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REVIEW

The evolving landscape in the therapy of acute myeloid leukemia

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ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of myeloid precursors arrested in their maturation, creating a diverse disease entity with a wide range of responses to historically standard treatment approaches. While significant progress has been made in characterizing and individualizing the disease at diagnosis to optimally inform those affected, progress in treatment to reduce relapse and induce remission has been limited thus far. In addition to a brief summary of the factors that shape prognostication at diagnosis, this review attempts to expand on the current therapies under investigation that have shown promise in treating AML, including hypomethylating agents, gemtuzumab ozogamicin, FLT3 tyrosine kinase inhibitors, antisense oligonucleotides, and other novel therapies, including aurora kinases, mTOR and PI3 kinase inhibitors, PIM kinase inhibitors, HDAC inhibitors, and IDH targeted therapies. With these, and undoubtedly many others in the future, it is the hope that by combining more accurate prognostication with more effective therapies, patients will begin to have a different, and more complete, outlook on their disease that allows for safer and more successful treatment strategies.

KEYWORDS acute myeloid leukemia, hypomethylating, FLT3, gemtuzumab ozogamicin

INTRODUCTION

Acute myeloid leukemia (AML) consists of a group of diverse hematopoietic neoplasms characterized by clonal proliferation of myeloid precursors with a reduced capacity to differentiate into more mature cellular elements. Beyond the heterogeneity of the biology of AML, the response to treatment and overall survival of patients with AML ranges widely but remains poor overall. Certain factors can help shape prognostic risk at diagnosis, including age, performance status, as well as chromosomal and molecular alterations, but the success of conventional chemotherapy has been limited. Although induction chemotherapy leads to a complete remission in the majority of patients, relapse rates are high, with only 40% of patients younger than 60 years, and 10%–20% of older patients, remaining in remission at 5 years after diagnosis (Lowenberg et al., 1996, 1998, 1999). Given the current state of therapeutic options, novel therapeutic approaches are urgently needed and are being actively investigated. Many new treatments in development are targeted at genetic and molecular alterations that are thought to mediate leukemogenesis and/or affect patient prognosis. This review, while aware of the effectiveness of conventional chemotherapy, understands its limitations and seeks to both lay the current state of therapeutic approaches for AML and highlight innovative treatments that will hopefully improve upon current outcomes.

CURRENT APPROACHES AT TREATMENT AND PROGNOSTICATION

During the past few decades, remission induction chemotherapy combining infusional cytarabine and an anthracycline, typically idarubicin or daunorubicin, has become the standard of care for most patients in the US who are not participating in a clinical trial (Yates et al., 1982; Dillman et al., 1991; Rowe and Tallman, 1997). In an attempt to improve the rate of complete remission and decrease relapse, studies have tested alternative and higher doses of agents, as well as novel combinations of conventional chemotherapies (Arlin et al., 1990; Bishop et al., 1990; Berman et al., 1991; Vogler et al., 1992; Wiernik et al., 1992; Bishop et al., 1996; Weick et al., 1996; Hann et al., 1997; Buchner et al., 1999; Estey et al., 2001; List et al., 2001; Lowenberg et al., 2003). However, in the majority of trials thus far, no alternative approach has proved itself definitively better

