Review

Genetic Abnormalities and Challenges in the Treatment of Acute Myeloid Leukemia

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Abstract

Acute myeloid leukemia (AML) is a hematopoietic disorder in which there are too many immature blood-forming cells accumulating in the bone marrow and interfering with the production of normal blood cells. It has long been recognized that AML is a clinically heterogeneous disease characterized by a multitude of chromosomal abnormalities and gene mutations, which translate to marked differences in responses and survival following chemotherapy. The cytogenetic and molecular genetic aberrations associated with AML are not mutually exclusive and often coexist in the leukemic cells. AML is a disease of the elderly, with a mean age of diagnosis of 70 years. Adverse cytogenetic abnormalities increase with age, and within each cytogenetic group, prognosis with standard treatment worsens with age. In the past 20 years, there has been little improvement in chemotherapeutic regimens and hence the overall survival for patients with AML. A huge unmet need exists for efficacious targeted therapies for elderly patients that are less toxic than available chemotherapy regimens. The multitude of chromosomal and genetic abnormalities makes the treatment of AML a challenging prospect. A detailed understanding of the molecular changes associated with the chromosomal and genetic abnormalities in AML is likely to provide a rationale for therapy design and biomarker development. This review summarizes the variety of cytogenetic and genetic changes observed in AML and gives an overview of the clinical status of new drugs in development.

Keywords

acute myeloid leukemia, genetic abnormalities, new drugs

Introduction

Acute myeloid leukemia (AML) is a clonal hematopoietic disorder resulting from genetic alterations in normal hematopoietic stem cells. These alterations disrupt normal differentiation and/or cause excessive proliferation of abnormal immature leukemic cells known as blasts. As the disease progresses, blast cells accumulate in the bone marrow, blood, and organs and interfere with the production of normal blood cells. This leads to fatal infection, bleeding, or organ infiltration in the absence of treatment within 1 year of diagnosis.[1-3] AML is characterized by more than 20% blasts in bone marrow. AML can arise *de novo* or secondarily either due to the progression of other diseases or due to treatment with cytotoxic agents (referred to as therapy-related AML). Up to 10% to 15% of patients with AML develop the disorder after treatment with cytotoxic chemotherapy (usually for a solid cancer). There are 2 main types of therapy-related AML. The "classic" alkylating-agent type has a latency period of 5 to 7 years and is often associated with abnormalities of chromosomes 5 and/or 7.[4] Exposure to agents, such as etoposide and teniposide, that inhibit the DNA repair enzyme topoisomerase II is associated with secondary AML with a shorter latency period, usually 1 to 3 years, with rearrangements at chromosome 11q23.[5] Drugs, such as chloramphenicol, phenylbutazone, chloroquine, and methoxypsoralen, can induce marrow damage

occur because of progression of myelodysplastic syndrome (MDS) or chronic bone marrow stem cell disorders, such as polycythemia vera, chronic myeloid leukemia, primary thrombocytosis, or paroxysmal nocturnal hemoglobinuria.[6,7] Secondary AML has a particularly poor prognosis and is not considered to be curable, with the exception of secondary acute promyelocytic leukemia (APL).[8] This is largely due to the high percentage of secondary AML associated with multidrug resistance (MDR) mechanisms: up to 70% of secondary AML patients show overexpression of P-glycoprotein (Pgp) or other MDR mechanisms.[9]

The genetic changes in leukemic blasts make them ineffective at generating mature red blood cells, neutrophils, monocytes, and platelets. In addition, these AML blasts also inhibit normal blasts from differentiating into mature progeny. Inhibition does not result from "crowding out" of normal blasts; rather, inhibition may be mediated by various chemokines produced by AML blasts.[10] AML progresses rapidly and is typically fatal within weeks or months if left untreated. The most common cause of death in AML is bone marrow failure, and the principal sign of marrow failure is infection. Potential fatal organ infiltration, most

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commonly involving the lung and the brain, becomes more likely as the disease progresses.

