review

Use of molecular markers to determine postremission treatment in acute myeloid leukemia with normal cytogenetics

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Hematol Oncol Stem Cell Ther 2015; 8(4): 143–149

DOI: http://dx.doi.org/10.1016/j.hemonc.2015.09.003

Most patients with acute myeloid leukemia can be induced into complete remission, but postremission treatment is required for cure. The choice of postremission therapy in a majority of nonelderly patients, between intensive chemotherapy and allogeneic hematopoietic cell transplantation, is largely determined by the results of conventional cytogenetic analysis. In 45% of patients with a normal karyotype, the presence or absence of specific molecular mutations should be used to determine the prognosis and postremission treatment. In addition, the identification of mutations may indicate a role for targeted intervention, including following transplantation.

KEYWORDS: Molecular markers; Acute myeloid leukemia; Cytogenetics

Acute myeloid leukemia (AML) is a disease of older adults; however, half of patients with AML are ⩽65 years of age at presentation.[1] The incidence of AML has increased to nearly 20,000 new cases per year, in a large part due to more patients surviving longer periods following chemotherapy and radiation treatment for other malignancies, and longer survival in general.[2] Older and treatment-exposed patients are at higher risk than the general population. While patients with AML fare poorly overall (<20% leukemia-free survival [LFS] at 5 years), nonelderly patients (<65 years of age) have better outcomes than their older counterparts, and most have a substantial chance for cure. Cytogenetic analysis is used to risk stratify patients, and is the most important factor in determining postremission treatment. The identification of specific molecular mutations is important in patients with normal cytogenetics for their appropriate risk categorization and for help in determining the postremission treatment. In addition to improvements in treatment and supportive care, which have significantly lowered the treatment-related mortality (TRM) with chemotherapy[3] and with allogeneic hematopoietic cell transplantation (allo-HCT),[4] these advances in risk stratification and in the development of evidence-based guidelines for choice of postremission treatment are responsible for improving outcomes.

TREATMENT OF AML

Induction

The modern treatment of AML in the nonelderly includes induction therapy, which achieves complete remission (CR) in >70% of patients, followed by postremission therapy designed to cure the patient. For most patients ⩽65 years of age and many healthy patients older than 65 years, the standard aggressive induction therapy in North America consists of 7 + 3, shorthand for 7 days of cytarabine and 3 days of an anthracycline. Some centers add etoposide or other drugs to this regimen, although prospective

studies have not demonstrated long-term benefits over 7 + 3. Recent trials have demonstrated improved outcomes with more intensive anthracycline dosing.[5,6]

POSTREMISSION THERAPY

The two main choices for postremission therapy in those patients who achieve CR following induction therapy are intensive chemotherapy, usually with three to four cycles of high-dose cytarabine (HiDAC), and allo-HCT. Patients who achieve CR with induction therapy, but who do not receive postremission therapy, are almost never cured because they retain leukemic stem cells that are not detectable by standard measures. In many instances, the use of sophisticated techniques (e.g., multiparametric flow cytometry[7] or next-generation sequencing[8,9]) may identify residual disease in patients in CR.

Autologous transplantation was performed frequently in AML as recently as a decade ago. Favorable outcomes have been reported with this approach, particularly in young patients with favorable-risk AML.[10,11] A meta-analysis has demonstrated no prolongation of survival after autologous transplantation compared to standard chemotherapy.[12] Thus, intensive chemotherapy and allo-HCT are the main alternatives for postremission treatment.

