 3.2 months	[45]
	Phase III, randomized (2:1), placebo-controlled		Treatment-naive	A: LAR 600 mg/m^2 (day 2) plus AraC 1.5 g/m^2/day (day 1–3)	A: CR: 35%; OS: 177 days	[46]
				B: placebo (day 2) plus AraC 1.5 g/m^2/day (day 1–3)	B: CR: 19%; OS: 128 days	
Clofarabine	Phase II, nonrandomized	21	Age >60 years; treatment-naive	CLO 20 mg/m^2 (day 1–5) plus DAU 50 mg/m^2 (days 1/3/5)	ORR: 38.1%; OS: 11.2 months	[47]
	Phase II, nonrandomized	57	Age 18–60 years, treatment-naive	CLO 22.5 mg/m^2 (days 1–5) plus IDA 6 mg/m^2 (days 1–3) plus AraC 750 mg/m^2 (days 1–5)	ORR: 79%; OS not reached	[48]
	Phase II, randomized	404	Treatment-naive, unfit for intensive treatment	A: CLO 20 mg/m^2/day (day 1–5)	A: ORR: 38%; 2-year OS: 13%	[49]
				B: AraC 20 mg/12 h (days 1–10)	B: ORR: 19%; 2-year OS: 12%	
Elacytarabine	Phase III, randomized	381	Relapsed–refractory disease	A: ELA 2000 mg/m^2 (days 1–5)	A: ORR: 23%; OS: 3.5 months	[50]
				B: investigator's choice	B: ORR: 21%; OS: 3.3 months	
	Phase II, nonrandomized	61	Age >18 years; failed two or more therapies	ELA 2000 mg/m^2 (days 1–5)	ORR: 34%; OS: 10.9 months	[51]
	Phase II, nonrandomized	43	Relapsed–refractory disease	ELA 2000 mg/m^2 (days 1–5)	ORR: 37.2%; OS: 4.7 months	[52]
	Phase II, nonrandomized	51	Persistent blasts postinduction	ELA 1000 mg/m^2 (days 1–5) plus IDA 12 mg/m^2 (days 1–3)	ORR: 41%	[53]
Vosaroxin	Phase II, single-agent, randomized	113	Age >60 years; treatment-naive, high-risk disease	A: VOR 72 mg/m^2 (days 1/8/15)	A: ORR: 41%; OS: 8.7 months	[54]
				B: VOR 72 mg/m^2 (days 1/8)	B: ORR: 29%; OS: 5.8 months	
				C: VOR 72 mg/m^2 (days 1/4)	C: ORR: 38%; OS: 7.3 months	
				D: VOR 90 mg/m^2 (days 1/4)	D: ORR: 25%	
	Phase Ib/II, randomized	69	Relapsed–refractory disease	VOR 80–90 mg/m^2 (days 1–4) plus: A: AraC (400 mg/m^2/24 h) or B: AraC (1 g/m^2/2 h)	A + B: ORR: 29%; OS: 6.9 months	[55]

AraC: Cytarabine; CLO: Clofarabine; CR: Complete response; CRi-CR: with incomplete blood count recovery; DAU: Daunorubicin, ELA: Elacytarabine; IDA: Idarubicin; LAR: Larmoustine; LDAC: Low dose cytarabine; ORR: Overall response rate; OS: Overall survival; VOR: Vosaroxin.

of increased relapse risk and an association with increased proliferation of myeloid precursors. Mutations of the activation loop of the FLT3 TK domain (FLT3-TKD mutations) are seen in a further 8–10% of AML patients, but their prognostic implications are less well established [110].

A number of FLT3 TK inhibitors have been the subject of extensive preclinical and clinical evaluation. Depending on the relative activity against the FLT3-ITD receptor over the wild-type (WT) and other TK receptors (e.g. ,c-kit and Ras, among others), these agents can be broadly classified as highly selective (e.g., quizartinib and sorafenib), intermediate (e.g., sunitinib and KW-2449) and less selective (e.g., lestaurtinib and midostaurin) [111].

