Recent Advances in Acute Myeloid Leukemia

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Our expanding knowledge of acute myeloid leukemia (AML) over the last four decades has resulted in major advances in the understanding and classification of this disease. What we consider as acute myeloid leukemia is now known to represent a number of distinct, but related diseases, some of which require unique therapeutic approaches.

The World Health Organization (WHO) classification of AML attempts to incorporate some features of traditional AML classification (particularly the French-American-British Cooperative Group Classification, or FAB), while also recognizing the significance of cytogenetic changes, the presence of multilineage dysplasia and prior therapy. The WHO classification of AML include major categories of AML with recurring cytogenetic abnormalities, AML with multilineage dysplasia, therapy related AML and myelodysplasia, and AML, not otherwise categorized. While only four recurring cytogenetic abnormalities are currently in the classification, hopefully this category will be expanded in future editions. The WHO classification, which also includes other hematopoietic tumors, is now widely accepted for pathologic diagnosis, and thus represents a major advance in AML classification. Despite this, the transition from the FAB classification to the WHO system for many pathologists and hematologists is a difficult one.

Table. World Health Organization (WHO) Classification of Acute Myeloid Leukemia

Acute myeloid leukemia with recurrent cytogenetic translocations
   Acute myeloid leukemia with t(8;21)(q22;q22); ($AML1(CBF\alpha)/ETO$)
   Acute myeloid leukemia with abnormal bone marrow eosinophils
   inv(16)(p13q22) or t(16;16)(p13;q22); ($CBF\beta/MYH11$)
   Acute promyelocytic leukemia (AML with t(15;17)(q22;q112) ($PML/RAR\alpha$)
   and variants
   Acute myeloid leukemia with 11q23 (MLL) abnormalities
Acute myeloid leukemia with multilineage dysplasia
Acute myeloid leukemia and myelodysplastic syndromes, therapy related
Acute myeloid leukemia not otherwise categorized
   Acute myeloid leukemia minimally differentiated
   Acute myeloid leukemia without maturation
   Acute myeloid leukemia with maturation
   Acute myelomonocytic leukemia
   Acute monoblastic and monocytic leukemia
   Acute erythroid leukemia
   Acute megakaryoblastic leukemia
   Acute basophilic leukemia
   Acute panmyelosis with myelofibrosis
   Myeloid sarcoma

WHO Categories of AML

AML with Recurrent Cytogenetic Abnormalities

AML with t(8;21)

The t(8;21)(q22;q22) is present in 8-13% of pediatric and adult AMLs. Some patients may present with extramedullary tumors (myeloid or granulocytic sarcomas). The t(8;21) results in fusion of the *RUNX1* gene (aka *AML1*) on chromosome 21 with the *ETO* gene on chromosome 18, and the resulting RUNX1/ETO chimeric protein disrupts normal function of the core-binding factor, a transcription factor complex that regulates normal hematopoiesis. Despite disruption of the core binding factor by RUNX1/ETO, this genetic abnormality alone is not sufficient to cause leukemic transformation.

Blast cells with abundant pink granules, and slightly basophilic cytoplasm are characteristic of this disease. The blasts usually show perinuclear clearing, or hofs, and a subset of the blasts have large, irregular pink (often termed salmon-colored) cytoplasmic granules. Thin Auer rods may also be present, but may be difficult to identify with some Wright stains. Eosinophils that are morphologically normal, are often increased in number in the marrow, but this feature is not specific. Maturing granulocytes may show pinker cytoplasm or mild nuclear irregularities, but this granulocytic "dysplasia" should not be interpreted as evidence of multilineage dysplasia, which is not seen most cases of de novo AML with t(8;21). The large number of cytoplasmic granules in the neoplastic cells of this leukemia subtype may raise concern for making a diagnosis of acute leukemia, as the cells may not be considered true blasts by some, but the WHO classification considers all cases with this cytogenetic abnormality to represent AML regardless of blast count. Cases of this type are usually classified as AML-M2 in the FAB scheme, but only represent a small subset of FAB M2 AMLs.