AML is the most common acute leukemia affecting adults, and its incidence increases with age.[1] Although the majority of patients under age 60 years achieve complete remission (CR) with traditional anthracycline- and cytarabine-based induction regimens, the long-term survival rates continue to be poor at approximately 30% to 40%.[11-13] The prognosis is even poorer for those with high-risk AML, such as those who are older, those who had preceding MDS or myeloproliferative disorders, or those with secondary AML from environmental exposures or prior chemotherapy. In such cases, CR is achieved in less than 40% of cases, with survival rates of less than 10%.[13] While 60% to 80% of younger patients achieve CR with standard therapy, only about 20% to 30% of the overall patient population has long-term disease-free survival.[3] Outcomes are worse for patients aged 60 years or over, with CR rates in the range of 40% to 55% and poor long-term survival rates.[3] Along with age, remission rates and overall survival depend on a number of other factors, including cytogenetics, previous bone marrow disorders such as MDS, and comorbidities.[3]

Epidemiology and Etiology of AML

AML accounts for approximately 25% of all leukemias diagnosed in adults, and the median age at diagnosis is 67 years.[13,14] In the United States, 43,050 new cases of leukemia were reported in the year 2010, of which 12,330 were new cases of AML. There were 21,840 patients who died in the year 2010 because of leukemia, of which 8,950 were attributed to AML.[15] The incidence of AML in the <65 years' age group is 1.8 cases per 100,000 patients, and the incidence in the >65 years' age group is 17.9 cases per 100,000 patients.[15] The incidence of AML is expected to increase in the future in line with the aging population, and along with its precursor myelodysplasia, AML prevalence appears to be increasing, particularly in the population older than 60 years of age, and represents the most common type of acute leukemia in adults. Table 1 shows the incidence and prevalence of AML in the United States and other developed countries.

Development of AML has been correlated with exposure to a variety of environmental agents, most likely due to links between exposure history and cytogenetic abnormalities. Radiation, benzene inhalation, alcohol use, smoking, dyes, and herbicide and pesticide exposure have all been implicated as potential risk factors for the development of AML.[16,17] Survivors of the atomic bombs in Japan had an increased incidence of myeloid leukemias that peaked approximately 5 to 7 years following exposure.[18] Therapeutic radiation also increases AML risk, particularly if given with alkylating agents such as cyclophosphamide, melphalan, and nitrogen mustard.

Table 1. Number of Incidence and Prevalence Cases of Acute Myeloid Leukemia (AML) in the Major Pharmaceuticals Markets in 2010

Markets	Incidence of new AML in 2010	Prevalence of AML in 2010
US	12,330	25,180
Europe	12,923	22,790
Japan	3,173	5,820
	28,426	53,790

Note: Incident cases are the new cases diagnosed within a particular time frame; prevalent cases are all cases present at a particular time. Prevalence is thus a function of incident cases and duration of disease.

Diagnosis and Classification of AML

Demonstration of the accumulation of blasts resulting from the block in differentiation, characteristic of AML, is the essential requirement of diagnosis.[19] The early signs of AML include fever, weakness and fatigue, loss of weight and appetite, and aches and pains in the bones or joints. Other signs of AML include tiny red spots in the skin, easy bruising and bleeding, frequent minor infections, and poor healing of minor cuts. The 2 systems commonly used in the classification of AML are the French-American-British (FAB) system and the World Health Organization (WHO) system. The FAB system is based on morphology and cytochemistry and recognizes 8 subtypes of AML, as shown in Table 2.[20] In 1999, the WHO classification was introduced to include newer prognostic factors, such as molecular markers and chromosome translocations, and lowered the blast minimum criterion to 20%, thus including many cases classified as high-grade MDS in the FAB system.[21] The WHO classification system identifies 4 AML subgroups: 1) AML with recurrent genetic abnormalities, 2) AML with multilineage dysplasia, 3) therapy-related AML and MDS, and 4) those that do not fall into any of these groups. This system created a minimum of 17 subclasses of AML, allowing physicians to identify subgroups of patients who might benefit from specific treatment strategies. Recently, a revised classification has been published as part of the fourth edition of the WHO monograph series.[22] The aim of the revision was to incorporate new scientific and clinical information to refine diagnostic criteria for previously described neoplasms and to introduce newly recognized disease entities.

