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Diagnosis and Therapy of Acute Myeloid Leukemia in the Era of Molecular Risk Stratification

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Abstract

The diagnosis and risk stratification of acute myeloid leukemia (AML) primarily rely on morphologic analysis and assessment of karyotype by chromosome banding analysis. For decades, standard AML induction therapy has utilized the combination of anthracyclines and cytarabine. Despite the use of postremission therapy, less than half of patients with AML will be cured of their disease. Allogeneic hematopoietic stem cell transplantation combines cytoreductive chemotherapy with adoptive immunotherapy and may cure patients who fail chemotherapy alone. Recent advances in next-generation sequencing have yielded important insights into the molecular landscape of AML with normal karyotype. Integrated prognostic models incorporating somatic mutation analyses may outperform prediction based on conventional clinical and cytogenetic factors alone. We review the evolution of risk profiling of AML from the cytogenetic to molecular era and describe the implications for AML diagnosis and postremission therapy.

Keywords

somatic mutation profiling; allogeneic hematopoietic stem cell transplantation; graft-versus-leukemia; induction chemotherapy; postremission therapy; next-generation sequencing

INTRODUCTION

We are now two centuries into the era since acute leukemia was first described, beginning with Peter Cullen's account of a patient with milky blood in 1811 (1) and followed by the more complete description by Alfred Velpeau in 1825 (2). Velpeau provided a detailed report of clinical findings in a 63-year-old florist, along with the first post mortem pathologic findings, including a gruel-like consistency of the blood and splenomegaly. In 1845, the famed pathologist Rudolf Virchow (3), just shy of his 24th birthday, published comprehensive clinical findings and a pathologic analysis of a 50-year-old woman with "*weisses blut*" or "*leukämie*" and further described the inversion of white and red blood cell

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production that characterizes the disease. Although these descriptions of the clinical syndrome of acute leukemia occurred prior to 1850, fewer than 1,000 manuscripts on leukemia were published prior to 1950 (4). In contrast, more than 170,000 manuscripts on the subject were published in 1950–2000, when the modern era of diagnosis and therapy began (4).

INDUCTION AND CONSOLIDATION CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA

The latter half of the twentieth century brought the first trial of cytotoxic agents in cancer, first as individual therapies and then in combinations. A critical transformation of acute leukemia therapy began with trials of pediatric acute lymphoid leukemia conducted by Frei and Freireich at the National Cancer Institute in the 1960s, which demonstrated for the first time reliable cures of previously fatal leukemias by the sequence of induction, consolidation, and maintenance therapies (reviewed in 5). The era of modern therapy for acute myeloid leukemia (AML) also began in that decade, with the observation that the anthracyclines (including daunorubicin) and the nucleoside analog cytarabine had activity as single agents. By the early 1970s, combinations of daunorubicin and cytarabine became the standard for AML induction therapy (i.e., therapy aimed at achieving an initial remission). In 1983, a landmark Eastern Cooperative Oncology Group (ECOG) study that compared outcomes using postremission (consolidation) therapy to an observation arm demonstrated definitively that postremission therapy was necessary in AML; the trial was halted early after nearly all patients in the postinduction observation arm relapsed at a median of four months (6). In contrast to acute lymphoid leukemia, where maintenance following consolidation was necessary for long-term disease-free survival, maintenance was found nonessential in early studies in AML (7). A subsequent ECOG study demonstrated that low-dose maintenance chemotherapy was inferior to intensive consolidation therapy consisting of high-dose cytarabine (HiDAC) and amsacrine (8). A classic study conducted by the Cancer and Leukemia Group B (CALGB) demonstrated in adults under age 60 that higher doses of cytarabine as consolidation (3 g/m^2 versus lower doses of 100 mg/m^2 and 400 mg/m^2) were superior; of note, this study included maintenance therapy in all arms (9). Critically, although modern consolidation usually consists of 3–4 cycles of cytarabine, it should be noted that typical results with this schedule are only modestly better than those seen in the 1998 US Intergroup study, wherein only one cycle of HiDAC was administered (10). It should also be noted that the benefits of HiDAC were limited or nonexistent in elderly subjects, who were much more likely to be poorly tolerant of intensive consolidation therapy (11).