AML with t(8;21) has a characteristic immunophenotype that can be helpful in making the diagnosis. The blast cells characteristically express CD13 and CD33, similar to other types of AML, but also usually express CD34 and the B-cell associated antigens CD19 and PAX5. A subset of cases may also express CD56, which is associated with a worse prognosis in some studies. Rare cases will not express CD13 or CD33, but will be positive for other myeloid-associated markers, such as myeloperoxidase. The detection of the distinctive granular blast cell population with perinuclear clearing and large pink cytoplasmic granules in the setting of myeloid antigen expression and CD34 and CD19 expression is highly predictive of the t(8;21), and molecular studies are warranted in this setting if the initial karyotype is normal. AML with t(8;21) is considered to have an intermediate to good prognosis with therapeutic regimens that include high-dose cytarabine.

AML with inv(16) or t(16;16)

Acute myeloid leukemia with inv(16)(p12q22) or t(16;16)(p13;q22) occurs in 5-10% of all AML cases and tends to occur in children or young adults; while only approximately 1-3% of older adult AMLs have this abnormality. Both cytogenetic abnormalities result in a fusion of the *CBFβ* and *MYH11* genes on chromosome 16,

which, similar to the t(8;21), disrupts the core binding factor transcription factor complex.

The bone marrow usually shows a proliferation of blasts with monocytic features and cytoplasmic granules as well as a population of abnormal eosinophils. This category generally corresponds to the AML M4Eo designation in the FAB classification. Not all cases, however, will show monocytic differentiation either by morphology or cytochemistry. The presence of an increase in normal-appearing eosinophils is not sufficient to suggest this diagnosis, as it can occur in a variety of AML subtypes. The maturing eosinophils must show abnormal basophilic granules that are large and coarse , which may give the appearance of basophils; however, there are readily identifiable background eosinophilic granules present in the cells as well. While the abnormal eosinophils are classically described as positive for chloroacetate esterase by cytochemistry, as opposed to normal eosinophils that are negative for chloroactetate esterase, this test is often only weakly positive and not usually necessary for the diagnosis. Rare cases will have numerous eosinophils and maturing monocytes, making the blast cell count less than 20%. However, the WHO scheme classifies this disease as acute leukemia regardless of blast cell count. Multilineage dysplasia is usually not present in this AML subtype, but subtle changes have been interpreted as dysplasia in at least one study. The detection of abnormal eosinophils is highly predictive of a chromosome 16 abnormality, which may be subtle on routine karyotype. Therefore, additional studies, such as RT-PCR or FISH should be performed on cases with abnormal eosinophils and an apparently normal karyotype. A significant percentage (up to 20%) of cases will not have abnormal eosinophils, and this cell type is often not present in the peripheral blood of patients with this disease. In cases without abnormal eosinophils, the precise diagnosis can only be made after cytogenetic or molecular genetic detection of an inv(16) or t(16;16).

AML with inv(16) or t(16;16) shows expression of the expected myeloid antigens (CD13 and CD33), and often monocyte-associated markers, such as CD4, CD14 and CD64. Immunophenotyping may show two distinct cell population, one with a more typical myeloblasts immunophenotype and one with a more monocytic phenotype, or only a single population may be detected. A subset of AML with inv(16) or t(16;16) cases will show aberrant expression of the T-cell associated marker CD2, but it is not specific for this disease.

AML with inv(16) or t(16;16) is generally associated with a favorable prognosis with high-dose cytarabine.

Acute Promyelocytic Leukemia

Acute promyelocytic leukemia (APL) represents 9-12% of AMLs and is frequently associated with disseminated intravascular coagulopathy (DIC). All molecular subtypes contain mutations of the retinoic acid receptor alpha (*RARα*) gene on chromosome 17. The t(15;17)(q22;q12) is the most common genetic aberration in APL and results in fusion of *RARα* with the *PML* gene on chromosome 15. This category generally corresponds to AML-M3 in the FAB classification.

A variety of morphologic types of APL are described, but they are most easily subdivided into hypergranular and hypogranular (or microgranular) forms of the disease. The classic *hypergranular* APL has "blast" cells that somewhat resemble normal

