Advances in Understanding the Biology and Genetics of Acute Myelocytic Leukemia

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Note: This article is also a continuation of the series on Neoplastic Hematologic Disorders that appeared in the previous issue of *Clinical Laboratory Science* (Fall 2004).

Acute myelocytic leukemia (AML) is a malignant neoplasm of hematopoietic cells characterized by an abnormal proliferation of myeloid precursor cells, decreased rate of self-destruction and an arrest in cellular differentiation. The leukemic cells have an abnormal survival advantage. Thus, the bone marrow and peripheral blood are characterized by leukocytosis with a predominance of immature cells, primarily blasts. As the immature cells accumulate in the bone marrow, they replace the normal myelocytic cells, megakaryocytes, and erythrocytic cells. This leads to a loss of normal bone marrow function and associated complications of bleeding, anemia, and infection. The incidence of AML increases with age, peaking in the sixth decade of life. In the United States, there are about 10,000 new cases of AML and 7,000 deaths in those with an AML diagnosis per year. Current molecular studies of AML demonstrate that it is a heterogeneous disorder of the myeloid cell lineage.

This paper will discuss the most recent understanding and research of the cellular origin of AML and associated common genetic mutations that fuel the neoplastic process. Also discussed are how these advances have impacted the classification, selection of therapy, and definition of complete remission in AML. Promyelocytic leukemia will be discussed in detail as this AML subtype reveals how our understanding of the biology and genetics of the disease has led to targeted therapy that results in a cure in up to 80% of patients.

ABBREVIATIONS: AML = acute myelocytic leukemia; APL = acute promyelocytic leukemia; ATRA = all trans retinoic acid; CBF = core binding factor; FAB = French-American-British; HDAC = histone deacetylase; ITD = internal tandem duplications; MDS = myelodysplastic syndrome; MLL = mixed lineage leukemia; NB = nuclear body; NCOR = nuclear corepressor; NK = natural killer; PML = promyelocytic leukemia; PTD = partial tandem duplications; RA = retinoic acid; RAR = retinoic acid receptor; TK = tyrosine kinase; WHO = World Health Organization.

INDEX TERMS: acute myelocytic leukemia; clonal genetic mutations; hematopoietic stem cells; lineage commitment; PML-RARA; promyelocytic leukemia.

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Focus Continuing Education Credit*: see pages 57 to 59 for learning objectives, test questions, and application form.*

LEARNING OBJECTIVES

1. Explain the cancer stem cell hierarchical model and how it applies to acute myelocytic leukemias (AMLs).
2. Correlate cytogenetic and molecular genetic findings in the diagnosis and prognosis of AML.
3. Compare and contrast class I and class II mutations in AML and give examples of each.
4. Explain the functions of the PML/RARA fusion protein in promyelocytic leukemia (PML).
5. Propose what will occur at the molecular level when ATRA is given to patients with PML and correlate with clinical findings in the patients.
6. Assess how advances in our understanding of the biology and genetics of hematopoietic neoplasms has affected the classification of these disorders.
7. Compare and contrast hematologic remission, cytogenetic remission, and molecular remission.

CELLULAR ORIGIN OF AML

Cancer is a clonal disease in that it is initiated by mutations in a single cell. The mutated cell produces progeny that form the

tumor. The mutations occur in genes that control cell proliferation, survival, or differentiation and act as dominant genes. These mutated genes are known as oncogenes. Their normal cellular counterparts are called proto oncogenes. Cancer can also result from mutations in tumor suppressor genes. These genes are recessive genes. In AML, the progeny of the original tumor cell are functionally heterogeneous. This raises two confounding questions: What is the target cell where the original mutation occurred and which tumor cells have the capacity to sustain or re-initiate the tumor? Understanding which progenitor cell is the target mutated malignant cell (leukemic stem cell) helps in understanding the cellular processes that are affected by the mutation and may help identify specific therapy that targets the mutated gene or protein.