Cytogenetic Abnormalities in AML

AML is characterized by a high degree of heterogeneity with respect to chromosome abnormalities, gene mutations, and changes in expression of multiple genes and microRNAs. Cytogenetic abnormalities can be detected in approximately 50% to 60% of newly diagnosed AML

Table 2. French-American-British (FAB) Classification of Acute Myeloid Leukemia (AML)

FAB subtype	Morphological classification	% of all AML cases
AML-M0	Undifferentiated acute myeloblastic leukemia	5
AML-M1	Acute myeloblastic leukemia with minimal maturation	15
AML-M2	Acute myeloblastic leukemia with maturation	25
AML-M3	Acute promyelocytic leukemia	10
AML-M4	Acute myelomonocytic leukemia	20
AML-M4 eos	Acute myelomonocytic leukemia with eosinophilia	5
AML-M5	Acute monocytic leukemia	10
AML-M6	Acute erythroid leukemia	5
AML-M7	Acute megakaryoblastic leukemia	5

patients.[23] The majority of AML cases are associated with nonrandom chromosomal translocations that often result in gene arrangements. Cytogenetics is the most important prognostic factor for predicting remission rate, relapse, and overall survival.[23] Several chromosomal abnormalities such as monosomies or deletions of part or all of chromosomes 5 or 7 (–5/–7 AML) and trisomy 8 are common in AML.[24] The chromosomal abnormalities also include the long arm of chromosome 11 (11q); balanced translocations between chromosomes 15 and 17 (t(15;17)); chromosomes 8 and 21 (t(8;21)); others such as (q22;q22), (q31;q22), and t(9;11); and inversion such as inv(16).[25] Table 3 shows the most frequent chromosomal aberrations and their corresponding fusion genes in AML. The translocation in t(15;17) is always associated with APL and leads to the expression of PML-RARα oncofusion gene in hematopoietic myeloid cells.[26] Generally, patients with APL t(15;17) phenotype represent a unique group characterized by distinct biological features and good prognosis, particularly when all-trans retinoic acid (ATRA) is used as part of remission induction.

Many of the gene rearrangements involve a locus encoding a transcriptional activator, leading to expression of a fusion protein that retains the DNA-binding motifs of the wild-type protein. Moreover, in many instances, the fusion partner is a transcriptional protein that is capable of interacting with a corepressor complex.[27] A commonly accepted paradigm is that through aberrant recruitment of a corepressor to a locus of active transcription, the fusion protein alters expression of target genes necessary for myeloid development, thus laying the groundwork for leukemic transformation.[28] Potential targeting of this interaction has become a major focus for the development of novel therapeutics. ATRA serves as a prototype: by altering corepressor interaction with the APL fusion protein, ATRA effectively induces remission and has become a mainstay of treatment of this previously fatal disease.[8] However, to date, APL represents both the most curable and the best-studied subtype of AML, while molecular data on other fusion proteins are limited or absent. Still, the work on PML-RARα has inspired the molecular analysis of many other AML-associated oncofusion proteins, especially AML1-ETO, CBFβ-MYH11, and MLL fusions.

Table 3. Acute Myeloid Leukemia (AML)–Associated Oncofusion Proteins

Translocations	Oncofusion protein	Frequency of occurrence(% of AML)
t(8;21)	AML1-ETO	10%
t(15;17)	PML-RARα	10%
inv(16)	CBF□MYH11	5%
der(11q23)	MLL-fusions	4%
t(9;22)	BCR-ABL1	2%
t(6;9)	DEK-CAN	<1%
t(1;22)	OTT-MAL	<1%
t(8;16)	MOZ-CBP	<1%
t(7;11)	NUP98-HOXA9	<1%
t(12;22)	MN1-TEL	<1%
inv(3)	RPN1-EVI1	<1%
t(16;21)	FUS-ERG	<1%

Oncofusion Proteins Associated with AML

A total of 749 chromosomal aberrations have been catalogued in AML.[29] The frequencies of the 4 most common translocations are between 3% and 10%, while for others, the prevalence is significantly smaller. The most frequent oncofusion proteins, PML-RARα, AML1-ETO, CBFβ-MYH11, and MLL fusions, are described below.

t(15;17), PML-RARα

The t(15;17) translocation is found in approximately 95% of APLs, a specific subtype of AML. The translocation results in the expression of the PML-RARα oncofusion gene in hematopoietic myeloid cells.[8] The PML-RARα oncofusion protein acts as a transcriptional repressor that interferes with gene expression programs involved in differentiation, apoptosis, and self-renewal.[8]

t(8;21), AML1-ETO

Approximately 10% of AML cases carry the t(8;21) translocation, which involves the AML1 (RUNX1) and ETO genes, and express the resulting AML1-ETO fusion protein. AML1 is a DNA-binding transcription factor crucial for hematopoietic differentiation,[30,31] while ETO is a protein harboring transcriptional repressor activities.[32] The fusion protein AML1-ETO is suggested to function as a transcriptional repressor that blocks AML1-dependent transactivation in various promoter reporter assays, suggesting it may function as a dominant-negative regulator of wild-type AML1.[33,34]

inv(16), CBFβ-MYH11

inv(16) is found in approximately 8% of AML cases. inv(16) fuses the first 165 amino acids of core binding factor β (CBFβ) to the C-terminal coiled-coil region of a smooth muscle myosin heavy chain (MYH11). CBFβ-MYH11 fusion protein is suggested to cooperate with AML1 to repress transcription.[35,36]