promyelocytes with abundant cytoplasmic granules. Numerous Auer rods are present in individual blasts (termed faggot cells). In contrast to the blasts of AML with t(8;21) or reactive promyelocyte proliferations, APL cells do not show differing stages of maturation or perinuclear clearing. *Hypogranular* APL is usually more difficult to recognize as a type of APL, and may be mistaken for a myelomonocytic leukemia. The blasts characteristically have folded or bilobed nuclei ("butterfly" nuclei) with very fine to undetectable cytoplasmic granules. Rare, hypergranular cells may be present with Auer rods, but they often require an extensive search to identify. Both types of APL show very strong myeloperoxidase positivity in virtually all blast cells, by both cytochemistry and flow cytometry, which is often helpful in differentiating hypogranular promyelocytes from myelomonoblasts or monoblasts. Some cases of APL contain a subset of blasts that are positive for non-specific esterase by cytochemistry. This could lead to confusion with an acute myelomonocytic leukemia, but features of APL take precedent over the cytochemical results. Multilineage dysplasia is not usually present in APL. While there are no well defined morphologic features to differentiate most of the cytogenetic variants of APL, cases with the *PLZF/RARα* molecular fusion are described as having distinctive features. These include more round blast cell nuclei with more variably sized cytoplasmic granules than other types of APL. In addition, circulating "Pelger-like" neutrophils are described with clumped chromatin and hyposegmented or unsegmented nuclei. However, this molecular subtype of APL is extremely uncommon, and the predictive value of these morphologic features is unclear.

APL cells express CD33 and CD13, although the latter may be weak and heterogenous. With CD45 gating, the blast cell area is expanded because of an increase in side scatter secondary to the cytoplasmic granularity of the leukemic cells. Unlike most types of AML, APL blasts are typically negative or only partially express HLA-DR. Other AML types, however, may show loss of HLA-DR, thus this immunophenotypic feature alone is non-specific. A subset of cases, usually of the hypogranular type, aberrantly express CD2, which is reportedly associated with a poorer prognosis.

The t(15;17) is usually detectable by karyotype analysis at diagnosis. Molecular detection of *PML/RARα* is usually performed by RT-PCR or FISH analysis, and these tests may be useful to rapidly confirm the initial diagnosis. However, the combined morphologic and immunophenotypic features can be extremely reliable in detecting this disease. Less commonly, chromosomal translocations of *RARα* on chromosome 17 may occur with other genes such as *PLZF* at 11q23, *NuMA* at 11q13, *NPM* at 5q35 and *STAT 5b* at 17q11. Cases that lack *RARα* translocations or harbor *RARα* translocations involving *PLZF* or *STAT 5b*, may not respond to all-*trans*-retinoic acid and thus require a different therapeutic approach. With current therapy, using all-*trans*-retinoic acid or arsenic trioxide, APL is considered to have a favorable prognosis.

AML with 11q23 abnormalities

AML with 11q23 (*MLL*) cytogenetic or molecular abnormalities is a specific subtype of AML in the WHO classification and represents 9-22% of pediatric AML cases, but probably only 4-5% of de novo adult AMLs. This disease is relatively common in infants. AMLs arising after topoisomerase II inhibitor therapy may have similar morphologic and genetic features, but would not be placed in this WHO category, since they are a subtype of therapy-related AML (see below). AML with 11q23

abnormalities usually have monocytic or myelomonocytic features and would usually be classified as AML M4 or M5 in the FAB scheme.

In the bone marrow, the blasts features are usually nonspecific but may have round to folded monocytoid nuclei with usually abundant, slightly basophilic and vacuolated cytoplasm. Promonocytes are often present, with more mature-shaped nuclei but retaining immature nuclear chromatin. More mature cells may predominate in the blood, which may suggest a chronic monocytic proliferation. Multilineage dysplasia is usually not present in the blood or marrow. Because the morphologic features are not specific for this molecular subtype of AML, there is no way to reliably predict an *MLL* abnormality without performing the appropriate cytogenetic and molecular assays.

The blasts may variably express myeloid-associated antigens CD13 and CD33, are usually CD34 and myeloperoxidase negative, and often express monocyte-associated markers such as CD4, CD14 and CD64. They may also express CD56.

This disease category is fairly heterogenous at the molecular level since over 40 different translocation partners have been reported to fuse with *MLL*. *MLL* genetic abnormalities that are not associated with balanced translocations may also occur. Despite the numerous translocation partners for *MLL*, the most common in AML are located on chromosomes 6q27 (*AF6* gene), 9p22 (*AF9*), 19p13.3(*ENL*), 19p13.1 (*ELL*), 19p13.3 (*EEN*), 16p13 (*CBP*) and 22q13 (*p300*). Partial tandem duplication mutations of *MLL* also occur in AML and are more common in patients with normal karyotypes or trisomy of chromosome 11. In general, the presence of *MLL* translocations or partial tandem duplications indicates an unfavorable prognosis, but the t(9;11) in childhood AML may actually confer a good prognosis.