The intensity of induction therapy in AML has long been studied. Early trials demonstrated that the use of intermediate- or higher-dose cytarabine during induction did not improve outcomes (12), in contrast to the clear benefit of cytarabine dose escalation during consolidation. Subsequent studies demonstrated relapse-free survival was improved by the use of HiDAC in induction, but overall survival (OS) was no better, due to treatment-related morbidity and mortality. The benefits of anthracycline dose escalation were long debated, but a definitive answer arrived with the publication of the ECOG 1900 (E1900) study, which

compared induction regimens containing daunorubicin at either 90 mg/m^2 or 45 mg/m^2 in combination with cytarabine in newly diagnosed AML patients under age 61. Individuals randomized to the higher daunorubicin dose had significantly higher remission rates (71% versus 57%) and longer median survival (24 versus 16 months) (13). Although they established the current standard of care for induction, these results remain sobering, as actuarial five-year survival remains <50% in the modern era. Further progress is clearly needed.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AS POSTREMISSION THERAPY

Given historically marginal rates of long-term disease-free survival, which diminish further with relapse, allogeneic hematopoietic stem cell transplant (HCT) approaches were developed to improve outcomes in AML. The initial rationale was that elimination of residual leukemia could best be achieved by the use of ablative doses of chemotherapy, with or without total-body irradiation. As previously reviewed, the first successful allogeneic HCT, using marrow as the stem cell source, was performed in 1968 (14). Early in the HCT era, transplantation was only available for patients with sibling donors, as unrelated-donor registries were not established until 1979; additionally, marrow obtained by operative harvest was the sole source of stem cells. Given the use of intensive conditioning, delayed engraftment with marrow harvests, and suboptimal supportive care practices of the time, treatment-related mortality (TRM) was high. However, randomized studies of allogeneic HCT as consolidation for AML in first complete remission (CR1) demonstrated non-inferiority of this approach. HCT was perceived as a high-risk therapy; as recently as 1992 the ECOG published results of a study of postremission therapy for AML wherein only individuals under the age of 41 with a sibling donor were assigned to consolidation with HCT, because at the time, HCT in older recipients and in those requiring a matched unrelated donor was considered too toxic to compare to HiDAC consolidation (8). As noted in the conclusion of that classic study, “decline in EFS over time after alloBMT is largely due to deaths from... complications..., because relapse rates are very low after alloBMT in first CR” (8).

HEMATOPOIETIC STEM CELL TRANSPLANT AS IMMUNOTHERAPY

The early rationale for HCT was that the infusion of allogeneic stem cells could rescue recipients from the intensive conditioning needed to eliminate residual leukemia; immunologic effects of the donor graft were largely associated with the unwanted complication of graft-versus-host disease (GVHD) and not with putative benefits (14). By the 1980s, attempts to eliminate the seemingly undesirable effects of the donor graft via syngeneic transplantation were associated with higher relapse rates. Questioning conventional wisdom, Gale & Champlin (15) noted that the actuarial rate of relapse in syngeneic recipients with AML in CR1 was a remarkably high 59% (versus just 18% in recipients of nonidentical sibling transplants) and concluded that success of HCT might “depend substantially on the immunotherapeutic effect of bone marrow.” Based on murine experiments, the antitumor effects of HCT independent of chemotherapy had been

postulated as early as 1956 (16); however, human T cells and B cells were not fully recognized as separate lineages of lymphocytes until 1968 (17–19), more than a decade into the era of human HCT (20).

The recognition that T cells were responsible for GVHD and the ability to mechanically deplete T cells from donor grafts led to analyses of the effects of T cell depletion on relapse rates. In a seminal analysis of >3,000 HCT recipients, international bone marrow transplant registry results confirmed that donor graft T cell depletion in the setting of HCT for AML in CR1 was associated with significantly increased relapse rates; similarly increased relapse rates were also seen in syngeneic recipients (20), as previously suggested (21). Further definitive evidence for the existence of a graft-versus-leukemia (GVL) effect came from trials of donor lymphocyte infusion following the relapse of patients with acute and chronic leukemias after HCT; sustained remissions were seen following donor lymphocyte infusion alone, suggesting that GVL effects were in some patients more potent than ablative chemotherapy (15). These and other collected observations cemented our understanding that the curative potential of HCT is dependent, in part, on immunotherapeutic effects and not simply on the ability to administer otherwise lethal doses of cytoreductive therapies.