Two models have been proposed to answer the above questions.[1] The first proposes that each tumor cell has the capacity to renew the tumor. The second model is based on a stem cell hierarchical framework in which the target cell is a cancer stem cell that may give rise to cells capable of reinitiating the tumor *and* cells that have limited capacity to differentiate. The extent to which the target cell progeny retain the functional and morphological properties of stem cells depends on the extent to which the target cell can differentiate. For example, if the leukemic cell progeny can differentiate into more mature cells such as in chronic myelocytic leukemia (CML), the more mature cells are not able to renew and appear morphologically different from the leukemic stem cell. In CML, the BCR-ABL gene translocation is in an early stem cell that has both lymphoid and myeloid differentiation capacity. This helps explain the fact that this disease may progress to either myeloid or lymphoid blast crisis.

Even though most AML patients go into hematologic remission with therapy, less than 50% are cured. This suggests that although therapy is effective at killing most leukemic cells, it does not kill the leukemic stem cell. It also suggests that the leukemic blasts identified by morphology and flow cytometry do not have the same cell markers as the leukemic stem cell since leukemic blast cells are not detected during complete remission.[2] If the cancer stem cell model is accurate, then the target mutated cell is probably at the top of the hierarchy and represents a minority of cells, as in the hematopoietic stem cell theory. Stem cells have a high self-renewal capacity and if dysregulated could provide unrestrained self-renewal (cancer cell). Stem cells are also long-lived so it would be easier for these cells to accumulate the number of mutations needed to develop into a cancer stem cell than it would be for more differentiated cells.

Support for the cancer stem cell theory comes from studies in mice.[3] One measure of self-renewal is the ability of a transplanted cell to form progeny. Primitive hematopoietic precursor cells have the following cell phenotype: CD34+, CD38-, CD71-, Thy1-. When transplanted into mice the CD34+, CD38- AML cell fraction initiated an AML-like disease. Yet these cells made up only 1/100 to 1/1000 of the leukemic cells found in the mice. CD34+, CD38+ cells, and CD34- cells did not initiate tumors, but constituted the bulk of the leukemic cells. Analysis of different AML FAB subtypes revealed that, despite morphological differences of the predominant immature hematopoietic cell, the repopulating cells are mostly quiescent and found in the CD34+, CD38- population. This suggests that the uncommitted progenitor cells in AML are the targeted mutant cells. The heterogeneity of AML may be explained by the occurrence of different mutations in the target mutant cell. The expression of their leukemic gene product influences lineage commitment and the degree of cellular differentiation. The growing body of knowledge of genetic mutations in hematopoietic neoplasms has led to a new classification of these disorders, which is largely based on specific abnormal cell karyotypes and gene mutations by the World Health Organization (WHO) classification.

GENETIC MUTATIONS IN AML

Over 300 recurring chromosome translocations have been identified in leukemia. About a third of these have been cloned and characterized giving implications as to their causal role in leukemia.[4] In most cases these translocations result in expression of a chimeric fusion protein. The fusion proteins result in abnormal functions of cell self-renewal, proliferation, differentiation, and/or apoptosis.

Cytogenetic analysis of AML

Cytogenetic analysis is considered the single most important factor in determining prognosis in AML. Therefore it is recommended that karyotyping be done on every patient at diagnosis.[5]

Clonal cytogenetic mutations can be identified in up to 50% of patients with *de novo* AML.[6] These mutations have not all been defined at the molecular level. However, they currently are used to define the overall survival risk status and to make treatment decisions. The Southwest Oncology Group and Eastern Cooperative Oncology Group studied the outcomes of 609 patients with AML after induction therapy. They identified four cytogenetic risk status groups: favorable, intermediate, unfavorable, and unknown (Table 1).[7] Seventy-one

percent achieved complete remission but the rate varied among the groups from 84% in the favorable group to 55% in the unfavorable group.

There was a significant interaction between the cytogenetic risk group and treatment effect on overall survival. Those patients in the favorable cytogenetic group had a better response to autologous bone marrow transplant and allogeneic bone marrow transplant than to chemotherapy alone. Those in the unfavorable cytogenetic risk group did better with allogeneic bone marrow transplant. Cytogenetic karyotypes involving core binding factor mutations have been shown to benefit from high-dose cytarabine.[8] The outcome of promyelocytic leukemia (PML), sometimes referred to as acute promyelocytic leukemia (APL), with the t(15;17) mutation is promising when treated with all-trans retinoic acid (ATRA). Thus, cytogenetic analysis is a very important parameter in diagnosis and treatment of AML.