Clinical trial data suggest that nonselective 'first-generation' FLT3 inhibitors, notably midostaurin (PKC412) and lestaurtinib (CEP701), exhibit only transient clinical activity that is generally limited to the clearance of peripheral blood blasts despite relatively good tolerability. Midostaurin was examined in a Phase IIb study of 95 relapsed–refractory patients with AML (89%) and a small proportion with high-risk myelodysplastic syndrome (MDS) [59]. Although no excess toxicities were noted, clinical activity was limited in depth, with transient >50% reductions in bone marrow blasts or hematological improvement seen in 70% of FLT3-mutated and 42% of FLT3-WT patients. Results are expected soon from an international Phase III RCT (RATIFY) comparing midostaurin and placebo given with standard induction (DA) and consolidation (HDAC) chemotherapy and as subsequent maintenance therapy in younger, treatment-naive patients with FLT3-mutated AML. Comparable outcomes were demonstrated in a Phase II study of lestaurtinib in 29 treatment-naive older patients deemed unfit for intensive chemotherapy, of whom only a minority had a FLT3 mutation (17%) [56]. Using a dose of 60 mg twice daily, a transient lowering of peripheral blood and bone marrow blast counts and transfusion independence was gained in three patients with a FLT3 mutation (60%) and five patients with FLT-WT status (23%). Crucially, sustained FLT3 inhibition was demonstrated to be necessary for a clinical response. The wider off-target FLT3 inhibitory activity of midostaurin and lestaurtinib is compounded by unfavorable pharmacodynamic properties resulting from high protein binding. There may, however, still be a future clinical role for drugs that inhibit multiple kinase targets, particularly in the setting of newly diagnosed FLT3-mutated AML, in which there is less addiction to FLT3 signaling and simultaneous suppression of other pathways may be beneficial [112].

Sorafenib (BAY 43-9006) is a multikinase inhibitor that is currently approved for patients with hepatocellular and renal cell carcinoma [113]. Modest clinical activity was demonstrated in a Phase II study of 52 older (aged >60 years) treatment-naive patients, all of whom were FLT3-ITD/TKD positive [62]. Sorafenib (400 mg/12 h for 28 days) was administered with standard induction–remission chemotherapy with DA and intermediate-dose AraC. Thereafter, consolidation with either allo-SCT or maintenance sorafenib was given. No unexpected toxicities were experienced with four patients dying within 30 days of starting the treatment. The overall CR rate was 61%, but with only a median follow-up of 4.5 months, further follow-up data are required for a full evaluation of survival. A similar study of 51 younger patients (aged < 60 years) with relapsed–refractory disease showed similar results, with better outcomes noted in FLT3-ITD (CR: 92%) and FLT3-TKD (CR: 100%) compared with FLT3-WT cases (CR: 66%) [60]. Sorafenib in the relapsed–refractory setting appears to be active, although this has not been examined formally. Recent published results from a compassionate-use program included clinical responses to sorafenib monotherapy in six relapsed–refractory AML patients, including three CRs, with two patients going on to allo-SCT [114]. Based on the currently available data, off-label use of sorafenib 400 mg twice daily can certainly now be considered as a valid treatment strategy in relapsed–refractory FLT3-mutated disease if recruitment to a suitable clinical trial is not possible [115].

Quizartinib (AC220) displays more highly FLT3-selective kinase inhibition with minor targets that include KIT, CSF1R, RET and PDGFR [116]. Of the FLT3 inhibitors that have been developed, quizartinib has shown the most promise, especially in the setting of monotherapy. At a dose of 90–135 mg/day, one study of 137 older (aged >60 years) relapsed–efractory patients showed beneficial activity of quizartinib independent of FLT3 status (CR: 33–44%) [63]. Concerns regarding quizartinib-induced QT-interval prolongation have generally led to little symptomatic significance, although ECG monitoring is still advised. The NCRI AML18 pilot study confirmed an acceptable safety profile of quizartinib when used at a dose of 40 mg/day for 14 days in combination with induction–consolidation chemotherapy (ADE and DA) in older newly diagnosed patients [117], and the AML18 study proper will assess the clinical efficacy of quizartinib in combination with chemotherapy and subsequently given as maintenance. Quizartinib is also the subject of an ongoing RCT in relapsed FLT3-driven disease in which patients are randomized between quizartinib monotherapy and a choice of salvage chemotherapy regimens (AC220-007 study).

Table 3. Selected studies of novel nonchemotherapeutic agents in acute myeloid leukemia.