than cytarabine-anthracycline standard therapy. The challenge then remains in determining the type and extent of consolidative or post-remission therapy as an effort to eliminate minimal residual disease and prevent relapse. The choice of therapy for consolidation is largely influenced by prognosis and risk stratification. Several clinical and biologic characteristics help predict the likelihood of disease-free survival for patients with AML. Age, performance status, cytogenetic and molecular features, history of exposure to cytotoxic agents or radiation therapy, and history of prior myelodysplasia or other hematologic disorders all influence prognosis and risk, and thus direct post-remission therapy (Estey, 2001; Sekeres et al., 2004; Olesen et al., 2005). In the majority of studies, advanced age (often defined as over age 60) correlates with lower rates of achieving complete remission and shorter disease-free survival, with a population characterized by stronger intrinsic resistance and lower tolerance to chemotherapy (Lowenberg et al., 1996; Leith et al., 1997; Lowenberg et al., 1998). This is an important consideration, as the mean age of AML is 66, and the attendant risk is oftentimes, though not always, linked to performance status and the presence of medical co-morbidities and organ dysfunction. A retrospective analysis of newly diagnosed AML reported increasing 30-day mortality rate with worsening performance status and increased age at diagnosis (Appelbaum et al., 2006). Patients $\leqslant$65 years had 30-day mortality rates of 5%, 4%, 9%, and 21% if they presented with an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, 2, or 3, respectively. Patients >65 years had rates of 13%, 16%, 35%, and 60%, respectively. Another study found 28-day mortality to range from 5% for patients under the age of 50 years with an ECOG performance status <3 to 57% for patients over age 69 with an ECOG performance status of $\geqslant$3 (Estey, 2001). Those older patients with a worse performance status were also less likely to obtain a complete remission. It is important to note that all of these estimates derive from results of clinical trials, which typically exclude individuals with organ dysfunction and substantially impaired performance status, and thus may underestimate the morbidity and mortality for a large fraction of older patients diagnosed with AML who may not tolerate current treatment options. Nevertheless, subsequent studies have reported lower rates of short-term mortality in older patients undergoing treatment, likely due to improvements in supportive care (Lowenberg et al., 2009).

Those diagnosed with secondary AML, including patients with a history of exposure to cytotoxic agents or radiation therapy and patients with underlying myelodysplasia or hematologic disorders, carry a considerably worse prognosis than those diagnosed with primary AML. Therapy-related myeloid neoplasms (t-MN), including AML, MDS, and myelodysplastic syndrome/myeloproliferative neoplasms (MDS/MPN) account for approximately 10%–20% of all cases of AML, MDS, and MDS/MPN (Smith et al., 1996). Multiple clinical and biological subsets of t-MN have been recognized, and these are correlated with the specific therapy administered for the primary disease. The most common type of t-MN is due to damage from alkylating agents, which react directly with DNA, leading to t-MN with unbalanced aberrations, primarily loss of chromosome material (characteristically, chromosomes 5 and/or 7) (Pedersen-Bjergaard et al., 2008). A second subset of t-MN occurs after therapy with DNA topoisomerase II inhibitors, such as etoposide, doxorubicin, mitoxantrone, etc., resulting in balanced translocations most often involving the MLL gene at 11q23 or the RUNX1 gene at 21q22 (Pedersen-Bjergaard and Philip, 1991; Pui and Relling, 2000; Libura et al., 2005). The prognosis of patients with t-MN is generally worse than for those with de novo AML, as it is more frequently impacted with high-risk cytogenetic and molecular features, rendering the disease more resistant to further therapies. Pre-existing myelodysplastic or myeloproliferative disorders are common in older patients who develop AML, occurring in 24%–40% of cases (Letendre et al., 1998; Estey, 2001; Wahlin et al., 2001; Bello et al., 2011). These pre-existing disorders are often associated with ineffective hematopoiesis and dysfunctional blood cells. By the time that AML emerges, these patients may be colonized by pathogenic flora, threatened by recurrent bleeding episodes, and dependent upon blood product transfusions, leading to worse outcomes than in de novo AML.

An important prognostic tool at diagnosis involves karyotypic evaluation of leukemic cells, which helps distinguish patients into low-, intermediate-, and high-risk groups. Classification into these subgroups can help select appropriate therapy, provide prognostic information, and help clarify underlying molecular pathogenesis so that improved targeted therapies may be developed. These prognostic groups are shown in Table 1. Favorable-risk cytogenetics, such as translocation 15;17 or those which impact core binding factor (CBF) (translocation 8;21 and inversion 16), are more frequently detected in younger patients and are associated with higher rates of complete remission and lower risk of relapse. Translocation of chromosomes 15 and 17, a feature of acute promyelocytic leukemia, portends a markedly good prognosis, and more than 90% of patients can be cured, given the exquisite sensitivity of this disease to differentiating agents, such as all-trans retinoic acid (ATRA) and arsenic trioxide. AML with concomitant alterations impacting a transcription factor, CBF, also have relatively improved outcomes with conventional induction therapy, with long-term survival of 60% or higher. For those with CBF-AML, the post-remission therapy of choice is 3–4 additional chemotherapy cycles with high doses of cytarabine. The high-risk group includes cytogenetic abnormalities that are complex ($\geqslant$3 unrelated abnormalities), monosomies of chromosome 5 or 7, deletion of the long arm of 5 or 7, and abnormalities of the long arm of chromosome 3. These abnormalities are associated with a survival rate of less than 20% at five years regardless of age, although they are more frequent in older patients and in those with secondary AML (Appelbaum et al., 2006). Given these poor outcomes, the recommendation for post-remission therapy in eligible patients with high-risk karyotypic features is consolidative allogeneic transplantation.