HiDAC requires brief hospitalizations with regular outpatient follow-up, and is safer and less expensive than allo-HCT. It is curative in selected patients. Allo-HCT is the other commonly employed postremission therapy and exerts a more powerful antileukemic effect, first through its use of myeloablative chemotherapy or combined chemo- and radiation therapy, at doses which could not be tolerated without hematologic rescue by donor hematopoietic cells, as well as the potent antileukemia effect of the donor immune cells, termed the *graft-versus-leukemia effect*. Originally reported by Thomas et al.[13] to cure some patients with advanced acute leukemia, this approach is now more commonly and effectively used in patients in first CR.[14,15] Allo-HCT often requires hospitalization in excess of 4 weeks, followed by close outpatient monitoring. Compared to intensive chemotherapy, this approach is much more expensive. Allo-HCT is associated with significantly higher rates of nonrelapse mortality (NRM), and may be complicated by chronic graft-versus-host disease, which compromises the quality of life of some long-term survivors. It should be noted, however, that NRM following allo-HCT has been substantially diminished over the past two decades.[4] Whereas 1-year mortality rates with allo-HCT of ~30% were commonly cited a decade ago, a recent Center for International Bone Marrow Transplant Registry study of well over a thousand patients with AML in first CR performed at >100 international centers demonstrated a 1-year mortality rate of 12% using what is now the most widely administered pretransplant conditioning regimen, intravenous busulfan and cyclophosphamide.[15] The dose adjustment of busulfan, based on plasma levels following the first dose, could further improve NRM by avoiding the variation in plasma levels among different individuals. The optimal plasma levels of busulfan in specific situations are uncertain; it is likely that these may vary by disease, stage, comorbidities, and other factors, and require further investigation.

Further, the potential application of allo-HCT to virtually all patients ⩽65 years (and many older than that) using alternative donors has had a substantial impact. Only 30% of patients have human leukocyte antigen (HLA)-identical sibling donors. Matched unrelated adult donors, cord blood, and haploidentical family donors (using post-transplant cyclophosphamide to prevent graft-vs.-host disease) have all yielded results approaching those achieved in patients with fully matched sibling donors.[16]

In addition to the use of alternative donors, the application of allo-HCT has been enhanced by the development of reduced-intensity regimens with less toxicity than myeloablative conditioning. These regimens have extended transplantation to older patients and to those with significant comorbidities. The consensus criteria to define regimen intensity have been reported.[17] A regimen of fludarabine and low-dose total body irradiation has minimal toxicity, permits the engraftment of cells from HLA-identical sibling donors, and relies on the graft-versus-leukemia effect.[18] Additional donor lymphocytes can be infused within a few months of transplantation to augment the antitumor activity. More powerful (compared to fludarabine/low-dose total body irradiation) reduced-intensity regimens with less toxicity than myeloablative therapy have been developed, including widely used regimens with less than ablative doses of busulfan in combination with fludarabine.[19,20] These regimens result in lower NRM and less toxicity than myeloablative regimens, but are associated with higher relapse rates. Generally, survival has not been shown to be significantly different from that achieved with myeloablative regimens; however, a myeloablative busulfan/fludarabine regimen was associated with better LFS and overall survival compared to a reduced-intensity regimen using the same drugs in an analysis of patients allografted in second CR by the European

Group for Blood and Marrow Transplantation.[21] The development[22] and refinement[23] of comorbidity indices designed to determine which patients could tolerate myeloablative allo-HCT, those who might fare better with reduced-intensity regimens, and those best served by not performing bone-marrow transplantation have been a major advance.

Categorizing AML patients by conventional cytogenetic analysis

The likelihood of cure in an individual with AML is influenced by a variety of factors, particularly cytogenetic analysis, which is the standard method used to categorize patients into favorable-, intermediate-, and adverse-risk groups.[24–26] Patients with favorable cytogenetics, including t(8;21), inv(16), or t(16;16), collectively called *core-binding factor AML*, are generally at low risk of relapse when treated with three to four cycles of HiDAC following the achievement of remission, and do not generally benefit from allo-HCT in first CR.[27] More than half of these favorable-risk patients are alive 5 years following treatment.[28] Roughly one-fourth of patients with core-binding factor AML carry a c-KIT mutation, and these individuals have a much higher risk of relapse.[29] It is uncertain whether this subset of patients would fare better with allo-HCT in first CR. In addition, the impact of targeted therapy with c-KIT inhibitors (e.g., dasatinib) is being explored in ongoing studies of patients with this mutation.