AML with Multilineage Dysplasia

AML with multilineage dysplasia is a disease that shows dysplastic changes in the non-blast marrow and peripheral blood elements. The WHO classification defines AML with multilineage dysplasia as 20% or more bone marrow or peripheral blood blast cells with 50% or more dysplastic cells in at least two cells lines (erythroid, granulocytic or megakaryocytic). The 50% cutoff for dysplastic changes is arbitrary and excludes some cases with definite associated dysplastic changes.

Dysplastic changes may be seen in both peripheral blood and bone marrow samples. Red cell changes manifest as anisopoikilocytosis of peripheral blood red cells, including hypochromic teardrop-shaped cells and macrocytes, dimorphic red cell populations of the blood, nuclear-cytoplasmic asynchrony of red cell precursors, megaloblastic changes and irregularities of red cell precursor nuclei. Megaloblastic changes differ from a "left-shift" of erythroid cells by the presence of more immature nuclear chromatin, often associated with more mature, red-staining erythroid cell cytoplasm. Nuclear irregularities, including multinucleation, nuclear blebs and irregular nuclear contours are commonly seen in dysplastic erythroid precursors. Granulocyte dysplasia is most easily recognized in the more mature granulocyte forms of the blood and marrow. These changes include uneven cytoplasmic granulation or completely agranular mature neutrophils. Nuclear changes include clumping of nuclear chromatin that is usually associated with abnormalities of nuclear lobation, particularly monolobated or bilobate nuclei ("pseudo Pelger-Huet" cells). Some nuclear abnormalities may be seen with drug therapy and do not constitute true dysplasia. These

are usually not accompanied by hypogranulation. Therefore, a requirement of both features in the granulocyte line for dysplasia seems warranted. Peripheral blood platelets may show variation in platelet size with hypogranular platelets. Megakaryocytes may show great variation in size with detached, hyperlobated nuclei or hypolobated or monolobated forms with hyperchromatic nuclei.

Multilineage dysplasia-associated AML does not show specific blast cell morphologic features, and can include all types of FAB AMLs, although FAB-M3 leukemia with multilineage dysplasia is unusual. Therefore, there are no specific cytochemical or immunophenotypic features of the blast cells for this disease category. AML with multilineage dysplasia may occur *de novo* or may follow known myelodysplasia. Both types show an increased frequency of complex cytogenetic abnormalities, deletions or chromosomes 5 or 7, trisomies, or abnormalities of chromosome band 3q21. AML with multilineage dysplasia is generally associated with a poor prognosis, and separation of *de novo* cases and those arising from myelodysplasia does not appear to have clinical relevance.

Therapy-Related AML

Therapy-related AML is further subdivided in the WHO classification into alkylating agent related and topoisomerase II inhibitor related types. Alkylating agent-related disease usually occurs 5-7 years after therapy, and shows morphologic and cytogenetic changes similar to AML with multilineage dysplasia with complex karyotypes that commonly include abnormalities of chromosome 5 and 7. Topoisomerase II inhibitor-related disease generally has a shorter latency period of 2-3 years, tends to have monocytic features and usually do not have associated multilineage dysplasia. Abnormalities of the *MLL* gene of 11q23 and *AML1* gene of 21q22 are commonly present in these cases, but a variety of other abnormalities, including inv(16) and t(15;17) may also be seen. Therapy-related AML is generally associated with a poor prognosis, but cytogenetic studies are still useful to identify prognostic disease groups. Many patients receive a combination of therapies that may include both alkylating and topoisomerase II inhibitors, or develop therapy-related AML following radiation or administration of other drug types and it is often difficult to obtain the specifics of the prior therapy at the time of diagnosis. For this reason, it is often not possible to subdivide therapy-related AMLs.