Agent	Study design	Patients (n)	Patient population	Treatment groups	Results	Ref.
Lestaurtinib	Phase II, nonrandomized	29	Treatment-naive, unfit for intensive treatment	LES 40–80 mg/12 h	ORR: 0%; BR: 19%; HemR: 11%	[56]
	Phase I/II, nonrandomized	14	Relapsed–refractory disease, FLT3-positive	LES 40–80 mg/12 h	PB/BM response < 5%: 36%	[57]
Midostaurin	Phase II, nonrandomized	20	Relapsed–refractory AML/MDS, FLT3-positive	MID 75 mg/8 h	PB blast count reduction >50%: 70% BM blast count reduction >50%: 70%	[58]
	Phase II, randomized	95	Relapsed–refractory or unfit for intensive treatment with AML/MDS	A: MID 50 mg/12 h	A: ORR: 67% (wild-type); 67% (mutant)	[59]
				B: MID 100 mg/12 h	B: ORR: 44% (wild-type); 76%(mutant) A + B OS: 130 days	
Sorafenib	Phase I/II, nonrandomized	51	Relapsed–refractory disease	Induction: SOR 400 mg/12 h (days 1–28) plus AraC 1.5 g/m^2 (days 1–3 or 4)	CR (overall): 78%	[60]
				Consolidation: SOR 400 mg/12 h (days 1–28) plus AraC 0.75 g/m^2 (days 1–3) plus IDA 8 mg/m^2 (days 1–2)	CR (FLT3-ITD): 92% CR (FLT3-TKD): 100% CR (FLT3-wild-type): 66%	
	Phase I/II, nonrandomized	37	Relapsed–refractory disease	SOR 400 mg/12 h (days 1–28) plus AZA 75 mg/m^2 (days 1–7)	CR: 27%; CRi: 16%; PR: 3%	[61]
	Phase II, nonrandomized	52	Age >60 years, treatment-naive, FLT3-ITD- and FLT3-TKD-positive	Induction: SOR 400 mg/12 h (days 1–7) plus AraC 100 mg/m^2 (days 1–7) plus DAU 60 mg/m^2 (days 1–3) Consolidation: A: allo-SCT B: SOR 400 mg/12 h (days 1–28) plus AraC 2 g/m^2 (days 1–5)	CR/CRi: 69%; CRi: 8%	[62]†
Quizartinib	Phase II, nonrandomized	137	Age >60 years, relapsed–refractory disease	QUI 90 mg/day (female), 135 mg/day (male; days 1–28)	FLT3$^+$ CR: 44%; OS: 23.1 weeks FLT3$^-$ CR: 34%; OS: 25.6 weeks	[63]†

†Published in abstract form.
AraC: Cytarabine; AZA: Azacitidine; BM: Bone marrow; BOR: Bortezomib; BR: Bone marrow response; CR: Complete response; CRi-CR: with incomplete blood count recovery; CRp: CR with incomplete platelet count recovery; DAU: Daunorubicin; DEC: Decitabine; HaemR: Haematological response, HMA: Hypomethylating agent; IDA: Idarubicin; LDAC: Low dose cytarabine; LEN: Lenalidomide; LES: Lestaurtinib; MID: Midostaurin; ORR: Overall response rate; OS: Overall survival; PB: Peripheral blood; PR: Partial response; TIP: Tipifarnib; TST: Tosedostat; VOL: Volasertib; VSTAT: Vorinostat; QUI: Quizartinib.

Table 3. Selected studies of novel nonchemotherapeutic agents in acute myeloid leukemia (cont.).

Agent	Study design	Patients (n)	Patient population	Treatment groups	Results	Ref.
SGI-110	Phase II, randomized	67	Relapsed–refractory or unfit for intensive treatment with AML/MDS	A: SGI-110 60 mg/m^2 B: SGI-110 90 mg/m^2	ORR: 25% Treatment-naive (n = 17) CR: 53% Relapsed–refractory (n = 50) CR: 16%	[64]†
Volasertib	Phase I/II, randomized	87	Unfit for intensive treatment with AML	A: VOL 350 mg/h (days 1/15) plus LDAC 20 mg/12 h (days 1–10) B: LDAC 20 mg/12 h	A: CR/CRi: 31% B: CR/CRi: 11%	[65]†
Decitabine	Phase III	485	Age >65 years, treatment-naive	A: DEC 20 mg/m^2 (days 1–5) B: supportive care/LDAC 20 mg/m^2 (days 1–10)	A: CR/CRp: 17.8%; OS: 7.7 months B: CR/CRp: 7.8%; OS: 5.0 months	[66]
	Phase I	30	Age < 60 years, treatment-naive	DEC 60–140 mg/m^2 (total daily dose) plus AraC 100 mg/m^2 (days 1–7) plus DAU 60 mg/m^2 (days 1–3)	CR : 57% PR : 33%	[67]
Decitabine/ Tosedostat	Phase II, nonrandomized	26	Age >60 years, treatment-naive, AML/high-risk MDS	TST 120 mg/day (days 1–21) plus: A: AraC 1 g/m^2 (days 1–5) or B: Dec 20 mg/m^2 (days 1–5)	Overall CR/CRi: 54%	[68]†
Vorinostat	Phase II, nonrandomized	39	AML/high-risk MDS	VSTAT 500 mg/8 h (days 1–3) plus IDA 12 mg/m^2 (days 4–6) plus AraC 1.5 g/m^2 iv. (days 4–7). Consolidation and maintenance (single-agent VSTAT)	Treatment-naive (n = 26) ORR: 88%; OS: 21.7 months Relapsed–refractory (n = 13) ORR: 30%; OS: 4.9 months	[69]†
	Phase II, randomized	37	Relapsed AML or treatment-naive (high risk factor and unsuitable for intensive treatment)	A: VSTAT 400 mg/24 h (days 1–21) B: VSTAT 200 mg/8 h (days 1–14)	A: CR: 0% B: CR: 4.5%	[70]
	Phase II, nonrandomized	75	Treatment-naive AML/high-risk MDS	VSTAT 500 mg/8 h (days 1–3) plus IDA 12 mg/m^2 (days 4–6) plus AraC 1.5 g/m^2 iv. (days 4–7). Consolidation and maintenance (single-agent VSTAT)	ORR: 85%; OS: 82 weeks	[71]