The convincing demonstration of the GVL effects led to a seismic shift in our approach to HCT. Whereas early approaches maximized the role of cytotoxic chemotherapy and/or irradiation, and thus largely limited HCT to patients below age 55–60, the late 1990s witnessed the development of reduced-intensity ablative conditioning (RIC) and even nonmyeloablative conditioning regimens in patients with hematologic malignancies. Combinations of purine analogs (including fludarabine) and alkylating agents (e.g., melphalan) were safely administered in individuals up to age 70 in these early studies, resulting in excellent disease-free survival in patients with AML in CR1, and dramatically reduced TRM (22). However, further attempts to minimize toxicity by the use of truly nonmyeloablative regimens in HCT for AML yielded higher relapse rates and reduced OS (23), despite a reduction in TRM; these results suggested the importance of conventional cytoreduction as a partner to donor graft–derived GVL effects. We now understand that the kinetics of malignant cell growth dictate the optimal intensity of conditioning; for instance, the more indolent growth behavior of follicular lymphoma, even when transplanted following multiple relapses, may facilitate excellent long-term relapse-free survival after HCT even when truly nonmyeloablative regimens are used (24).

The advent of RIC, the use of peripheral blood stem cells, and general improvements in transplant care (including advances in nursing care and management of infections) have together facilitated dramatic reductions in HCT TRM. Leading academic centers will now consider HCT in patients up to age 70–75 without severe comorbidities. In general, the decision to pursue HCT depends on four factors:

1. Disease risk, especially likelihood of cure with non-HCT approaches.
2. Age of the recipient and comorbidities that may increase HCT TRM.
3. Availability of a donor. Siblings and matched unrelated donors yield similar outcomes; alternative donor approaches, including haploidentical and cord blood SCT, have dramatically improved but are still associated with higher TRM.

4. Psychosocial factors, including the ability to comply with much more complex therapies and follow-up, the presence of a dedicated caregiver(s), and adequate social and financial support.

THE IMPORTANCE OF RISK STRATIFICATION

As reviewed above, we now know that the best therapy for most patients with AML consists of induction therapy, using standard doses of cytarabine combined with intensified doses of anthracyclines. We have also learned that consolidation therapy (but not long-term maintenance) is necessary to achieve cures. Although we know that outcomes in relapsed disease are poor, and relapsed AML requires HCT to achieve a cure, we have historically had a difficult time predicting which patients will be cured with conventional induction and consolidation therapy alone. Approaches to HCT have dramatically improved, enabling application even to those who are older and have modest comorbidities, but transplantation is still associated with significantly increased early TRM, and it requires much more patient commitment (including the need for intensive medical therapy and compliance with caregiver support) than chemotherapy alone. Furthermore, even the augmented benefit of GVL associated with HCT cannot overcome the risk of ultimate relapse in a subset of patients with aggressive or resistant AML.

Optimal risk stratification would allow us to defer early HCT when chemotherapy alone is likely to result in cure; conversely, patients with high-risk AML might not only benefit from transplantation in CR1 but also be candidates for novel induction approaches and/or sequential transplantation and post-transplant therapies. An additional group of patients might fare better with novel induction strategies that include targeted therapies, with or without standard HCT consolidation. Pragmatically, there may be patients who have uniformly poor outcomes regardless of initial therapy and/or HCT; ideally, these patients would be spared the added risks and compromises in quality of life associated with transplantation and be considered for novel clinical trials and/or palliative strategies focused on prolonging quality of life.

CYTOGENETIC RISK STRATIFICATION

By the late 1980s, analyses of leukemic blast karyotype by chromosome banding methods (25) had been demonstrated to have clear prognostic significance in AML (26). It was recognized that the likelihood of obtaining an initial remission and the probability of OS were associated with the presence of favorable cytogenetic abnormalities, including t(8:21), inv(16), and t(15:17). At the other end of the spectrum, deletions of chromosomes 5 or 7 or the presence of complex karyotype (i.e., multiple, unrelated cytogenetic abnormalities) were associated with poorer prognosis; individuals with diploid karyotype were classified as having intermediate risk. In the next decade, the prognostic value of cytogenetic classification was confirmed in the context of large clinical studies, including the Medical Research Council AML 10 trial of >1,600 younger (i.e., <55-year-old) subjects with newly diagnosed de novo or secondary AML (27). The value of cytogenetics was further validated in large cooperative group studies examining various postremission therapies in younger patients with newly diagnosed AML (28–30). Similar results have been seen in studies of

older (i.e., >60-year-old) subjects with AML (31). Although these earlier studies definitively addressed the importance of more frequently found cytogenetic abnormalities, the Medical Research Council conducted a more comprehensive analysis of 5,876 younger adult subjects, in order to assess the potential prognostic significance of rarer abnormalities (i.e., those with individual incidence <2%, but collectively seen in ~10% of AML cases) (32). This analysis confirmed prior adverse markers, e.g., t(3;3), inv(3), del(5q), −5, and −7, but also identified additional cytogenetic abnormalities with adverse prognostic implications, including –17 and abnormalities of 17p (associated with loss of *TP53*) (33). In patients without a clearly associated adverse karyotype, the presence of four or more unrelated abnormalities was also linked to poor prognosis (33).