In contrast to those with favorable-risk AML, patients with deletions of chromosome 5 or 7, del (5q), abnormalities of 3q, or complex abnormalities are classified as having adverse-risk AML. These patients, even after obtaining CR, have poor outcomes with standard intensive postremission chemotherapy and exhibit a 5-year survival of <15%. Allo-HCT improves results for this population substantially, more than doubling the rate of sustained survival.[27,30]

Because genetic studies are fundamental in risk stratifying and determining the appropriate postremission treatment for AML patients, the diagnostic bone-marrow examination in patients suspected to have AML must include cytogenetic and molecular studies. The European LeukemiaNet has developed a standardized system for reporting cytogenetics and molecular alterations in adults with AML, and confirmed its prognostic significance.[31] While patient age, presenting white-blood count, extramedullary leukemia, and other factors may influence outcome, the specific genetic abnormalities in the leukemia cells comprise the predominant factor predictive of the outcome.

AML with a normal karyotype

Approximately 45% of patients with AML do not have detectable cytogenetic abnormalities and, until recently, were categorized together within an intermediate-risk group.[24–26] Overall, these patients have a 5-year survival of 30–35% following postremission chemotherapy.[24,27] A large prospective trial, unusual in that 82% of patients "randomized" to allo-HCT on the basis of having an HLA-matched sibling donor actually underwent transplantation, demonstrated clearly superior LFS among intermediate-risk-group patients undergoing allo-HCT compared to those receiving postremission chemotherapy.[30] Meta-analyses have demonstrated similar results.[27] Based on these data, allo-HCT was widely and reasonably recommended for patients in either the intermediate or adverse cytogenetic risk groups.

Next-generation sequencing has molecularly refined the characterization of AML, and defined the biologic and prognostic significance of identified mutations.[9] These advances have permitted the subcategorization of patients with normal cytogenetics, and have better defined best postremission therapy among these patients. Molecular markers are now used to categorize patients with a normal karyotype, into molecularly favorable or unfavorable group.[31,32]

Molecular mutations in AML with normal cytogenetics

FMS-related tyrosine kinase 3

FMS-related tyrosine kinase 3 (FLT3) is a transmembrane tyrosine-kinase receptor, which, when activated by the FLT3 ligand, stimulates cell proliferation. The FLT3 internal-tandem-duplication (ITD) mutation causes constitutive activation of the receptor tyrosine kinase. It occurs in approximately one-third of AML patients with normal cytogenetics, and bestows an unfavorable outcome.[33] Patients with high mutant-to-wild-type ratios (>0.50)[34] and those in whom the ITD insertion occurs in the β1 sheet of the tyrosine-kinase domain have a particularly dismal prognosis.[35] Patients with FLT3–ITD mutation, regardless of the presence of additional mutations, fare poorly with postremission intensive chemotherapy, and have significantly better results with allo-HCT.[32] While patients with FLT3–ITD experience better outcomes with allo-HCT than with standard therapy, even with allo-HCT, FLT3–ITD is an adverse prognostic factor.[36] The addition of FLT3 inhibitors following transplantation is well tolerated and appears to lower the incidence of relapse.[37] Its use in this context is being further investigated.

Nucleophosmin 1

Nucleophosmin 1 (NPM1) is a phosphoprotein integral to the assembly of ribosomes and protein transport. Mutations of NPM1 impair the transport of proteins to the nucleus; they occur in one-half of normal-karyotype AML patients. Mutations in NPM1, in the absence of the FLT3–ITD abnormality, confer a favorable prognosis on patients with AML. AML patients with an isolated NPM1 mutation fare well with postremission chemotherapy.[32] Interestingly, a prospective-donor-versus-no-donor analysis demonstrated significantly longer LFS among patients with mutated NPM1 who underwent allo-HCT.[38] These results differed from previous studies. The overall survival was not improved, however, likely due to responsiveness to therapy in relapsed patients.

CCAAT/enhancer-binding-protein alpha

CCAAT/enhancer-binding-protein alpha (CEBPA) is a critical transcription factor for myeloid-cell development. CEBPA mutations result in a block in granulocyte differentiation. Patients with double CEBPA mutations are less common than those with the FLT3–ITD or NPM1 mutations, and comprise fewer than 10% of patients with cytogenetically normal AML. These patients (with double mutations) have a favorable prognosis with postremission chemotherapy. While relapse-free survival appears to be improved in patients with double-mutant CEBPA who receive allo- or auto-HCT in CR as compared to chemotherapy, relapsed patients still have a favorable outcome after reinduction and allo-HCT.[39]