11q23, MLL Rearrangements

Mixed lineage leukemia (MLL) is implicated in at least 10% of acute leukemias of various types. In general, the prognosis is poor for patients harboring MLL translocations.[37] In these patients, the MLL protein fuses to 1 of >50 identified partner genes, resulting in an MLL fusion protein that acts as a potent oncogene.[38] The amino-terminal portion of MLL serves as a targeting unit to direct MLL oncoprotein complexes to their target loci through DNA binding, whereas the fusion partner portion serves as an effecter unit that causes sustained transactivation.

Gene Mutations in AML

Approximately 40% to 50% of patients with AML have a normal karyotype and represent the largest subset of AML.[39] All such cases of cytogenetically normal AML are currently categorized in the intermediate-risk group; yet, this group is quite heterogeneous, and not all patients in this subset have the same response to treatment. This is likely a result of the large variability in gene mutations and gene expression in this population. These alterations appear to fall into 2 broadly defined complementation groups. One group (class I) comprises mutations that activate signal transduction pathways and thereby increase the proliferation or survival, or both, of hematopoietic progenitor cells. The other complementation group (class II) comprises mutations that affect transcription factors or components of the cell cycle machinery and cause impaired differentiation.

Class I Mutations

Mutations in KIT, FLT3, and NRAS fall into the class I mutations.

KIT mutations. Although patients with AML and inv(16) and t(8;21) in general have a more favorable prognosis, there remains a significant failure rate, and the long-term disease-free survival rate is approximately 60%. Studies have shown that activating KIT mutations in approximately 30% to 40% of patients with inv(16) are associated with higher incidence of relapse and significantly lower survival. In those with t(8;21), the incidence of KIT mutations appears to be variable.[40]

FLT3 mutations. Fms-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase that plays a key role in cell survival, proliferation, and differentiation of hematopoietic stem cells.[41,42] It is frequently overexpressed in acute leukemias. FLT3 mutations occur in approximately 30% of AML patients and confer a poor prognosis. The 2 major types of mutations that occur are internal tandem duplication (ITD) mutations of the juxtamembrane region and point mutations in the tyrosine kinase domain (TKD), which frequently involve aspartic acid 835 of the kinase domain. Both mutations result in constitutive activation of the receptor's tyrosine kinase activity in the absence of ligand.[41] The incidence of FLT3 mutations also increases with age, but the FLT3 ITD mutations have less prognostic impact in patients >60 years of age possibly because other adverse prognostic factors are more prevalent.

RAS mutations. Mutations in NRAS and KRAS occur in approximately 10% and 5% of AML patients, respectively. IRASS mutations occur only rarely in conjunction with FLT3 mutations and do not appear to have a significant impact on AML survival.[43]

Class II Mutations

In addition, mutations in MLL, brain and acute leukemia gene (BAAL), Wilms tumor gene (WT-1), CCAAT/enhancer-binding protein α (CEBPα), and nucleoplasmin 1 (NPM1) have also been observed in AML patients.[44-46] Recently, mutations in DNA methyltransferase gene *DNMT3A* have been identified in one third of patients with *de novo* AML with intermediate-risk cytogenetics.[47] *DNMT3A* represents 1 of 3 human genes that encodes DNA methyltransferase that catalyzes the addition of methyl groups to cytosine within CpG dinucleotide, resulting in repression of nearby genes. Genomes with *DNMT3A* mutations commonly harbored additional mutations in *FLT3*, *NPM1*, and *IDH1*. The presence of any *DNMT3A* mutation,

Table 4. Acute Myeloid Leukemia (AML) Cytogenetic Risk Groups

Karyotype	Frequency, %	Complete remission, %	Event-free survival, %
Favorable			
t(8;21)	5-10	90	60-70
inv(16)	5-10	90	60-70
t(15;17)	5-10	80-90	70
Intermediate			
Diploid, –Y	40-50	70-80	30-40
Unfavorable			
−5/–7	20-30	50	5-10
+8	10	60	10-20
11q23, 20q-, other	10-20	60	10