Table 1. Cytogenetic risk groups based on complete remission rates with therapy according to Southwest Oncology Group and Eastern Cooperative Oncology Group definitions[7]

Favorable
inv/del(16)
t(16;16)
t(15;17)
t(8;21) without del(9q) or complex karyotypes
Intermediate
normal
Trisomy 8
Trisomy 6
-Y
del(12p)
Unfavorable
11q/23 mutations
del(5q)
-5
-7
del(7q)
abn3q
9q
11q
20q
21q
17p
t(6;9)
t(9;22)
complex karyotypes (>3)

Although 50% of AML patients have no identifiable karyotype abnormalities, molecular mutations may be present. Examples of this are the partial tandem duplication of the mixed lineage leukemia (MLL) gene and length mutations of FMS-like tyrosine kinase 3 (FLT3) detected by molecular methods but not by cytogenetics. AML that is secondary (occurs in a patient with previous myelodysplastic syndrome [MDS] or myeloproliferative disorders [MPD]) or that is therapy-related, may show cytogenetic abnormalities distinctly different from those of primary AML.

Molecular genetics of AML

It is a well-accepted concept by hematologists that, for development of the full neoplastic process of myeloproliferation and de-differentiation in AML, two broad, cooperating mutations are necessary, Class I and II. Class I mutations give a proliferative and/or survival advantage to the mutated myeloid precursors (Table 2). These mutations have no effect on differentiation. They result in constitutive activation of tyrosine kinase receptors or downstream effectors. Class II mutations complement Class I mutations and impair myeloid differentiation (Table 2). By interfering with terminal differentiation and apoptosis, Class II mutations also provide a survival advantage. Class II mutations involving core binding factors (CBF) are the most common in acute leukemia but this mutation alone is not sufficient to cause leukemia. Mouse models show that leukemia is accelerated in animals with CBF mutations if chemical mutagens are given.[9,10] This supports the need for additional mutations for the leukemic process to develop.

Common Class I mutations

Tyrosine kinases (TKs) are normally involved in regulation of hematopoiesis (cell proliferation, migration, differentiation, and survival) as well as other cell functions. Several growth factor receptors have TK activity (TK receptors). These TK receptors have an extracellular domain that binds ligands, a transmembrane domain, and an intracellular tyrosine kinase domain (Figure 1). When a growth factor binds the extracellular domain of the receptor, the TK becomes active and transfers phosphate from ATP to tyrosine residues on intracellular proteins. Phosphorylation is a common mode of regulating the activity of intracellular proteins.

Many TK genes are proto oncogenes. When mutated, they are constitutively activated and can induce uncontrolled cellular proliferation, inhibit differentiation and apoptosis, and decrease adhesion.[11] Recently, a second role for TKs in cancer was identified.[12-14] Tumors that express these mutated proteins are resistant to chemotherapy and irradiation. There are at least four mechanisms through which the TKs can become constitutively activated. These include chromosome translocation, truncation, over expression, and activating mutations.[11] The mutations remove the inhibitory domains or induce a configuration that activates the TK. The first TK oncogene identified in a hematopoietic neoplasm was BCR/ABL found in CML.

The FLT3 gene codes for a receptor with TK activity that is involved in the proliferation and differentiation and/or survival of hematopoietic stem cells. The FLT3 ligand (FL, also known as stem cell factor/SCF) is expressed in bone marrow stroma in both membrane-bound and soluble forms. Binding of a ligand to the normal FLT3 receptor enhances the effect of colony stimulating factor on hematopoietic progenitor cells in synergy with other colony stimulating factors. The receptor is involved in activating several signal transduction pathways as well as tyrosine phosphorylation. It is expressed on immature hematopoietic precursors (CD34+).

FLT3 is the most common mutated gene in AML. It is highly expressed in both AML and ALL.[15] Up to 41% of AML cases have activating alleles of FLT3.[15] FLT3 internal tandem duplications (ITD) are most common. Missense or in-frame deletion of critical residues, most often Asp 835, is less common and associated with a higher WBC count and higher blast count than FLT3 (ITD). When mutated, FLT3 protein is a constitutively activated cell receptor through autophosphorylation (growth factor independent). This results in a proliferation and survival advantage to the mutated cell. It is believed that FLT3 mutations work together with other gene mutations to cause AML. The FLT3 mutation by itself causes a myeloproliferative phenotype but does not result in AML. The FLT3 mutations are present in patients with t(15;17), t(8;21), inv(16), and 11q23 rearrangements.