The remainder comprises >50% of patients and are char-

Table 1. Risk stratification in AML

	Clinical characteristics	Cytogenetics	Molecular abnormalities
Low		inv(16), t(16;16), t(8;21), t(15;17)	Normal cytogenetics: with NPM1 mutation or isolated CEBPA mutation in the absence of FLT3
Intermediate		normal cytogenetics, +8, t(9;11)	c-KIT mutation with concurrent t(8;21) inv(16), t(16;16): FLT3-ITD mutation with NPM1 mutation
High	Advanced age (age >60), ECOG performance status ⩾3, history of exposure to cytotoxic agents/radiation therapy, history of prior myelodysplasia or other hematologic disorder	Complex karyotypes (⩾3 clonal chromosomal abnormalities), -5, -7, 5q-, 7q-, 11q23 other than t(9;11) inv(3), t(3;3), t(6;9), t(9;22)	Normal cytogenetics: with FLT3-ITD mutation in the absence of NPM1 mutation

acterized as having an intermediate risk of relapse. This group is the most heterogeneous of all, characterized by myeloblasts with a normal karyotype or cytogenetic abnormalities not included in the definitions of low or high risk. While patients with cytogenetically normal AML have traditionally been included in the intermediate risk group, more sophisticated analyses, including gene mutation and microRNA expression studies, suggest that this group is much more heterogeneous than previously thought. The accumulation of molecular information will provide additional prognostic and therapeutic information within the cytogenetic strata. Gene expression profiling studies can subdivide the large group of patients with normal karyotypes into different biological subsets that appear to have different outcomes (Haferlach et al., 2005).

Although a multitude of genes are currently under investigation regarding their prognostic value, it is currently routine to test for alterations impacting the genes FLT3, NPM1, and CEBPα at diagnosis. Studies have shown that patients with CEBPα and NPM1 mutations in the absence of an FLT3-internal tandem duplication (ITD) mutation have a favorable prognosis, whereas an isolated FLT3-ITD mutation undoubtedly confers a poor prognosis, due to significantly higher risk of relapse (Kottaridis et al., 2001; Preudhomme et al., 2002; Barjesteh van Waalwijk van Doorn-Khosrovani et al., 2003; Bienz et al., 2005; Marcucci et al., 2007; Grimwade et al., 2010). However, recent studies have shown that many patients with AML do not harbor any of these currently recognized gene mutations that are thought to contribute significantly to the pathogenesis of the disease (Shen et al., 2011; Patel et al., 2012). While more efforts are being focused on better classification and prognostication, particularly of the intermediate risk group, none of the current schemes are optimal, suggesting a more complete understanding of the genetic and epigenetic changes relevant to the pathogenesis of AML is necessary. A recently published study analyzed the genome of 200 adult cases of de novo AML (Ley et al., 2013). By organizing mutated genes in AML into functionally related categories, they revealed many potentially important relationships. Notably, certain categories produced a pattern of mutual exclusivity for mutations, suggesting that one mutation in these pathways is adequate for AML pathogenesis. Though not completely understood, this new dataset will undoubtedly be a platform and framework for future studies that attempt to classify both risk and response to therapy in patients with AML. A better understanding of the mechanisms by which these mutations, or lack thereof, produce heightened or blunted sensitivity to treatment has the potential to allow more rational and selective applications of chemotherapy and transplantation. In addition, there is the hope that by identifying critical genes in AML, new therapeutic agents will be able to either specifically target these genes or modify their expression in attempts to improve clinical outcomes (Mrozek et al., 2007).