HCT was traditionally applied in individuals with relapsed disease and unfavorable cytogenetic status. However, a seminal meta-analysis, despite including early studies of HCT with higher-than-reasonable TRM (by modern standards), demonstrated the benefit of HCT in intermediate-risk AML; in this analysis, the higher TRM with HCT was outweighed by the significantly reduced relapse risk (34).

INITIAL STUDIES OF MOLECULAR MARKERS FOR PROGNOSIS

Until the past decade, cytogenetic analysis and clinical features (e.g., age, response to initial therapy, de novo versus secondary disease) were the primary disease features available to predict relapse risk and to guide the choice of postremission therapy. However, a majority of patients (estimated at 55%) have no clonal cytogenetic abnormalities, making therapeutic decision making in intermediate-risk AML challenging. A 2008 study shed new light on the value of somatic mutational analysis in assigning risk in 872 patients with AML presenting with normal karyotype (NK-AML) (35). The authors sequenced five genes implicated in leukemic transformation: nucleophosmin 1 (*NPM1*), Fms-like tyrosine kinase 3 internal tandem duplication (*FLT3*-ITD), CCAAT/enhancer binding protein α (*CEBPA*), the mixed-lineage leukemia gene (*MLL*), and the neuroblastoma RAS viral oncogene homologue (*NRAS*). Among 438 patients with complete mutation data, at least one mutation was identified in 369 (84%) patients. In the four trials studied, all patients with an HLA-matched related donor were assigned to HCT. Further analyses suggested that a subset of patients (e.g., those with mutated *NPM1* without *FLT3*-ITD) derived no benefit from HCT, based on donor/no-donor comparisons, but others (except those with mutant *CEBPA*) had improved survival when transplanted (35). This landmark study demonstrated the independent prognostic value of molecular analyses in NK-AML and led to the incorporation of molecular analyses into a revised expert risk-stratification guideline (36) (Table 1).

Also in 2008, Ley et al. (37) used the technique of massively parallel sequencing to characterize the whole genome of leukemia blasts and healthy skin cells in a previously healthy woman in her 50s who presented with de novo AML with diploid cytogenetics, and who eventually relapsed and died. Analysis by next-generation sequencing revealed a total of 10 nonsynonymous somatic mutations. Two were well-characterized AML-associated mutations (including *FLT3*-ITD and mutation of *NPM1*). In contrast, the other eight somatic mutations were single-base changes not previously detected in an AML genome; four were in gene families implicated in cancer pathogenesis. Notably, mutations were present in both

the original and relapsed specimens, suggesting chemotherapy resistance that may have contributed to relapse.

CORRELATING THE MOLECULAR LANDSCAPE WITH RESPONSE TO THERAPY

To better define the landscape of somatic genetic alterations in AML, Patel et al. (38) performed a mutational analysis of 18 genes in 398 younger adults with AML who had previously been treated in the E1900 study (13) examining the clinical impact of anthracycline dose intensity. Nearly all subjects (97.3%) had at least one somatic alteration identified in these 18 genes. In descending order, the most common mutations were in *FLT3* (ITD or tyrosine kinase domain mutation), 37%; *NPM1*, 29%; and *DNMT3A*, 23%; other abnormalities were found in ≤10% of cases. Figure 1 shows frequencies of mutations and a Circos plot demonstrating patterns of co-occurrence of mutations in the entire cohort. Several abnormalities, including *MLL* partial tandem duplication (*MLL*-PTD), *FLT3*-ITD, and mutations in *ASXL1* and *PHF6*, were associated with reduced OS. In contrast, mutations in isocitrate dehydrogenase 2 (*IDH2*) and *CEBPA* were associated with improved OS. Critically, the favorable effect of *NPM1* mutations was restricted to patients who had concurrent mutations of either *IDH1* or *IDH2* along with mutated *NPM1* (38). Genetic predictors improved risk stratification independent of classic clinical risk factors (e.g., age, extent of white blood cell count, induction dose, and postremission therapy); furthermore, predictive value was confirmed in a validation cohort (38).