Most studies reveal that the duration of remission and overall survival are shorter in patients with the FLT3 mutation than in those who lack the mutation.[16,17] The occurrence of FLT3(ITD) was found to be lower in children than in adults with AML.[18] However this may be related to the smaller normal cytogenetics subgroup in children (15% to 30%). It is found in all FAB subtypes of AML.

Class II mutations

Core binding factors, CBF, are transcription factors with two subunits. One subunit binds DNA (CBFα) and the other is a non-binding DNA subunit (CBFβ). The CBFs are involved in several hematopoietic cell pathways and are essential for normal hematopoietic development. In mice, knockout of both CBFα and CBFβ genes results in blocked hematopoiesis.[19-22]

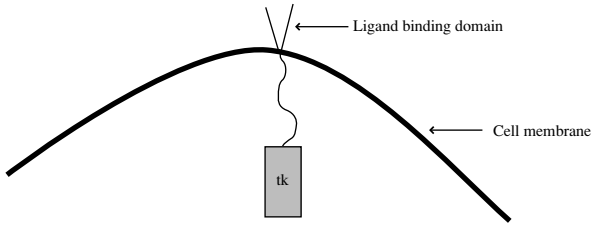
A gene encoding a CBFα, known as RUNX1, CBFA, or AML1 and another encoding a CBFβ subunit, CBFB, are

Table 2. Mutation classes

Class I mutations found in AML that confer a proliferative or survival advantage to leukemic cells:
- ABL
- FES
- Flt3
- c-Kit
- RAS
- TEL-PDGFRβ

Class II mutations found in AML that confer a survival advantage to leukemic cells by interfering with differentiation or apoptosis:
- AML1-ETO
- CBFB-MYH11
- CEBPα
- AML1 mutations
- MLL rearrangements

Figure 1. A tyrosine kinase receptor has an extracellular domain that binds ligand, a transmembrane domain, and the intracellular tyrosine kinase domain. When a ligand binds to the receptor, it activates the kinase which in turn phosphorylates proteins, a common way to regulate protein function. When mutated, these kinases may be constitutively activated.



frequently mutated in leukemia, particularly in acute lymphoblastic leukemia. The AML1 gene appears to influence cell maintenance and expansion or survival of hematopoietic stem cells or more differentiated progenitor cells.[23] In t(8;21), one of the most frequent genetic mutations in AML, the AML1 gene on chromosome 8 is fused to the ETO gene (also called MTG8 or CBF2T1) on chromosome 21. The AML1-ETO fusion protein inhibits normal function of CBF by repressing transcription, thus inhibiting myeloid differentiation. The mutation leads to an acute leukemia with FAB classification M2 morphology. It is associated with a long-term CR and disease free survival if treated with high dose cytarabine. The t(8;21) mutation can be detected by karyotyping, fluorescent in situ hybridization (FISH), and polymerase chain reaction (PCR). Another frequent mutation involving a CBF is pericentric inversion of chromosome 16, inv16(p13q22). In this mutation, there is a CBFB/MYH11 fusion. Although the molecular pathogenesis of this mutation is similar to t(8;21), it leads to a different leukemia morphology (FAB classification, M4eos).[24] Patients with CBF mutations are usually less than 60 years of age and enter a complete remission with standard chemotherapy.[25,26]

Translocation of the mixed lineage leukemia (MLL) gene (ALL1 or HRX) is found in both ALL and AML. It is located in the chromosome band 11q23 with a region of about 100 kb of DNA. It is associated with leukemia in infants as well as in mixed lineage leukemia. The wild type gene is thought to affect the proliferation and commitment of the hematopoietic stem cell to progenitor cells. Forty genes have been identified that can partner with MLL producing a fusion gene. The mutated gene is found in t(11q23) abnormalities, a common mutation point in AML and in 90% of patients with trisomy 11 (+11). MLL oncogenic fusion proteins increase the growth potential of cells. Molecular analysis reveals duplications of the MLL gene in about 10% of AML patients with normal karyotypes.[27] It was the first molecular mutation identified in AML patients with a normal karyotype.[28] The significance of this mutation is not understood as partial tandem duplications (PTD) of MLL can be identified in the blood and bone marrow of healthy individuals.[29]