†Published in abstract form.
AraC: Cytarabine; AZA: Azacitidine; BM: Bone marrow; BOR: Bortezomib; BR: Bone marrow response; CR: Complete response; CRi-CR: with incomplete blood count recovery; CRp: CR with incomplete platelet count recovery; DAU: Daunorubicin; DEC: Decitabine; HaemR: Haematological response, HMA: Hypomethylating agent; IDA: Idarubicin; LDAC: Low dose cytarabine; LEN: Lenalidomide; LES: Lestaurtinib; MID: Midostaurin; ORR: Overall response rate; OS: Overall survival; PB: Peripheral blood; PR: Partial response; TIP: Tipifarnib; TST: Tosedostat; VOL: Volasertib; VSTAT: Vorinostat; QUI: Quizartinib.

Table 3. Selected studies of novel nonchemotherapeutic agents in acute myeloid leukemia (cont.).

Agent	Study design	Patients (n)	Patient population	Treatment groups	Results	Ref.
Tosedostat	Phase I/II, nonrandomized	18	Age >60 years, relapsed–refractory AML/high-risk MDS	TST 120–180 mg/day plus: A: AZA 50 mg/m^2 (days 1–7) or B: LDAC 7.5 mg/m^2 (days 1–10)	ORR: 33%; OS: 3.1 months	[72]†
	Phase II, randomized	73	Age >60 years, relapsed–refractory disease	A: TST 120 mg/day for 6 months (n = 38) B: TST 240 mg/day for 2 months then TST 120 mg/day for 4 months (n = 35)	A: CR/CRi: 5%; OS: 175.5 days B: CR/CRi: 14%; OS: 88 days	[73]
Tipifarnib	Phase III, randomized	457	Age >70 years, treatment-naive	A: TIP 600 mg/12 h (days 1–21) B: best supportive care	A: ORR: 11%; B. ORR: < 1%; OS HR: 1.02 (p = 0.843)	[74]
	Phase II, nonrandomized	252	Relapsed–refractory AML	TIP 600 mg/12 h (days 1–21)	CR: 4%	[75]
Lenalidomide	Phase II, nonrandomized	33	Age >60 years, treatment-naive	LEN 50 mg/day (day 1–28) for two cycles, then LEN 10 mg/day	CR: 30%; CRi: 53%; OS: 4 months	[76]
Bortezomib	Phase II, nonrandomized	95	Age 60–75 years, treatment-naive	BOR 1.3 mg/m^2 (days 1/4/8/11) plus AraC 100 mg/m^2 (days 1–7) plus DAU 60 mg/m^2 (days 1–3)	CR: 65%; CRp: 4%	[77]

†Published in abstract form.
AraC: Cytarabine; AZA: Azacitidine; BM: Bone marrow; BOR: Bortezomib; BR: Bone marrow response; CR: Complete response; CRi-CR: with incomplete blood count recovery; CRp: CR with incomplete platelet count recovery; DAU: Daunorubicin; DEC: Decitabine; HaemR: Haematological response, HMA: Hypomethylating agent; IDA: Idarubicin; LDAC: Low dose cytarabine; LEN: Lenalidomide; LES: Lestaurtinib; MID: Midostaurin; ORR: Overall response rate; OS: Overall survival; PB: Peripheral blood; PR: Partial response; TIP: Tipifarnib; TST: Tosedostat; VOL: Volasertib; VSTAT: Vorinostat; QUI: Quizartinib.