Approximately 70% of younger patients typically enter complete remission after one or two induction treatments. However, the therapeutic course and options for older patients is less clear, with poorer outcomes and minimal advances over the years. Although 40%–65% will achieve complete remission, 85% will relapse within 3 years. This is a large barrier to advancing the field of AML treatment, as the great majority of patients with AML are older than 60 years. As mentioned above, older age is not only associated with worse prognosis based on functional status and comorbidities, limiting patients' abilities to undergo intensive and more successful regimens, but is also associated with unfavorable cytogenetics and reduced chemotherapy sensitivity. As we are better able to prognosticate and risk-stratify patients, is there a way to alter treatment to more safely administer effective therapies in higher-risk patients? The wealth of emerging molecular data will undoubtedly continue to evolve and will hopefully lead to innovative and successful treatment algorithms. Allogeneic stem cell transplantation, which comprises a large part of AML therapy, is beyond the scope of this review, as the remainder will focus on novel strategies pursued in recent years and which may be the beginning of a new landscape in the therapy of AML.

Hypomethylating agents

An established approach to the treatment of AML involves the targeting of epigenetic mechanisms that are important in the regulation of cellular processes mediating hematopoietic cell growth and differentiation. DNA cytosine methylation, which modifies gene expression, has been demonstrated to be abnormal in AML, with varying degrees of hypermethylation in

promoter regions identified in different subgroups correlating with the development of myelodysplasia, leukemogenesis, and drug resistance (Leone et al., 2002; Bullinger et al., 2010; Figueroa et al., 2010a). Methylation of cytosine in the CpG dinucleotide by DNA methyltransferase leads to transcriptional silencing of genes during normal development, and it has emerged as a significant mechanism for the loss of tumor suppressor gene expression in human cancers, including AML (Issa et al., 1997; Santini et al., 2001).

Decitabine and 5-azacitidine, hypomethylating agents, are incorporated into DNA during S phase and irreversibly inhibit DNA methyltransferase, resulting in loss of methylation and reactivation of silenced genes. In a preclinical study, with decitabine at a concentration of 0.5 μmol/L, leukemic cells exhibited significant myeloid differentiation compared to vehicle treated cells, which persisted with immature morphology, suggesting that AML cells are in at least some aspects maturation progressed from normal $CD34^+$ precursors, allowing them to be targeted specifically (Negrotto et al., 2012). Azacitidine and decitabine are currently approved for the treatment of MDS on the basis of advanced phase studies that demonstrated improved outcome compared to supportive care alone (Kantarjian et al., 2006; Fenaux et al., 2009).

Multiple studies have also highlighted the utility of both decitabine and 5-azacitidine as appropriate therapeutic alternatives for patients with AML who are perhaps unable to tolerate or decline standard cytotoxic chemotherapy. A large phase III prospective, randomized clinical trial evaluated the effectiveness of 5-azacitidine in treatment of patients with higher-risk myelodysplastic syndrome compared to conventional care, which included best supportive care, low-dose cytarabine, or intensive chemotherapy as selected before randomization (Fenaux et al., 2009). Thirty-two percent of participants met the current WHO criteria for AML. At last follow-up, 82 patients treated with 5-azacitidine had died compared to 113 in the conventional care group. At 2 years, based on Kaplan-Meier estimates, 50.8% of patients in the 5-azacitidine group were alive versus 26.2% in the conventional care group ($P < 0.0001$), a survival advantage seen across all prognostic subgroups. Across the whole study, the median overall survival (OS) was 24.5 months in the 5-azacitidine group versus 15.0 months for the conventional care groups. These results form the basis for the use of 5-azacitidine as therapy for AML.