Several critical conclusions emerged from Patel et al.'s (38) molecular characterization of the E1900 cohort. First, the benefits of anthracycline dose intensification in improving OS appeared to be confined to patients with *DNMT3A* or *NPM1* mutations or *MLL* translocations (three-year OS 44% with high-dose daunorubicin versus 25% for standard dose), but not in those with wild-type *DNMT3A* and *NPM1* and no *MLL* translocations (three-year OS 35% for high dose and 39% for standard dose), suggesting intensification of induction benefited distinct genetically defined subgroups. Furthermore, the addition of genetic profiling further delineated risk subgroups in a group classically defined as intermediate risk by karyotype. Within these subjects, the presence of mutant *NPM1* along with either mutant *IDH1* or *IDH2*, in the absence of *FLT3*-ITD, defined a favorable subgroup. In contrast, even with *FLT3*-ITD negativity, the presence of mutant *TET2*, *MLL*-PTD, *ASXL1*, or *PHF6* conferred an unfavorable prognosis; in *FLT3*-ITD-positive patients, only co-occurrence of mutant *CEBPA* conferred intermediate risk. In contrast, patients with *FLT3*-ITD positivity and mutant *TET2*, *MLL*-PTD, *DNMT3A*, or trisomy 8 (all without mutant *CEBPA*) also had an unfavorable prognosis. An integrated risk profiling approach, demonstrating the additive value of molecular profiling as an adjunct to cytogenetic risk stratification based on the approach of Patel et al. is presented in Table 2. However, these data do not inform the use of HCT, and additional data are needed to delineate whether transplantation improves outcome in the context of molecularly defined risk-classification schema.

WIDER GENOMIC AND EPIGENOMIC ANALYSES

The remarkable evolution of next-generation sequencing approaches has yielded a rapidly expanding understanding of genetic and epigenetic alterations in AML, and the time required to sequence a genome has shrunk from two years in 2008 to weeks at present. In 2013, the Cancer Genome Atlas Research Network published the results of analyses of 200 clinically annotated adult cases of de novo AML using whole-genome sequencing (in 50 cases) or whole-exome sequencing (in 150 cases) along with RNA and microRNA sequencing and DNA-methylation analyses (39). The results revealed that although AML genomes had fewer mutations than most other cancer genomes in adults, an average of 13 mutations were still seen; of these, five were in genes that are recurrently mutated in AML. Significant mutations were noted in 23 genes, and another 237 were mutated in more than one subject (39). As in previous lower-throughput studies (e.g., 38), the most commonly mutated genes were *NPM1*, *FLT3*, *DNMT3A*, and *IDH1* or *IDH2*. Besides mutations in *NPM1* (in 27% of subjects), classes of genes most commonly involved included DNA signaling genes (59%), methylation-related genes (in 44%), chromatin-modifying genes (30%), myeloid transcription-factor genes (22%), transcription-factor fusions (18%), tumor suppressors (16%), spliceosome-complex genes (14%), and cohesin-complex genes (13%). Critically, several of these classes of genes have not been integrated into large-scale prognostic models as of this writing. Further scrutiny revealed patterns of both cooperativity (e.g., between mutations in *NPM1*, *FLT3*, and *DNMT3A*) and exclusivity (e.g., *MLL*-containing fusions and *PML-RARA* were mutually exclusive of mutations in *NPM1* and *DNMT3A*, and *RUNX1* and *TP53* mutations were mutually exclusive of *FLT3* and *NPM1* mutations) (39).

RECURRENT MUTATIONS

The following is a brief summary of a subset of genes recurrently and more commonly mutated in adult AML.

Nucleophosmin 1 (NPM1)

NPM1 is a nucleolar protein whose diverse functions include ribosome biogenesis, DNA repair, and regulation of apoptosis. *NPM1* mutation is among the most common genetic mutations in AML, seen in 25–35% of patients and 45–64% of NK-AML cases. In the absence of *FLT3*-ITD, *NPM1* mutations are associated with improved survival in NK-AML. The beneficial effect may be associated with co-occurrence of mutations in *IDH1* or *IDH2.* NPM1 has no specific inhibitors.

Fms-Like Tyrosine Kinase 3 (FLT3)

FLT3 is a class III family receptor tyrosine kinase and is the receptor for the cytokine FLT3-ligand. Internal tandem duplication (ITD) is common, found in 20% of patients with AML and 28–34% of patients with NK-AML. In 28% of cases, mutations occur in the tyrosine kinase domain. The presence of *FLT3*-ITD is associated with increased relapse (with chemotherapy and with HCT) and decreased OS, and is dependent on the allelic ratio of mutant to wild-type FLT3. Tyrosine kinase domain mutations are associated with

particularly poor prognosis. Inhibitors of FLT3 include the first-generation compounds sorafenib, sunitinib, midostaurin, and lestaurtinib, and the second-generation compound quizartinib among others.