BIOLOGY AND GENETICS OF PROMYELOCYTIC LEUKEMIA (PML)

Promyelocytic leukemia is the best understood AML. It is the first AML in which a specific molecular mutation was identified, the cellular pathway affected was identified, and a targeted molecular therapy was developed. PML is characterized by chromosomal translocations involving 17q21. This leads to rearrangements of the gene that codes for RARα (RARA), a nuclear hormone receptor involved in modulating myelopoiesis. The most common fusion partner (found in >95% of PML cases) is PML on chromosome 15 (t[15;17][q22;q12-21]) forming the PML/RARA fusion gene and protein. This mutation results in two recombinant chromosomes, 15q+ and 17q-. The PML protein is a nuclear protein that accumulates in distinctive subnuclear domains together with several other proteins. These domains are called the PML nuclear bodies (PML-NBs). PML protein localization in the NBs is thought to be essential for PML to modulate transcription. The fusion protein PML/RARA, can disrupt and delocalize PML protein from the NBs in the promyelocytic leukemic cells and thus, affect transcription of target genes.

Target mutant cell

The target mutated cell in some cases of promyelocytic leukemia (FAB classification M3) may be different than the mutated progenitor cell in other groups of AML. Some investigations of clonality and the PML/RARA expression suggest that the mutation is restricted to a range of more committed progenitor cells.

In one study, the PML clone with the PML/RARA fusion gene arose in CD34+ and CD38+ progenitors (more differentiated myeloid cells).[30] CD34 was expressed at the granulocyte-monocyte precursor stage but was absent on the promyelocyte. There was no involvement of the CD34+ CD38- cells (primitive hematopoietic progenitor cells).

In another study, when the PML-RARA mutation was expressed in very early progenitors and in myelocytes and mature neutrophils, the mutation did not result in APL.[31] However, in the case of early progenitors, the PML-RARA fusion protein was not detected in the hematopoietic compartment. Thus the fusion protein may not have been expressed at a high enough level to cause disease. These findings suggest that the target mutation occurs in a cell that has not differentiated beyond the granulocyte-monocyte precursor cell.

The immunophenotype of PML cells is characterized by homogeneous expression of CD33, heterogeneous expression of CD13, HLA-DR-, CD34-, CD11b-, CD15-, CD9+, and absence of lymphoid antigens (except CD19). In some studies, there is atypical expression of both lymphoid and myeloid markers in PML blasts that suggest the mutation occurred in an undifferentiated stem cell. Some harbor the PML/RARA fusion in cells that express CD34 and CD56

(natural killer, NK cell), CD19 (B-cell marker), and CD2 (T-cell marker) but are CD38-. The CD34 cells with lymphoid markers are associated with the hypogranular variant of PML (FAB classification, M3v).[32]

The association of lymphoid markers with myeloid markers raises the debate of lineage infidelity or lineage promiscuity. In the lineage infidelity model, the coexpression of myeloid and lymphoid markers on the same cell may be due to a deregulation of the lineage-associated genes during leukemic transformation.[33] In the lineage promiscuity model, the coexpression of these markers may reflect the immunophenotype of the mutated progenitor cell and perpetuated in the cell's progeny.[34]

More recent studies of murine bone marrow indicate that myeloid and lymphoid genes are both expressed on pluripotential progenitor cells prior to commitment of the cell to one lineage or the other.[35,36] When cells become committed to the myeloid lineage, there is progressive silencing of the lymphoid and NK cell genes. On the other hand, when progenitor cells become committed to the lymphoid cell line, there is progressive silencing of the myeloid genes. Further, a study of leukemic transformation as a result of fusion genes involving MLL showed that the leukemic phenotype is dependent on the targeted mutated cell.[37] If the hematopoietic stem cells and multipotential progenitors were targeted, the result was AML, ALL, and biphenotypic leukemia. However if the committed myeloid progenitor cells or committed lymphoid progenitors were targeted, the result was the formation of only myeloid or lymphoid colonies respectively. Although the typical immunophenotype of PML blasts are CD34-, the majority of CD34+cells in these cases, harbor the PML/RARA fusion gene suggesting the mutation occurs in an earlier progenitor cell. Thus, in at least some cases, it appears that the mutated target cell in PML is the noncommitted hematopoietic progenitor cell.