In a phase II trial evaluating decitabine, older patients with previously untreated AML who were either not candidates for or who refused standard intensive chemotherapy were treated with decitabine 20 mg/m^2 for 10 days per cycle (Blum et al., 2010). Notably, 91% of participants had at least two of the following features: age greater than 70 years, secondary AML, unfavorable karyotype, or performance status greater than 2. Forty-seven percent achieved complete remission, with an additional nine people achieving complete remission with incomplete cell recovery, making an overall response rate of 64%. Median overall survival was 55 weeks (95% CI 36–72 weeks). To give these results some perspective, in a similar patient population treated with low-dose cytarabine, the comparative standard of care, the complete remission rate was 17%, with a median survival of only 3–4 months (Burnett et al., 2007). A separate multi-center, phase II trial evaluated a comparable patient population with decitabine for 5 days per cycle (Cashen et al., 2010). In fifty-five patients with a mean age of 74 years, all with intermediate or poor-risk cytogenetics without previous treatment, there was a complete response rate of 24% and an overall median survival rate of 7.7 months. The induction death rates were similar between the five-day and ten-day regimens—the increased treatment duration did not appear to increase early death and showed an improvement in complete response and overall survival. In a population where typically 64% of patients with AML greater than age 65 years are not treated and subsequently have a median survival time of 1.7 months, hypomethylating therapy does offer a promising therapeutic alternative (Menzin et al., 2006).

Beyond being a potential agent used as first line therapy for patients unable to tolerate cytotoxic therapy, as described above, decitabine may have potential as an epigenetic priming agent given prior to cytotoxic chemotherapy (Issa et al., 2004; Blum et al., 2007; Lubbert et al., 2007). A recent phase I trial evaluated the safety and biologic activity of epigenetic priming with decitabine before standard induction therapy in AML patients with unfavorable risk. The population was predominantly composed of older subjects (67% >50 years) with adverse molecular characteristics (57%) and at least one adverse risk feature (83%). Decitabine was administered for 3, 5, and 7 days of priming 24 h prior to the standard 7 + 3 induction therapy. The authors concluded that the overall complete remission rate of 83% would suggest that decitabine could be effective as a chemosensitizer by reactivating tumor suppressor gene expression during the window of exposure to cytotoxic induction therapy. This possibility will need to be explored further.

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin is a humanized anti-CD33 monoclonal antibody linked to a semi-synthetic derivative of calicheamicin, a potent cytotoxic antibiotic (Hinman et al., 1993). CD33 is expressed on approximately 90% of AML myeloblasts and is downregulated as the myeloid lineage matures, resulting in low-level expression on peripheral granulocytes and tissue macrophages, thus making it a prime target in the treatment of AML. Initial development and data highlighted gemtuzumab ozogamicin as a new therapeutic agent that could be efficacious in newly diagnosed AML, and several phase III studies were designed to test the drug in this setting. The drug was approved under the FDA accelerated approval program in 2000 for treatment as a single agent of patients older than 60 years with AML in first relapse who were not candidates for aggressive chemotherapy (Bross et al., 2001). Approval was based on the results of a phase II study of 142 patients with AML in first relapse—the complete response (including those with incomplete platelet recovery) was 30%, with an overall response

rate of 26% in patients over 60 years (Sievers et al., 2001). However, gemtuzumab ozogamicin was withdrawn in 2010 after a phase III comparative controlled trial by, Southwest Oncology Group (SWOG), revealed a toxicity rate that was significantly higher in the gemtuzumab ozogamicin combination therapy group vs. the standard therapy group (16/283 = 5.7% vs. 4/281 = 1.4%; P = 0.01) (Petersdorf et al., 2013). Since being withdrawn from the market, some studies have supported the efficacy of gemtuzumab ozogamicin in newly diagnosed AML with acceptable toxicity (Burnett et al., 2011, 2012). In an effort to exploit the science behind the drug and minimize toxicities, a recent randomized, open-label, phase III study performed in France studied gemtuzumab ozogamicin, employing fractionated dosing (Castaigne et al., 2012). Instead of the initially studied dosing on days 1 and 14, the study chose a new regimen based on the repetition of a lower dose of 3 mg/m^2 (max dose 5 mg) on days 1, 4, and 7. In patients with newly diagnosed AML, aged 50–70 years, event-free survival, the primary endpoint, was estimated as 17.1% (10.8–27.1) in the control group versus 40.8% (32.8–50.8) in the gemtuzumab ozogamicin group (hazard ratio 0.58, 0.43–0.78; P = 0.0003). Secondary endpoints were similarly better with overall survival at 41.9% (33.1–53.1) versus 53.2% (44.6–63.5), respectively (0.69, 0.49–0.98; P = 0.0368), and relapse free survival at 22.7% (14.5–35.7) versus 50.3% (41.0–61.6), respectively (0.52, 0.36–0.75; P = 0.0003). As mentioned, initial safety concerns regarding gemtuzumab ozogamicin were based on trials using two doses of gemtuzumab ozogamicin at 9 mg/m^2, with increased incidence of hepatic veno-occlusive disease. The impressive survival data collected from recent trials in using fractionated dosing, and a lower incidence of liver toxicity, offer a compelling case for possible reconsideration of gemtuzumab ozogamicin as a therapeutic option in AML.