Epigenetic modifiers: hypomethylating agents & histone deacytylase inhibitors

Altered gene expression independent of changes in gene sequence is a key driving factor in AML. Aberrant DNA hypermethylation and/or histone deacetylation are important epigenetic mechanisms that have been demonstrated to play a part in the silencing of key genes critical to growth, differentiation, angiogenesis, signaling and DNA repair [118–120].

A significant proportion of patients with AML are deemed unsuitable for intensive treatment due to confounding comorbities, poor performance status and extreme age. Many of these patients will also exhibit a poor cytogenetic profile. The aim for these patients is therefore to optimize efficacy without increasing treatment-related toxicity and mortality. The 'standard of care' for low-intensity induction over the last several decades has been LDAC, which resulted in a CR rate of 18% and improved OS when compared with supportive care and hydroxycarbamide in a RCT [121]. LDAC is, however, almost never effective in patients with unfavorable cytogenetics [6]. Attempts to improve outcomes have gathered pace with the hypomethylating agents 5-azacitidine (AZA) and, to a lesser extent, decitabine appearing to challenge LDAC as the standard of care.

The Phase III AZA-001 study compared AZA (75 mg/m^2 for 7 days every 28 days) with the best available therapy in patients with high-risk MDS [122]. In an unplanned subgroup analysis of 113 patients with 20–30% bone marrow blasts, a 18% CR rate with a survival benefit in favor of AZA was seen (24.5 vs 16 months; $p < 0.005$), including a higher 2-year survival rate (38 vs 0%; $p < 0.01$) in patients with adverse cytogenetics. However, 'best available therapy' included supportive care measures, LDAC and conventional chemotherapy, and hence caution should be exercised when interpreting these results. Similar results were reproduced in the CALGB 9221 study [123]. Whether the blast percentage prior to AZA therapy is more important than other prognostic factors is unknown. An Italian study analyzed 82 patients who received AZA enrolled on a compassionate-use program [124]. Independent factors determining overall response on multivariate analysis were white cell count (>10 × 10^9/l: 10 vs < 10 × 10^9/l: 45%; p = 0.008) and prior treatment (yes: 19%, no: 48%; p = 0.04). Bone marrow blast count was not correlated with response.

In 55 older treatment-naive patients deemed unfit for intensive treatment, decitabine (135 mg/m^2/72 h every 6 weeks) showed an ORR of 26% and a median OS of 5.5 months [125]. A similar investigation of 154 older patients using a 10-day decitabine schedule (20 mg/m^2) reported a CR rate of 40% and a comparable median OS of 6 months in a relapsed–refractory cohort, which improved to 11 months (n = 102) in treatment-naive patients (n = 52) [126]. A randomized Phase III trial compared decitabine (20 mg/m^2 over five days, repeated every four weeks) and best available therapy (supportive care or LDAC 20 mg/m^2 for 10 days) in 485 older patients (aged >65 years) [66]. Results were modest compared with those seen with AZA, with a nonsignificant increase in OS (7.7 vs 5.0 months; p = 0.108), but with a significant increase in CR/CRp (17.8 vs 7.8%; p = 0.001). In contrast to the AZA-001 study, a subgroup analysis identified better outcomes in patients with >50% bone marrow blasts (hazard ratio: 1.355; p = 0.0045) [127]. Attempts have also been made to combine decitabine with standard induction therapy. In a Phase I study of 30 treatment-naive younger patients (aged < 60 years), decitabine was administered in increasing cumulative doses (60–140 mg/m^2) with DA induction [67]. An overall CR rate of 83% was observed, although increased grade >3 gastrointestinal toxicity was noted. Greater hypomethylation was noted in patients receiving decitabine in a pulsed regimen rather than via continuous infusion, although the degree of hypomethylation did not accurately predict clinical response.

Preclinical studies using the histone deacetylase inhibitor vorinostat demonstrated DNA damage and cell cycle arrest leading to apoptosis [128] as important antileukemic mechanisms of action. Vorinostat is the furthest clinically developed histone deacetylase inhibitor and is currently licensed for the treatment of relapsed–refractory cutaneous T-cell lymphoma.

In a Phase II trial of vorinostat in 37 patients with relapsed or treatment-naive high-risk AML, due to a lack of response or physician preference, only 11 patients received more than two cycles [70],. Vorinostat (500 mg/8 h) was combined with idarubicin a Phase II study of 39 patients with FLT3-ITD AML [69]. Treatment-naive patients (n = 26) had superior outcomes to those with relapsed–refractory disease (n = 13; ORR: 88%, OS: 21.7 months vs ORR: 30%, OS: 4.9 months). Using the same regimen, 75 untreated patients with AML and high-risk MDS attained similar results [71].