Mutations in these three genes (FLT3, NPM1, and CEBPA) can be thus used to stratify patients with AML characterized by normal cytogenetics. In 2010, an international expert panel provided evidence and expert-opinion-based recommendation for the management of AML, including an updated genetic stratification.[31] Under these guidelines, patients with normal cytogenetics and mutated NPM1 without FLT3–ITD or mutated CEBPA are classified in the favorable group along with patients with core-binding-factor leukemia. Patients with FLT3–ITD, regardless of NPM1 mutations, and those with wild-type NPM1 and FLT3 are classified as intermediate.[31,40] Although intermediate I and II groups were formulated, empirical data suggest that this discrimination may not be useful clinically.[40,41]

Additional mutations are of interest because they influence prognosis and the effectiveness of specific treatments. Advances in integrated genetic profiling in AML promise to contribute further to risk stratification and to aid therapeutic decision making.[42]

DNA (cytosine-5)-methyltransferase 3 alpha mutation

Approximately one-third of patients with AML with normal cytogenetics have DNA (cytosine-5)-methyltransferase 3 alpha mutations.[43] The DNA methyltransferases maintain DNA methylation, which silences specific genes. Although some studies have reported an adverse impact on survival, others have suggested varying impact of different mutation types. Codon R882 mutations are associated with worse relapse-free survival, while all other mutations are associated with favorable survival. Interestingly, the unfavorable impact with lower doses of anthracycline induction was not seen when high anthracycline doses (which are now standard) were utilized.[42]

Isocitrate dehydrogenase 1 and 2

Mutations in isocitrate dehydrogenase (IDH) 1 or IDH 2 occur in approximately one-fourth of cytogenetically normal AML. These mutations impair the DNA hydroxymethylation and cause aberrant DNA methylation. A favorable effect is conferred in patients with IDH 2 R140 mutations, which occur largely in patients with NPM1 mutations, where these IDH 2 mutations further improve prognosis.[42]

The prognostic import of several other genetic mutations in AML has been similarly explored. None appears to have the clear impact that FLT3–ITD, NPM1, and double CEBPA mutations have on prognosis and on directing postremission treatment. Thus, normal-karyotype AML patients with a wild-type FLT3 gene who have an NPM1 mutation or double CEBPA mutations have a favorable prognosis, and are best treated with HiDAC or other intensive chemotherapy once they achieve CR. It is important to note that, as discussed previously here, all patients with normal cytogenetics were categorized previously as at intermediate risk of relapse and, based on evidence from prospective randomized trials, deemed to fare better with allo-HCT. Molecular characterization thus spares a significant number of these patients the risk of allo-HCT in first CR. Moreover, by excluding these patients who do not benefit from allo-HCT, the positive impact of allo-HCT on survival in the remaining patients with normal cytogenetics should be magnified. Patients with FLT3–ITD mutations and patients with none of the three mutations achieve a significant survival benefit with allo-HCT.[32] In 45% of patients with AML who have normal cytogenetics, molecular markers thus provide

the best mechanism to determine the postremission treatment.

Secondary AML

Patients with AML following an antecedent hematologic disorder (secondary AML [s-AML]) or prior chemotherapy or radiation treatment (therapy-related AML), including those with a normal karyotype, fare poorly with standard chemotherapy compared to patients with de novo disease with similar cytogenetics. Allo-HCT appears to provide better outcomes.[44–46] Stone et al.[47] recently confirmed the adverse prognostic impact of s-AML and therapy-related AML (median overall survival 7 months). By analyzing rigorously defined cases of s-AML, Lindsley et al.[48] defined a core group of mutations specific to AML developing after myelodysplastic syndrome or chronic myelomonocytic leukemia, and found that adults with clinically defined de novo AML with these mutations had similarly poor outcomes.[49] Whether the presence of these mutations in clinically de novo AML should influence the choice of allo-HCT as postremission treatment, similarly to a clinical diagnosis of s-AML, is unproven, but merits investigation.