The target mutated cell in PML may affect the biological characteristics and clinical presentation of PML. In patients with hypergranular PML (FAB classification M3), the BCR1 PML breakpoint is common. These cases most often express the wild-type FLT3 and the PML cells lack lymphoid markers. In the hypogranular variant of PML (FAB classification M3v), the BCR3 PML breakpoint is more common, the cells most often have activating mutations of FLT3 and the PML cells co-express lymphoid antigens. These findings suggest that the variants of PML are determined by the progenitor targeted and the related genetic mutations.

Genetic mutations

As discussed above, the genetic mutation diagnostic of PML is PML/RARA fusion gene and protein, a mutant of one of the retinoic acid receptors. This is a class II mutation that affects the ability of the cell to differentiate. The RARA part of the fusion protein retains both the DNA binding domain and the carboxy terminal E domain. This mediates interaction between the ligand, retinoic acid (RA), and the retinoid acid receptor (RAR) which is needed for high-affinity binding at the retinoid response elements.

The normal activity of PML protein (growth inhibitor and regulator of apoptosis) is disrupted in the fusion PML/RARA protein. PML-NBs are involved in transcriptional regulation. PML does not bind DNA directly but may regulate transcription through interactions with other proteins. Recently it has been found that tumor suppressor proteins also are located in the NBs, including p53. PML can activate transcription of p53 in an NB-dependent process. P53 is activated post-translationally by acetylation. Although PML does not have acetyltransferase activity, it may be important in the stability of p53-acetylation complex. PML may also regulate p53 phosphorylation. When PML is fused with RARA, the NBs are disrupted and PML function is deregulated which may give the leukemic PML cell a survival advantage.[38]

The following activities have been ascribed to the PML/RARA fusion protein:

- At physiologic levels of retinoic acid (RA), PML/RARA fusion protein complexes with nuclear co repressors (NCOR) and histone deacetylase (HDAC) with a higher affinity than wild-type RARA. This complex interferes with the transcription of downstream retinoid target genes. In addition, DNA methyltransferases are recruited and methylate key promoters which represses transcription of these target genes. This is thought to mediate the block in differentiation beyond the promyelocyte stage.
- PML/RARA forms a dimer with the wild type PML protein causing structural disruption of the PML nuclear body. This may interfere with signal transduction, apoptosis, and DNA transcription.

In rare cases the promyelocytic leukemia zinc finger (PLZF;11q23) fuses with RARA to form PLZF-RARA (t11;17)(q23;q21).[38] Promyelocytic leukemia zinc finger is a DNA binding transcription repressor and regulates apoptosis and cell proliferation. In PML patients with this translocation the prognosis is poor and there is no response

to ATRA treatment. Several other genes may fuse with RARA resulting in PML including nucleophosmin (NPM), t(5;17)(q35;q12-21), nuclear matrix associated (NuMA), t(11;12)(q13;q12-21) and STAT5b, del(17q).[39]

Many hematologic malignancies have both primary and secondary genetic abnormalities. Primary genetic changes are usually specific and required for the phenotype. An example is the PML/RARA mutation required for PML. Secondary genetic changes are non specific and can be found in different kinds of cancers. An example is loss of function of p53 or 5q-. The primary mutation may cause genomic instability and lead to the secondary mutations. In PML/RARA transgenic mouse models, 100% develop a myeloproliferative disease but less than 20% develop PML after a long latency period. The leukemic transformation event often occurs when there are other non-random chromosomal aberrations.[40-42] In mice, expression of the reciprocal fusion RARA/PML results in more mice developing PML but does not alter the latency of PML development. This suggests that additional mutations are necessary for the development of PML. (About 80% of human patients with PML express the reciprocal fusion RARA/PML in addition to PML/RARA.) The most common recurring secondary abnormalities in humans with PML are +8 or partial trisomy of 8q, and ider(17)t(15;17). The PML/RARA mutation may cause genetic instability resulting in the secondary mutations. The significance of these secondary mutations is not known as they are not associated with prognosis and do not predict response to therapy with ATRA.