DNA-Methyltransferase 3A (DNMT3A)

DNMT3A is an epigenetic regulator mediating DNA methylation at cytosine residues. *DNMT3A* mutation is common (seen in 20–25% of NK-AML) and often found in concert with *NPM1* and *FLT3* mutations. Its presence seems to predict poorer prognosis, although Patel et al. (38) described an adverse effect only in tandem with *FLT3*-ITD; an additional study failed to demonstrate prognostic significance in treatment-related and secondary AML (40). Azacitidine and decitabine are DNMT inhibitors and are used both in initial therapy and to prevent relapse after HCT (41, 42).

RAS (NRAS, KRAS)

NRAS and KRAS are signaling proteins involved in hematopoiesis, with critical roles in normal cell signaling and differentiation and with constitutive activation common in cancers. *NRAS* mutations occur in 25% of NK-AML and *KRAS* mutations in 15%. Their prognostic significance is unclear, although the RAS/MEK/ERK pathway in malignant transformation is well characterized. Direct therapeutic inhibition of the RAS pathway by farnesyl inhibitors was ineffective. Healthy lymphocytes are dependent on RAS/MEK/ERK activation. First- and second-generation inhibitors (e.g., selumetinib, trametinib) have been shown to attenuate alloreactivity mediating GVHD after murine HCT. Trials that use MEK inhibitors for dual purpose (e.g., inhibition of GVHD and limiting relapse) are being considered (43).

Isocitrate Dehydrogenase (IDH1 and IDH2)

IDH1 and IDH2 are NADP-dependent enzymes converting isocitrate to α-ketoglutarate. *IDH1* and *IDH2* mutations confer sensitivity to BCL-2 inhibition (44). The incidence of mutations is 15–30% in AML, higher in NK-AML. Their prognostic significance appears to be favorable when they co-occur with *NPM1* mutations, but possibly unfavorable in their absence and with wild-type *FLT3*. AG-221, a small-molecule inhibitor of mutant *IDH2*, has shown promise in models and is in human clinical trials; susceptibility to BCL-2 dependence may be targeted by ABT-199, a specific inhibitor of BCL-2.

Tet Methylcytosine Dioxygenase 2 (TET2)

TET2 is involved in epigenetic regulation. Recently, *FLT3*-ITD and TET deficiency wereshown to cooperatively induce DNA hypermethylation and leukomogenesis (45). Mutations occur in ~20% of AML and 10% of NK-AML. The prognostic significance of *TET2* mutation in AML has been unclear in clinical studies; it is apparently mutually exclusive with *IDH1* or *IDH2* expression, and preclinical evidence indicates cooperativity with *FLT3*-ITD. Studies in myelodysplasia suggest that *TET2* mutations predict response to hypomethylating agents, including azacitidine (46).

Runt-Related Transcription Factor 1 (RUNX1)

RUNX1 is a transcription factor that regulates hematopoietic stem cell differentiation. Approximately 5–13% of NK-AML cases manifest *RUNX1* mutation. Gene fusions involving *RUNX*, e.g., t(9;21), are favorable, but mutations involving *RUNX1* are more frequent in older subjects, associated with *ASXL1* mutations and with dismal prognosis. The small molecule AI-10–49 selectively binds to CBFβ-SMMHC and disrupts its binding to RUNX1, and delays leukemia in mice (47).

Tumor Protein 53 (TP53)

TP53 is a prototypic tumor suppressor gene. Mutations in *TP53* are extraordinarily common (>50%) across the spectrum of human cancers, ~8% in AML, and very common in AML with complex karyotype (up to 70%). Mutations are commonly seen in older adults with AML and associated with extremely poor prognosis. They result in loss of function and so are impossible to target directly.

Additional Sex Combs-Like 1 (ASXL1)

ASXL1 is an epigenetic regulator. The incidence of mutations is approximately 5–16% in NK-AML. Mutations are seen more frequently in secondary AML and in older recipients and are associated with poor prognosis. No specific inhibitors are in clinical trials.

Mixed-Lineage Leukemia (MLL)

MLL functions as a histone methyltransferase. Mutations occur in approximately 8–10% of NK-AML cases. *MLL* partial tandem duplication (*MLL*-PTD) was the first mutation found to confer adverse prognostic significance in the setting of NK-AML (48). Pharmacologic interactions of MLL with Menin are inhibited by small-molecule inhibitors MI-463 and MI-503 and have activity in preclinical models (49).