FLT3 tyrosine kinase inhibitors

FMS-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase that acts as a key mediator of early hematopoiesis. Approximately 23% of patients with AML are found to have an internal tandem duplication (ITD) mutation of the FLT3 gene, which causes constitutive activation of the tyrosine kinase, offsetting negative regulatory functions and causing suppression of apoptosis and dysregulated cell proliferation. As mentioned above, the FLT3-ITD alteration designates an extremely high-risk group with a markedly poor prognosis—studies demonstrating markedly higher rates of relapse and worse disease-free and overall survival rates (as low as 15% at 5 years) (Kottaridis et al., 2001; Thiede et al., 2002; Levis and Small, 2003; Gale et al., 2008). Targeting the constitutively active FLT3 in these patients with potent tyrosine kinase inhibitors may hold promise in the treatment of a large group of AML patients with an otherwise extremely poor prognosis.

Studied FLT3 inhibitors have had widely varying degrees of success, with the majority of earlier agents producing, at best, temporary reductions in peripheral myeloblasts, with the duration of response ranging from 2 weeks to 5 months (Fiedler et al., 2003; Smith et al., 2004). Clinical trials have established that clinical response strongly correlates with both sustained and effective inhibition. This knowledge has led to a newer generation of FLT3 inhibitors that, in general, exhibit a greater relative specificity for and potency against FLT3, and have improved pharmacokinetics. These agents appear to hold greater promise, especially in the setting of relapsed disease, wherein leukemic cells have been characterized as having a greater mutant FLT3 allele burden, and more addicted to and driven by the constitutively active FLT3 (Pratz et al., 2010). In such a setting, specific and potent FLT3 inhibitors, such as quizartinib (currently in clinical trials), may hold great promise. Preclinical testing of quizartinib revealed both selectivity and potency superior to other FLT3 inhibitors, as well as a longer half-life of approximately 1.5 days allowing for sustained inhibition (Chao et al., 2009; Zarrinkar et al., 2009). In a phase II study of patients with relapsed/refractory AML, preliminary data revealed a composite complete remission (CRc) rate of 45% (Cortes et al., 2011; Levis et al., 2012). Cohort 1 consisted of patients older than 60 years with relapsed or refractory disease to 1st line chemotherapy, and Cohort 2 consisted of patients greater than 18 years with relapsed or refractory disease to 2nd line chemotherapy or hematopoietic stem cell transplantation (HSCT). Of all those refractory to any prior therapy, the CRc rate was 62%, albeit the remission rate consists of mostly those with incomplete peripheral recovery. Quizartinib allowed 8% of patients in Cohort 1, and 30% of patients in Cohort 2, to be successfully bridged to a potentially curative HSCT. Interestingly, responses to quizartinib monotherapy were seen in both FLT3-ITD positive and negative patients, with a CRc rate of 44% and 34%, respectively, likely related to off-target effects of the agent (Bennett et al., 2010). Regardless, this data supports quizartinib as the agent with the highest clinical efficacy amongst all FLT3-target therapy thus far, with acceptable safety parameters. Further studies with quizartinib monotherapy and in combination with other agents are necessary, but this initial data suggests effective and clinically meaningful outcomes in patients with refractory or relapsed AML.