Early data from a study examining vorinostat (200 mg/8 h) in combination with AZA (75 mg/m^2) in AML patients with poor baseline characteristics (ECOG performance status >2 and renal/hepatic impairment, among other factors) have been more promising [129]. Although the study is ongoing, of the 17 evaluable patients, an ORR of 41% was gained, with only one induction death (5%). Recruitment is also ongoing in a randomized Phase II study (RAvVA;

NCT01617226) comparing AZA alone with AZA in combination with vorinostat. These studies may support the combined use of epigenetic modifiers with possible synergy between hypomethylating agents and histone deacetylase inhibitors being possible.

Other histone deacetylase inhibitors (e.g., romidepsin, pracinostat and panabinostat) remain in early development. Overall, clinical progress with histone deacetylase inhibitors has been hindered by systemic side effects, most notably gastrointestinal toxicities and asthenia.

Farnesyltransferase inhibitors

The *ras* group of proto-oncogenes encodes a number of membrane-associated G-proteins that are central to the survival of haemopoeitic cells via the activation of Raf, MEK-1 and ERKs [130]. Inhibition of the enzyme farnesyltransferase, which facilitates membrane ras attachment by the addition of a 15-carbon farnesyl group to ras, would in theory restrain the transduction of proliferative signals [131], making farnesyltransferase inhibition an attractive therapeutic option, especially in the 15–25% of AML patients who have mutations or abnormal expression of *ras* [132]. Translational work in the clinical setting using the farnesyltransferase inhibitor tipifarnib has, however, failed to deliver meaningful results, at least when used as a monotherapy. A Phase II trial of tipifarnib 600 mg twice daily in 252 patients with relapsed–refractory AML demonstrated a CR in only 11 patients (4%) and a CRp in two patients (0.8%) [75]. Results from a larger Phase III RCT comparing tpifarnib with best supportive care (including hydroxycarbamide) at the same dose in 457 elderly patients (aged >70 years) yielded a similar CR rate (8%) and no improvement in OS (hazard ratio: 1.02; p = 0.843) [74]. The NCRI AML16 tipifarnib and LDAC trial arm was prematurely closed after recruiting 45 older patients (age range: 62–86 years), citing no effect on response, toxicity or survival [133]. Whether further farnesyltransferase inhibitors will emerge in light of such results is unclear.

Polo-like kinase 1 inhibitors

Polo-like kinases are a distinct group of enzymes involved in a number of regulatory cell cycle processes: Polo-like kinase 1 (PLK1) is a key player in the cell cycle at the G_2/M transition [134] and in mitosis, while PLK2 and PLK3 regulate the G_1 and early S phases [135]. In many neoplasms, however, downregulation of PLK3 [136] and possible tumor suppression by epigenetic inactivation of PLK2 [137] suggest a complex set of independent and opposed actions. In comparison to normal healthy $CD34^+$ and peripheral blood leukocyte cells, overexpression of PLK1 has been demonstrated in AML cell lines [138], although the exact pathological role of PLK1 in AML remains unknown. In one Phase I/II RCT, 87 patients deemed unfit for intensive treatment received LDAC with or without the PLK1 inhibitor volasertib (BI 6727) at a dose of 350 mg over a 1-h infusion on days 1 and 15 of each cycle [65]. The addition of volasertib to LDAC significantly improved CR/CRi rates (31.0 vs 11.1%; p = 0.0277). An increase in grade ≥3 gastrointestinal and infection adverse events (95.2 vs 68.9%) was not reflected in a rise in overall mortality. Further clinical investigation of volasertib is currently ongoing, while other PLK1 inhibitors have yet to come to clinical development.