AN EMERGING UNDERSTANDING OF CLONAL EVOLUTION TO AML AT ONSET AND AT RELAPSE

In addition to our broader understanding of molecular events governing prognosis of overt AML, we are now beginning to derive a better understanding of the earliest stages of leukemia development and the mutations that underlie transformation. By analyzing sequence data derived from 2,728 subjects within the Cancer Genome Atlas, Xie et al. (50) discovered 77 blood-specific mutations in cancer-associated genes; 83% of these mutations were in 19 leukemia- and lymphoma-associated genes. Recurrently mutated genes included *DNMT3A*, *TET2*, *JAK2*, *ASXL1*, and *TP53.* The authors concluded that 2% of healthy individuals (and 5–6% of those over age 70) had pre-existing mutations, without overt disease, that might predispose them to subsequent malignant diseases. Jaiswal et al. (51) characterized sequence data from 17,182 subjects and found somatic mutations were rare in younger subjects but increased in frequency in septuagenarians (9.5%) and older subjects, with mutations seen in 18.4% of individuals aged 90–108. The majority of variants occurred in three genes associated with AML (*DNMT3A*, *TET2*, and *ASXL1*). In a simultaneously published report, Genovese et al. (52) confirmed these findings and additionally reported a

high frequency of mutations in the *PPM1D* gene in healthy subjects. Together, these reports suggest that isolated mutations in genes associated with AML are rare early in life but accumulate with age, and that cooperativity between mutations is needed for overt hematologic illness to develop.

We are also beginning to understand how signatures of clinically distinct subsets of AML may differ, as shown by a recent analysis of somatic mutation frequency in individuals presenting with therapy-related AML, secondary AML, and de novo AML. Lindsley et al. (53) found that unique somatic mutations were characteristic of and highly specific for secondary AML; furthermore, a subset of elderly patients with de novo AML shared a similar molecular signature, and this was associated with a lower remission rate and decreased event-free survival, highlighting the potential value of molecular characterization over historically defined clinical syndromes.

SUMMARY AND RECOMMENDATIONS

Two hundred years into the era of acute leukemia diagnosis, traditional diagnostic approaches based on morphologic and cytogenetic analyses are finally yielding to comprehensive assessments of somatic mutations in leukemic blasts. Molecular analyses may now be used to guide selection of postremission therapies including conventional chemotherapy and HCT, which combines malignant cytoreduction and adoptive immunotherapy. Given the rarity of AML, it will remain difficult to confidently assess how some molecular signatures will influence prognosis, especially when less commonly encountered mutations (or complex combinations) are found, given a growing array of therapeutic options.

Despite these limitations, we have sufficient data to recommend that at diagnosis all AML patients have their primary leukemia specimens assessed, at a minimum, for common somatic mutations that may stratify risk and inform decisions regarding the optimal postremission therapy, including the likely benefit of HCT in CR1. Given the increasing safety of HCT in individuals up to the age of 70 and beyond, we recommend HLA typing at diagnosis. HCT using a matched sibling or well-matched unrelated donor should be considered for patients with an integrated cytogenetic/molecular profile that confers intermediate and/or unfavorable risk (using the criteria outlined in Table 2).

Although the use of targeted therapies at initial diagnosis and the routine use of post-HCT maintenance therapies (e.g., with hypomethylating agents and/or targeted inhibitors) should for now be primarily confined to clinical trials, our increasing wealth of information should facilitate the design and testing of risk-targeted sequential strategies of induction, consolidation, and HCT (when appropriate). We are also likely to see the expansion of trials examining maintenance therapies in individuals at highest risk of relapse. Given the rapid reduction of the cost of performing molecular analyses and the potential to use this knowledge to develop more rational treatment strategies, the potential to achieve long-term survival in far more than 50% of adult patients with AML may finally be approaching.

Glossary

HiDAC	high-dose cytarabine
HCT	hematopoietic stem cell transplant
TRM	treatment-related mortality
CR1	first complete remission
GVHD	graft-versus-host disease
GVL effect	graft-versus-leukemia effect
NK-AML	acute myeloid leukemia with normal karyotype
ITD	internal tandem duplication
HLA	human leukocyte antigen
PTD	partial tandem duplication