Immunophenotyping

The clinical consequences of untreated PML are life threatening. Thus rapid identification of patients with this disease is important so that targeted therapy with ATRA can begin as soon as possible. Currently, morphology is the primary means of diagnosis. Morphology shows good correlation with the t(15;17) karyotype but morphology alone may miss the M3v variant of PML. The results of cytogenetics and molecular testing are not available for days. Thus, immunophenotyping may be helpful in providing timely results in cases in which morphology is equivocal. One study showed that morphology, cytochemistry with myeloperoxidase, and immunophenotyping using anti-PML-RARA antibodies is definitive and provides timely information for an accurate diagnosis.[43] Another study suggests using immunophenotyping for rapid discrimination using heterogeneous expression of CD13, the existence of a single major blast cell population, and a CD34/15 phenotype.[44]

Therapy

A new treatment strategy for acute leukemia is molecular targeting of genes by agents that induce the cell to differentiate and undergo apoptosis. The first such agent, ATRA, was introduced for treatment of PML in 1986.[45] Treatment with pharmacologic doses of ATRA is combined with chemotherapy. Complete remission is achieved in 90% to 95% of patients who receive ATRA with chemotherapy. However, up to 30% of patients relapse and become resistant to ATRA. At pharmacologic concentrations of ATRA, the following occurs:[46]

- ATRA binds to mutated RAR, causes degradation of the PML/RARA protein, and restores the cell's ability to differentiate.
- ATRA induces relocalization of PML in the nucleus and restores the natural structure of PML-NBs. This may restore the PML-NB related-function, including transcription of the p53 tumor suppressor gene.
- In the presence of pharmacological doses, binding of ATRA to PML/RARA results in a conformational change in the fusion protein. This causes nuclear corepressors to dissociate from the repressive complex and coactivator is recruited. This results in renewal of transcription of target genes and subsequent cell differentiation that ends with apoptosis.

More recently arsenic trioxide has been used in treatment of PML, particularly in patients with relapse PML.[39] Clinical remission can be achieved for at least 18 months but it is not clear if its use increases the five year survival rate. It has also been used in post-remission therapy to prevent disease recurrence. Arsenic can be carcinogenic or have antitumor effects. Arsenic induces apoptosis and degrades the PML-RARA oncoprotein.[47] It also causes PML to localize in the nucleus where it is degraded by proteosomes. Arsenic inhibits transcription of the telomerase gene. Telomerase is expressed in most cancer cells but not somatic cells after birth. Telomerase is important to maintain the length of chromosome ends that would shorten after cell division without it. When the telomeres reach a critical short length, the cells stop dividing and become senescent. Most cancer cells that lack telomerase have slow growth and death rates. Thus, therapy aimed at inhibiting telomerase is being investigated as a possible target therapy not only in PML but also in other cancers.

In one study, arsenic trioxide was given to patients who relapsed after achieving remission with ATRA and chemotherapy.[48] The patients were divided into two groups. One group was given ATRA plus arsenic and the other was given

only arsenic. In both groups there was an 80% remission rate. Thus, arsenic may be another promising therapy for PML.

REVISIONS IN CLASSIFICATION OF AML

The specific genetic mutations in AML have an impact on clinical behavior of the disease. Some mutations are specific for a particular type of AML and assist in classification while others have prognostic or treatment implications. AMLs with genetic abnormalities, distinct clinical features, and characteristic morphology are now classified as separate categories within the WHO classification system. Except for PML, these new AML categories do not correlate with the FAB classification, a morphology based classification system. However, morphology is still used to help initially identify AML that then is confirmed with genetic analysis. There are four main groups of AML in the WHO classification:

1. AML with recurrent cytogenetic translocations.
2. AML with myelodysplasia-related features.
3. Therapy-related AML and MDS.
4. AML not otherwise categorized.

The categories with distinct genetic aberrations are described in Table 3. These AMLs are excluded from the FAB classification. Patients with the t(8;21), inv(16), or t(15;17) genetic aberrations are diagnosed with AML regardless of the bone marrow or peripheral blood blast count. Severe multilineage dysplasia, prior therapy, and/or prior MDS are poor prognostic factors in AML.[49] It is suggested that these AMLs probably have a common pathogenesis. AMLs secondary to alkylating-therapy are associated with specific cytogenetic abnormalities (3q-, -5, -5q, -7, -7q, +8, +9, 11q-, 12p-, -18, -19, 20q-, +21, t[1;7], t[2;11]), and complex karyotypes. AML secondary to therapy with topoisomerase II inhibitors show cytogenetic abnormalities similar to those found in *de novo* AML and should be considered as distinct from alkylating-therapy AMLs. The most common abnormalities associated with topoisomerase II inhibitors are 11q23, t(8;21), inv(16), and t(15;17).