Aminopeptidase inhibitors

Numerous cellular processes are influenced by the cleavage of amino acid terminal residues from signaling proteins, a process catalyzed by aminopeptidases. Tosedostat is a first-in-class aminopeptidase inhibitor, with preclinical data suggesting its antiproliferative action to be derived from the blockage of protein recycling, a process that shows synergy with bortezomib, AraC and all-*trans*-retinoic acid in AML proliferation assays [139]. A small study examined the combination of tosedostat (120 mg/day) with AraC (1 g/m^2) or decitabine (20 mg/m^2) in older (aged >60 years) treatment-naive patients that included those with high-risk MDS [68]. Early results from 26 patients indicated activity with a CR/CRi of 54% and ten (42%) patients removed from the study due to a lack of response or disease progression. Response rates for each group were not specified. Preliminary results from a similar Phase I/II study comparing tosedostat (120–180 mg/day) combined with either AZA (50 mg/m^2) or LDAC (7.5 mg/m^2) were also recently published [72]. With only 18 relapsed–refractory older (aged >60 years) patients, which included those with high-risk MDS, an ORR of 33% was observed across both groups. Cardiac toxicity was evident, which included 50% QTc prolongation (6% grade >3) and one fatal acute coronary event. The small size of both studies limits the strengths of the conclusions regarding tosedostat activity. In OPAL, a larger Phase II RCT, 73 older (aged >60 years) patients, again with relapsed–refractory AML, were randomized to two single-agent tosedostat dosing regimens (120 mg/day for 6 months vs 240 mg/day for 2 months then 120 mg/day for 4 months) [73]. Modest response rates were demonstrated, which were higher in the larger dose (CR/CRi: 14% [240 mg] vs 5% [120 mg]). Toxicity was approximately equal across both groups, with no unexpected adverse events. Results from an extension of the OPAL study (TOPAZ; NCT01180426) are awaited. The clinical development of tosedostat

continues: it is currently being examined in combination with conventional chemotherapy (DA) in first-line therapy (EUCTR2009-014455-68-NL) and in combination with LDAC in the NCRI LI-1 study (ISRCTN-40571019).

Lenalidomide

Lenalidomide is an immunomodulatory analog of thalidomide that influences the cellular and humoral limbs of the immune system, as well as having anti-angiogenic properties [140]. It is currently licensed for the treatment of multiple myeloma and of transfusion-dependent low- or intermediate-risk MDS with isolated chromosome 5q deletion. Using a dose of 10 mg/day, transfusion independence (56%) and complete cytogenetic response (29%) was observed in a large Phase III RCT of patients with isolated 5q- MDS [141]. Common adverse events associated with lenalidomide are myelosupression and venous thromboembolism. Experience of lenalidomide in AML is, however, limited. A small Phase II study demonstrated activity when using a larger dose (50 mg/day) in 33 treatment-naive patients over 60 years of age [76]. Two cycles (28 days) were given, followed by a maintenance dose of 10 mg, resulting in an overall CR/CRi of 30% and an OS of 4 months, which was significantly longer for patients who were able to complete high-dose therapy (11 months). The majority of patients (91%) experienced a grade ≥3 adverse event, with myelosupression and infection being most common. Two UK-based trials – a Phase I study in relapsed patients post-allo-SCT (Viola; ISRCTN-98163167) combining AZA and lenalidomide and the NCRI LI-1 study in *de novo* older patients using lenalidomide with LDAC – are both currently open to recruitment.

Bortezomib

Bortezomib is a reversible proteasome inhibitor that is licensed for the treatment of multiple myeloma and mantle cell lymphoma. Inhibition of NF-κB activity, altered degradation of cell cycle proteins, altered balance of pro- and anti-apoptotic proteins, endoplasmic reticulum stress and inhibition of angiogenesis and DNA repair have all been reported to contribute to the antitumor effect of bortezomib [142]. Although bortezomib is certainly an exciting anticancer drug, experience in AML is limited. In a Phase I study, bortezomib at escalating doses of 0.7–1.5 mg/m^2 was combined with AraC and idarubicin in 31 patients with relapsed–refractory disease with good tolerability and a CR rate of 61% [143]. A recent Phase II study examined bortezomib with DA induction therapy in 95 patients (aged 60–75 years) with treatment-naive AML [77]. A comparable CR rate of 65% with a median follow-up time of 22 months and OS of 17.5 months was achieved. Treatment was again well tolerated, with neuropathy being both minimal and nonsevere.

Table 4. Selected ongoing studies of novel agents in acute myeloid leukemia.