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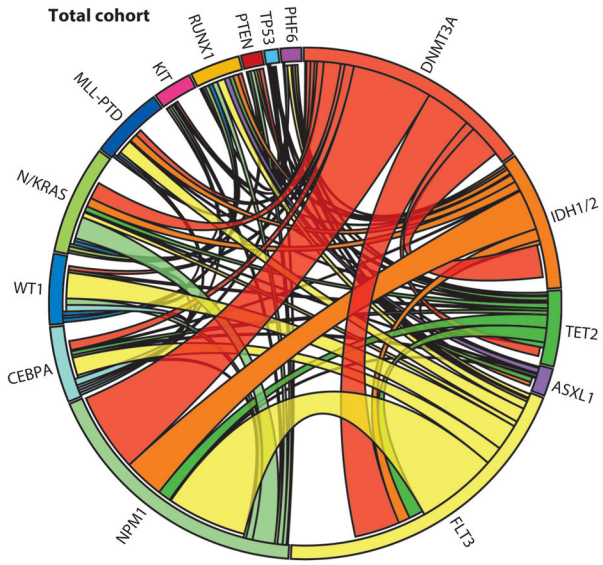

Gene	Overall frequency (%)
FLT3 (ITD, TKD)	37 (30, 7)
NPM1	29
DNMT3A	23
NRAS	10
CEBPA	9
TET2	8
WT1	8
IDH2	8
IDH1	7
KIT	6
RUNX1	5
MLL-PTD	5
ASXL1	3
PHF6	3
KRAS	2
PTEN	2
TP53	2
HRAS	0
EZH2	0

Figure 1.

Somatic mutations associated with acute myeloid leukemia. From Reference 38 with permission.

Table 1

European LeukemiaNet prognostic risk groups 36)

Genetic Group	Cytogenetics	Molecular Genetics
Favorable	t(8;21)(q22;q22) inv(16)(p13.1;q22) or t(16;16)(p13.1;q22) Normal karyotype (NK-AML)	Mutated *NPM1* without *FLT3*-ITD (NK-AML) or bi-allelic mutation of *CEBPA* (NK-AML)
Intermediate I	Normal karyotype (NK-AML)	Mutated *NPM1* and *FLT3*-ITD (NK-AML) Wild-type *NPM1* and *FLT3*-ITD (NK-AML) Wild-type *NPM1* without *FLT3*-ITD (NK-AML)
Intermediate II	t(9;11)(p22;q23) Cytogenetic abnormalities not classified as favorable or adverse	Not applicable
Adverse	inv(3)(q21;q26.2) t(6;9)(p23;q34) t(v;11)(v;q23) −5 or del(5q); −7; abnormal (17p) Complex karyotype[a]	Not applicable

[a]Defined as three or more chromosomal abnormalities in absence of WHO-designated recurring translocations or inversions: t(15;17), t(8;21), inv(16) or t(16;16), t(9;11)(v;q23), t(6;9), inv(3) or t(3;3).

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ITD, internal tandem duplication; NK-AML, normal-karyotype acute myeloid leukemia.

Table 2

Integrated cytogenetic and mutational profiling of Patel et al. (Ref. 38), applied to the ECOG 1900 cohort

Cytogenetic classification(incidence, survival)	**Mutational analysis**	**Integrated classification (incidence, survival)** ***Recommended postremission therapy***
Favorable (19% of cohort; 3-yr OS: 58%)	Any	Favorable (26% of cohort; 3-yr OS: 64%) *Consider alloHCT only at relapse*
Normal karyotype or intermediate-risk cytogenetic abnormalities (63% of cohort; 3-yr OS: 36%)	Favorable (*FLT3*-ITD negative, mutant *NPM1* and *IDH1* or *IDH2*) 3-yr OS: 85%	Intermediate (35% of cohort; 3-yr OS: 42%) *Consider alloHCT in CR1*
	Intermediate (*FLT3*-ITD negative; wild-type *ASXL1*, *MLL*-PTD, *PHF6*, and *TET2*) (*FLT3*-ITD negative or positive; mutant *CEBPA*) (*FLT3*-ITD positive; wild-type *MLL*-PTD, *TET2*, and *DNMT3A* and no trisomy 8) 3-yr OS: 42%	
	Unfavorable (*FLT3*-ITD negative; mutant *TET2*, *MLL*-PTD, *ASXL1*, or *PHF6*) (*FLT3*-ITD positive; mutant *TET2*, *MLL*-PTD, *DNMT3A*, or trisomy 8, without mutant *CEBPA*) 3-yr OS: 13%	Unfavorable (39% of cohort; 3-yr OS: 12%) *Consider alloHCT in CR1*
Unfavorable (18% of cohort; 3-yr OS: 11%)	Any	

Abbreviations: alloHCT, allogeneic hematopoietic stem cell transplant; CR1, first complete remission; ITD, internal tandem duplication; OS, overall survival; PTD, partial tendem duplication; yr, year.