Additional factors determining treatment

While cytogenetics and molecular profiling provide a fundamental platform for determining postremission therapy, the assignment of an individual to transplantation or chemotherapy is complicated by the patient's health. Comorbidities may preclude transplantation or make it more dangerous.[22,23] Even patients at high risk of relapse, but who have high risk of TRM based on multiple significant comorbidities, may be best and most safely treated with chemotherapy rather than allo-HCT.

In addition, donor source is important. Approximately 30% of patients have an HLA-identical sibling donor, the ideal donor for transplant. The other 70% of patients can utilize matched unrelated adult donors, cord-blood donors, or haploidentical family donors, all of which appear to carry slightly higher risk of TRM. The long-term survival after allo-HCT from alternative donors is beginning to approach that of allo-HCT using HLA-identical siblings. Results vary, however, according to donor characteristics. For example, cord-blood units that are well matched and consist of high cell doses yield better outcomes than less well-matched units with lower cell doses. The results of haploidentical transplantation with nonmyeloablative regimens are favorable;[50] whether similarly favorable results are achieved with ablative regimens is less certain. These and other factors merit careful consideration in determining optimal postremission therapy in patients with AML.

Mutation-targeted therapy in AML

In addition to playing an important role in risk stratification, molecular abnormalities have emerged as potential targets for therapy in AML. FLT3 inhibitors have been in development for a number of years as potential therapeutic options for patients with the ITD mutation and other activating FLT3 mutations. Small molecules that inhibit the FLT3 receptor have been tested as single agents in the setting of relapsed/refractory AML. Initial studies using the relatively nonspecific tyrosine-kinase inhibitor (TKI), sorafenib, showed high response rates in patients with the FLT3–ITD mutation.[51,52] Quizartinib, a newer FLT3 TKI that is currently under evaluation in a Phase 3 study, has shown efficacy as a single agent in early studies of relapsed/refractory AML patients and elderly AML patients harboring the FLT3–ITD mutation.[53–55] While FLT3 inhibitors, including quizartinib, have been able to bridge some AML patients to allo-HCT, these agents are not thought to have curative potential as single-agent therapies because AML emerges in a multistep process that involves more than one mutation. Remissions with TKIs are often short lived due to the multiple extrinsic, receptor intrinsic, and cell-intrinsic resistance mechanisms that can aid a leukemia cell in evading FLT3 inhibition.[56]

Chemotherapy combinations involving FLT3 inhibitors have been tested in various settings. Incorporating sorafenib into induction chemotherapy for elderly AML patients was associated with higher TRM and lower CR rates.[57] By contrast, a study of sorafenib and the hypomethylating agent, azacitidine, in AML patients, the vast majority of whom had relapsed/refractory FLT3–ITD AML, showed an encouraging response rate of 46%.[58] Other combination therapies, in the upfront, relapsed/refractory, and post-transplant settings, are being investigated at present. In addition to the ongoing studies involving quizartinib, other new FLT3 inhibitors—including crenolanib, PLX-3397, and ASP-2215—are currently being evaluated in clinical trials.

Targeted drug development is underway for other molecular abnormalities occurring in AML. In particular, IDH 1 and IDH 2 mutations are thought to represent appealing targets for AML therapy.[59] In a Phase 1 study of patients with IDH 2 mutation-positive advanced hematologic malignancies, AG-221 appears to be a tolerable IDH 2 inhibitor.[60] Moreover, the majority of patients on this clinical trial have

exhibited responses, some of which have been durable for months. If the efficacy of IDH inhibitors is reproduced in future studies, these agents will likely be investigated in combination with other AML treatment regimens in various settings.

CONCLUSION

AML is a remarkably heterogeneous disease with various somatic alterations responsible for differing pathophysiologies and a wide range of presentations, treatment responses, and outcomes. Specific mutations identified in individual patients can be used to categorize patients into risk groups and to identify the best choice of postremission treatment. In the sizable group of patients with normal cytogenetics, mutations in FLT3, NPM1, and CEBPA define risk of relapse and identify patients who are best treated with either postremission intensive chemotherapy or allo-HCT. Further advances in integrated mutational analysis promise to improve care by identifying those patients who should receive targeted therapy (e.g., FLT3 inhibitors), as well as to further refine the selection of those patients who should undergo allo-HCT and those who should receive HiDAC in first CR.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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