Investigational agent	Regimen	ClinicalTrials.gov identifier	Phase	Patient population	Status
Cabozantinib	Single agent	NCT01961765	I	Relapsed–refractory disease	Recruiting
VOR	AraC/DAU or Ara-C/IDA vs Ara-C/VOR	NCT01802333	III	Age 18–60 years, treatment-naive	Recruiting
VOR	VOR/AraC vs placebo/AraC	NCT01191801	III	First relapse, refractory disease	Recruitment completed
BOR/SOR	ADE vs ADE/bortezomib vs ADE/sorafenib	NCT01371981	III	Age < 29 years, treatment-naive, FLT3-positive	Recruiting
DAP/DEC	SAP/DEC vs DEC	NCT01303796	III	Age >70 years, treatment-naive	Recruiting
VOL	Low-dose Ara-C/VOL vs low-dose AraC/placebo	NCT01721876	III	Age >65 years, treatment-naive	Recruiting
VOX (VALOR trial)	VOX/AraC vs VOX/placebo	NCT01191801	III	Ineligible for intensive treatment	Recruiting
VOR/AraC (RAvVA trial)	AZA/VOR vs AZA	NCT01617226	III	Ineligible for intensive treatment	Recruiting

ADE: Daunorubicin and cytarabine plus etoposide; AraC: Cytarabine; AZA: 5-azacitidine; BOR: Bortezomib; DAU: Daunorubicin; DEC: Decitabine; IDA: Idarubicin; SAP: Sapacitabine; VOL: Volasertib; VOR: Vorinostat; VOX: Voxarosin.

There is a current lack of bortezomib RCT data in AML, although a Phase III RCT (NCT01371981) is currently underway comparing ADE chemotherapy alone and in combination with sorafenib and bortezomib in newly diagnosed AML patients aged less than 29 years.

Conclusion

There remains a desperate need to develop safe and effective treatments for AML in all age groups. Despite intense clinical trial efforts, the '3 + 7' combination of daunorubicin and AraC remains the backbone of AML therapy, although anthracycline dose intensification and particularly the addition of GO to induction regimens for those with favorable- and intermediate-risk disease have recently suggested potential short-term shifts in what is considered the 'standard of care'. Promising results have also been obtained from the addition of cladribine to standard induction and with the novel liposomal agent CPX-351, with both approaches continuing to undergo clinical investigation. For patients deemed unsuitable for intensive therapy, hypomethylating therapies continue to challenge LDAC as the 'standard of care' and continue to be the subject of ongoing randomized evaluations.

Future perspective

Growth in the understanding of AML pathogenesis has provided a wealth of potential molecular therapeutic targets. Translating this knowledge and the ensuing explosion of novel therapeutic compounds into meaningful outcomes for patients will require a concerted, coordinated international effort. Efficient clinical study design is vital to streamlining drug development by identifying promising agents earlier and facilitating their swifter progression to later-phase studies and wider clinical use. Novel trial designs including multifactorial randomization are key to the assessment of the plethora of new agents. The inclusion of translational biological assays and molecular stratification to trial design in order to highlight the patients who are most likely to benefit from specific therapies is also highly pertinent in light of the considerable heterogeneity of this disease and the varied specificities of emerging treatments.

A number of ongoing clinical investigations exploring a wide range of compounds, some of which have progressed to Phase III studies, are currently underway (Table 4). Exploitation of the complex interaction of dysfunctional immunoregulatory processes and the manipulation of T cells and natural killer cells is an area of particular future potential. Technologies such as adoptive T-cell therapy (e.g., chimeric antigen receptor technology), monoclonal antibody targeting of leukemic stem cells (e.g., CD44, CD47 and CD123) and vaccination have so far been limited to early-phase investigation. The incorporation of such novel immunological approaches into established chemotherapy protocols alongside the integration of novel targeted agents will provide fascinating challenges to clinical trialists over the next decade

Executive summary

Induction–consolidation strategies

- The combination of daunorubicin and cytarabine remains the standard of care for induction–consolidation regimens. The addition of gemtuzumab ozogamicin and cladribine, as well as anthracycline dose intensification, hold considerable promise but require further clinical investigation.
- Uncertainly remains concerning the optimal strategies of intensive consolidation therapy using high-dose cytarabine, notably regarding the dose and number of cycles, as well as the role of maintenance and the optimal management of minimal residual disease.

Nonintensive treatment approaches

- Hypomethylating agents, notably azacitidine, are challenging low-dose cytarabine as the historical standard of care in patients who are unfit for intensive therapy.

Novel agents

- Several 'novel' chemotherapeutic agents have shown early promise, but yielded disappointing Phase III trial results in recent years.
- A number of novel nonchemotherapeutic agents have shown promise in early-stage clinical trials, notably the FLT3 inhibitors sorafenib and quizartinib, although large-scale randomized data are limited and their optimal use remains undefined.

Clinical trial development

- Novel trial designs, including stratified molecular signature-driven protocols and international collaborations, will be vital to evaluating the growing number of emerging targets and agents.

Future role of immunotherapies

- Although early in development, immunologically based approaches using a range of monoclonal antibody targets, as well as T-cell and natural killer cell manipulation technologies, carry considerable promise.

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