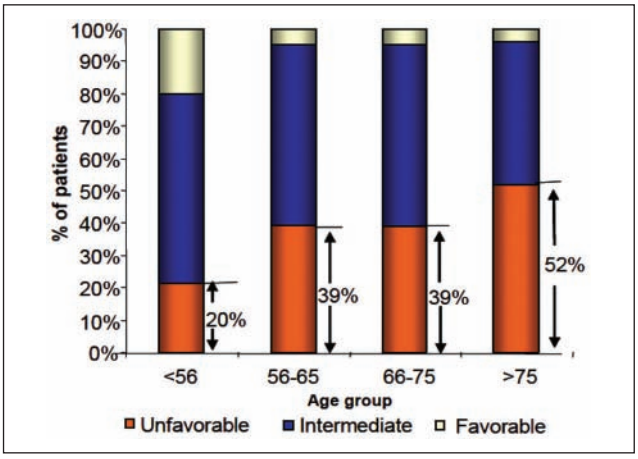

Figure 1. Cytogenetic risk group by age group. Adapted with permission from Appelbaum FR, Gundacker H, Head DR, *et al.* Age and acute myeloid leukemia. Blood. 2006;107:3481-5.

either alone or in combination with *FLT3* ITD mutation, is associated with significantly shorter overall survival (OS).[47]

Prognostic Factors in AML

Prognostic factors can be divided into those associated with treatment-related death occurring before response can be assessed and those associated with resistance to treatment. The predictor of treatment-related death is the patient's performance status. Therapy-related AML or AML arising after MDS is usually more resistant to treatment than *de novo* AML.[48] However, age and cytogenetics are the most important prognostic factors for predicting remission rate, relapse, and OS in AML. Risk stratification based on cytogenetics divides patients into 3 main groups: patients with favorable, intermediate, and unfavorable cytogenetics depending on the presence or absence of specific chromosomal abnormalities (Table 4). Studies have shown that the 5-year survival rate was 55% for patients with favorable cytogenetics, 24% for patients with intermediate risk, and 5% for patients with poor-risk cytogenetics.[24] Adverse cytogenetic abnormalities increase with age, and within each cytogenetic group, prognosis with standard treatment worsens with age.[3] A recent study demonstrated that the percentage of patients with unfavorable cytogenetics has been shown to increase from 35% in patients below 56 years of age to 51% in patients over 75 years (Fig. 1).[49]

Treatment of AML

The primary objective of therapy for AML is to achieve and maintain CR. CR is defined as a marrow with less than 5% blasts, a neutrophil count greater than 1,000, and a platelet count greater than 100,000. CR is the only response that leads to a cure or at least an extension in survival. The probability of AML recurrence sharply declines to <10% after 3 years in CR.[50] For the past 30 years, treatment of AML has consisted of the combination of an anthracycline, such as daunorubicin or idarubicin, and cytarabine.[51] Treatment of AML is divided into 2 phases: 1) remission induction therapy (with possible postinduction) and 2) postremission therapy.[52] Generally, AML treatment includes at least one course of intensive induction chemotherapy followed by an additional course of intensive consolidation therapy and then maintenance therapy.

Remission Induction Therapy

In induction therapy, the goal is to achieve a marked reduction in the number of malignant cells in order to establish normal hematopoiesis. A standard form of induction therapy consists of a standard dose of cytarabine (SDAraC, 100-200 mg/m^2), administered by continuous infusion for 7 days and combined with an anthracycline administered intravenously for 3 days (referred to as 7 + 3 regimen). With standard induction regimens, remission is achieved in about 65% to 85% of younger patients but in less than 50% of patients over 60 years of age.[2,53] This approach results in a long-term disease-free survival of approximately 30%, with treatment-related mortality of 5% to 10%. A number of studies have been conducted to improve the CR rate by use of alternative anthracyclines, incorporation of high-dose AraC (HDAraC), or addition of other agents such as etoposide, fludarabine, or cladribine. However, presently, there is no conclusive evidence to recommend one 7 + 3 induction regimen over another. However, these studies clearly support the conclusion that further intensification of the induction regimen is not associated with an increased CR rate.

In patients who fail to achieve CR following induction therapy, postinduction therapy is recommended. Postinduction therapy with standard-dose cytarabine is recommended in patients who have received standard-dose cytarabine induction and have significant residual blasts.[52] In other cases, postinduction therapy may consist of hematopoietic stem cell transplantation if a suitable donor can be found

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