Other novel approaches under study

A series of other novel approaches have been studied in patients with advanced AML (Table 2). Antisense oligonucleotides are short, synthetic stretches of DNA designed to bind complimentary mRNA strands that correspond to target genes. By binding to the mRNA, the antisense oligonucleotides prevent the translation of the target gene into a protein—the resultant DNA-mRNA complexes are degraded by a ribonuclease (Bennett et al., 2010). Using this mechanism, many genes are currently being investigated as potential targets for treatment in AML. X-linked inhibitor of apoptosis protein (XIAP) is an inhibitor of caspases 3, 7, and 9, protein mediating the last stages of apoptosis, and has been found to be overexpressed in AML, contributing to chemoresistance (Lacasse et

Table 2. Established and emerging therapies in AML

Conventional high-dose chemotherapy	-Cytarabine and anthracycline-based induction -High-dose cytarabine	-Traditional approach reserved for younger patients or those sufficiently robust to tolerate intensive chemotherapy -High rates of remission, but high rates of subsequent relapse
Low-dose chemotherapy	-Low-dose cytarabine	-Offer disease control for older patients or those who cannot tolerate intensive induction -Low rates of remission
Hypomethylating agents	-5-azacitidine -Decitabine	-Improved survival data with 5-azacitidine for those with high-grade MDS or AML with <30% marrow blasts -High overall response rate, but low CR rate
Antibody-drug conjugates	-Gemtuzumab ozogamicin	-Newer dosing approaches have suggested better tolerance and efficacy
FLT3 kinase inhibitors	-Sorafenib -Quizartenib -Midostaurin	-Potent FLT3 inhibition can lead to peripheral and marrow responses -Still under clinical study
Emerging novel approaches	-Aurora kinase inhibitors -Antisense agents -MTOR/PI3K inhibitors -PIM kinase inhibitors -HDAC inhibitors -IDH targeted therapies	

al., 2005). AEG35156 is an antisense agent targeting XIAP and has shown promise in preclinical and clinical trials. A phase I/II clinical trial of escalating doses of AEG35156 in combination with high-dose cytarabine and idarubacin reinduction therapy was performed in patients with relapsed and refractory AML (Schimmer et al., 2009). Of those who received the highest dose of AEG35156, 47% were reported to have achieved CR. However, the study's participants varied greatly in the prior regimens they had received, with some patients being refractory to greater than 2 induction regimens. Notably, of those who achieved complete remission, the majority were patients with primary refractory or first-relapse disease. High levels and frequency of target knockdown were observed in patients whose disease had rapidly relapsed or in those refractory to at least 2 induction regimens, but it appears that knockdown of XIAP was not sufficient to result in clinical responses in these patients. In summary, the addition of AEG35156 to reinduction chemotherapy was well tolerated with some suggestion of efficacy in first salvage, but further studies are needed to fully understand and optimize the pharmacokinetics of this and other antisense therapies.

Other novel approaches in treatment are under vigorous study. These include the targeting of various other upregulated pathways and kinases, including aurora kinases, mTOR, PI3 kinase, and PIM kinase. Aurora kinases are a family of proteins known to be integral to the regulation of mitosis and chromosomal segregation, and mutations of all subtypes have been identified in the oncogenic process of both solid and liquid tumors (Meraldi et al., 2004). Alisertib, an aurora A kinase inhibitor, and barasertib, an aurora B selective small molecule inhibitor, have shown promise in early studies (Walsby et al., 2008; Oke et al., 2009; Kelly et al., 2011). FLT3, c-KIT, and RAS mutations, amongst many others, result in constitutive activation of pathways leading to uncontrolled proliferation, a hallmark of all cancers, including AML. Given that many of these mutations cause activation of common downstream pathways, there is growing interest in inhibition of common downstream effectors, including mTOR and PI3 kinase. The PI3-K/Akt/mTOR signal transduction cascade is crucial for appropriate cell cycling, replication, and death, with PI3-K, a kinase near the cell surface, being activated by a number of receptor tyrosine kinases (FLT3, EGFR, HER2/neu, etc.) (Witzig and Kaufmann, 2006). Constitutive activation of this pathway has been detected in 50% to 70% of patients with AML and is often seen as a downstream effect with FLT3-ITD mutations, providing rationale for the therapeutic targeting of one or more members of this cascade (Brandts et al., 2005; Grandage et al., 2005; Martelli et al., 2006; Yee et al., 2006). Multiple early phase studies have evaluated novel agents that inhibit mTOR in AML, such as rapamycin, temsirolimus, sirolimus, and deforolimus, revealing that these agents are effective in suppressing phosphorylation of downstream targets of mTOR but have modest clinical activity as monotherapy (Mohi et al., 2004; Recher et al., 2005; Callera et al., 2008; Rizzieri et al., 2008). Subsequent trials are now attempting to evaluate the clinical efficacy and safety of mTOR inhibitors combined with traditional cytotoxic agents in patients with poor-risk AML. The serine/threonine kinase PIM (proviral integration site for Moloney murine leukemia), a downstream target of FLT3 found to be upregulated in AML, is also currently under extensive investigation (Kim et al., 2005, 2006). Recent data implicate PIM as an integral component of the FLT3 signaling complex in FLT3-ITD cell lines and inhibition of PIM appears to be directly and preferentially cytotoxic to FLT3-ITD AML cell lines (Fathi et al., 2012).