DEFINING COMPLETE REMISSION IN AML

As clinical research into new therapy for AML patients progresses and techniques to detect minimal residual disease (MRD) improve, the criteria for clinical remission must be redefined. The recommendations of the International Working Group are an attempt to standardize the design and report of clinical trials by revising guidelines for assessing patient response to therapy.[5] Cytochemical and phenotypic analysis should be done on all AML or suspected cases of AML. Cytogenetics performed at diagnosis is important in directing therapy and for prognosis. After therapy or with disease progression, karyotypes often vary from karyotypes at diagnosis. Immunophenotyping will help differentiate AML from ALL. Molecular studies are important in the development of targeted therapies and, in the case of PML-RARA, in delivering targeted therapy.

The goal of therapy in AML is to achieve a complete remission. In AML, there are three types of remission: hematologic, cytogenetic, and molecular. In hematologic remission, peripheral cell counts are normal, the differential count is normal, and the bone marrow has less than 5% blasts. The number of blasts in the peripheral blood is not significant if the bone marrow blast count is less than 5% blasts. In cytogenetic remission, all cells examined (usually 20) have a normal karyotype. In molecular remission, there is no evidence of a mutation at the molecular level. Molecular remission is considered most sensitive as it will identify abnormal cells at a sensitivity level of 1 in 10,000 to 1 in 100,000 cells. The recommended criteria for defining these various types of remission are listed in Table 4.[5] In cytogenetic remission, it is important to define the criteria for a normal karyotype including the number of metaphases required and technique used, i.e., fluorescence in situ hybridization is more sensitive than conventional banding. Molecular remission criteria also must be defined according to the molecular markers studied and the sensitivity of the assay used.

SUMMARY

Advances in biology and genetics have led to new insights into our understanding of AML. The molecular target for the original genetic mutation appears to be the primitive hematopoietic stem cell. The heterogeneity of AML is probably related to the specific mutation in the target cell. Two muta-

Table 3. Specific categories of AML with distinct genetic aberrations according to the WHO classification[50]

AML with t(8;21)(q22;22), AML1(CBFA/ETO)
APL t(15;17)(q22;q11-12)
AML with abnormal eosinophils, inv(16)(p13;q22) or t(16;16)(p13;q22), CBFB/MYH11
AML with 11q23(MLL) abnormalities

Table 4. Criteria for defining complete remission (CR). Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in AML[5]

Early treatment assessment
- Made 7 to 10 days after last dose of initial therapy
- <5% blasts in bone marrow

Morphologic leukemia-free state
- <5% blasts in bone marrow
- Absence of Auer rods
- Absence of extramedullary disease
- Absence of unique immunophenotype that was found in the pretreatment specimen

Morphologic CR
- <5% blasts in peripheral blood*
- Neutrophil count >1 x 10^9/L
- Platelets >100 x 10^9/L
- Transfusion independent
- No extramedullary leukemia
- Absence of dysplasia if present at diagnosis
- <5% bone marrow blasts
- No requirement for bone marrow cellularity

Cytogenetic CR (for clinical research studies only)
- Morphologic CR and reversion to normal karyotype

Molecular CR
- Morphologic CR and cytogenetic CR
- Absence of mutations by molecular methods in patients who had a detectable mutation at diagnosis
- Absence of aberrant phenotypes using multidimensional immunophenotyping by flow cytometry

* persistence of blasts in peripheral blood may be indicative of AML relapse

tions are thought to be necessary for development of AML. One mutation affects the proliferative potential of the cell and the other blocks the differentiation of the cell. Some mutations have a poorer prognosis than others. As these mutations are identified at the molecular level, new drugs are being developed to target the specific cellular aberration. Treatment of PML with the PML/RARA mutation is an example of a drug targeted specifically at the abnormal protein produced by the fusion gene. These advances have also led to new classifications of AML and definitions of remission.

The laboratory's role in identifying leukemic blasts by morphology and cytochemistry and differentiating ALL from AML by immunophenotyping, remains important for initial diagnosis. However, as the molecular basis for these malignancies are identified, the laboratory's role will expand to include more molecular testing for diagnosis and for identifying molecular remission following treatment.

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