Other approaches which target the epigenetic landscape are under study. Histone deacetylase, an enzyme that re-

moves acetyl groups from lysine residues leading to formation of condensed and transcriptionally silenced chromatin, and thus silenced gene expression, is an interesting new target in AML. RUNX1-ETO and CBFb-MYH11 are well characterized fusion genes in AML that cause the aberrant recruitment of histone deacetylase to promoters, resulting in a block in differentiation and uncontrolled proliferation of malignant cells (Kosugi et al., 1999; Amann et al., 2001; Gottlicher et al., 2001; Hiebert et al., 2003; Yang et al., 2004). HDAC inhibitors effectively work as epigenetic modulators, overriding the block in differentiation imposed by the aforementioned fusion genes and inhibiting proliferation of tumor cells by inducing cell arrest, differentiation, and/or apoptosis (Gelmetti et al., 1998).

Finally, mutations of isocitrate dehydrogenase (IDH) genes represent a recently discovered and unique constellation of point mutations in AML. These mutations retard oxidative decarboxylation of isocitrate to alpha-ketoglutarate, conferring novel enzymatic activity and creating excess 2-hydroxyglutarate (2-HG), a recognized oncometabolite (Leonardi et al., 2012). As more is understood about IDH1/2 mutations, it appears that their prognostic impact may vary according to the specific mutational locus, and the presence of other concurrent mutations of other genes, such as NPM1 and FLT3 (Boissel et al., 2010; Green et al., 2010; Marcucci et al., 2010; Paschka et al., 2010; Schnittger et al., 2010; Green et al., 2011). All IDH mutations have shown commonality in the excess production of the metabolite 2-hydroxyglutarate (2-HG). Data suggest that upregulation of 2-HG can lead to aberrant DNA hyper-methylation and epigenetic remodeling, an important consideration for the development of therapeutic agents (Figueroa et al., 2010a; Ward et al., 2010; Lu et al., 2012). There is ongoing interest therefore in the development on novel agents which target altered IDH proteins.

CONCLUSION

Progress in the treatment and outcomes for patients with acute myeloid leukemia has been limited. This is especially challenging as this is a disease of predominantly older patients, and many older adults, some of whom may exhibit chronic multi-organ dysfunction, or poor performance status, cannot tolerate intensive chemotherapy and harbor leukemic cells that are inherently more resistant. However, in recent years, gene expression profiling has been extensively studied as enhancing prognostication and innovating therapeutic strategies, with the hope that this will ultimately expand therapeutic options for AML. As patients' individual diseases are better understood, a more comprehensive and nuanced understanding of disease and prognosis can lead to more tailored treatments. Many of the new agents under development and techniques stemming from genetic analysis will undoubtedly have a role in future therapy for AML. This is especially important given the heterogeneous and pleiotropic nature of AML, the course and progression of which depends on the interplay of many compensatory cellular mechanisms, with progressive mutations leading to drug resistance and relapse. Although this raises many challenges, there are similarly countless opportunities for the development of novel and effective therapies.

ABBREVIATIONS

ATRA, all-trans retinoic acid; CBF, core binding factor; ITD, internal tandem duplication; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; OS, overall survival; t-MN, therapy-related myeloid neoplasms

COMPLIANCE WITH ETHICS GUIDELINES

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This article does not contain any studies with human or animal subjects performed by the any of the authors.

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