AML, Not Otherwise Categorized

The WHO classification of AML includes a "not otherwise categorized" (NOC) disease group that includes ten different subgroups. Many of these have similarities to the original FAB disease groups, but differ in several ways. The WHO, NOC category does not include cases with the four recurring cytogenetic abnormalities mentioned above, therapy-related AML or AMLs with evidence of multilineage dysplasia, factors that would not exclude a case from being subtyped into one of the FAB categories. Also, the WHO classification for AML, NOC defines acute leukemia as bone marrow proliferations with 20% or more blasts as opposed to a 30% or higher cutoff for the FAB classification.   The details of each subtype are not included here, and there is little if any clinical significance to most of the AML, NOC categories. The most confusing categories are acute erythroid leukemia and acute megakaryoblastic leukemia.

Acute erythroid leukemia consists of at least two different categories in the WHO classification that roughly correspond to the original Di Guglielmo's syndrome and disease. Erythroleukemia or erythroid/myeloid leukemia of the WHO corresponds to FAB M6. It is a myeloid blast cell proliferation that occurs in association with erythroid hyperplasia. By definition, erythroid precursors must represent 50% or more of the bone marrow cells, and blast cells are 20% (in the WHO) or more of the *non-erythroid elements* for this diagnosis. Therefore, cases with very high bone marrow erythroid precursor numbers and relatively low blast cell counts might be diagnosed as acute leukemia. The vast majority of cases that fulfill criteria these criteria also show multilineage dysplasia and would therefore not qualify as a subtype of the WHO AML, NOC category. These cases would be considered to represent cases of AML with multilineage dysplasia or myelodysplasia by some authors, as discussed previously. Pure erythroid leukemia is a second type of acute erythroid leukemia in the WHO AML, NOC category. It is defined as a bone marrow proliferation of over 80% neoplastic, immature erythroid cells without a significant myeloblasts component. These cases are also frequently associated with multilineage dysplasia and could be considered as types of myelodysplasia. Pure erythroid leukemia is often referred to as M6b, but it was not included in the FAB classification. A third type of erythroid leukemia, sometimes referred to as M6c, has also been proposed. This is defined as a proliferation of 30% or more bone marrow erythroid precursors with 30% or more blasts among the non-erythroid cells. This category is not included in either the FAB or WHO classification and is not used by most hematopathologists. All of these erythroid leukemia types are associated with complex cytogenetic abnormalities and/or abnormalities of chromosomes 5 and 7, similar to AML with multilineage dysplasia or myelodysplasia.

Acute megakaryoblastic leukemia corresponds to FAB M7, and the WHO AML, NOC category would include the cases associated with t(1;22) and Down's syndrome described below. The majority of cases of adult M7, however, is associated with multilineage dysplasia and would be considered as AML with multilineage dysplasia in the WHO classification. The WHO defines acute megakaryoblastic leukemia as an acute leukemia in which 50% or more of the blasts are of megakaryocytic lineage. The morphologic, immunophenotypic and ultrastructural features are similar in all types. The blast cells often have moderately abundant, finely granular cytoplasm. Cytoplasmic blebs are frequently described as characteristic, but are too non-specific to be used as a sole diagnostic feature. The diagnosis has traditionally required either electron microscopy or immunophenotypic confirmation of the megakaryocyte lineage, the latter being the method of choice in most laboratories. The blasts are usually myeloperoxidase negative, but are positive for acid phosphatase and alpha-naphthyl acetate esterase (sodium fluoride resistant). They often express myeloid-associated antigens, such as CD13 and CD33 and should express at least two megakaryocyte-associated antigens, such as CD41, CD42 or CD61. Platelet adherence on other blast types, which has been reported to give false positive results for megakaryocyte markers by flow cytometry, has lead some laboratories to confirm megakaryocyte lineage of the blasts by immunocytochemistry. These cases are commonly associated with marked marrow fibrosis and a "dry tap" making diagnosis difficult in many cases.

Acute megakaryoblastic leukemia of Down's syndrome is clinically distinct from the transient myeloproliferative syndrome of Down's syndrome, and may occur one to

two years after the resolution of a transient neonatal proliferation. Approximately 70% of Down's syndrome-associated AML are acute megakaryoblastic leukemias. These leukemias usually occur around two years of age and have a generally favorable prognosis, but the prognosis worsens with advancing age.

AML Types not Specified in the WHO Classification

Despite the advances of the WHO classification, a number of clinically significant cytogenetic and molecular genetic types of AML are not included. These will certainly increase as more is learned about AML and only a select group is discussed.

Recurring Cytogenetic Abnormalities in de novo AML

Acute Megakaryoblastic Leukemia with t(1;22)
There appear to be at least three different types of acute megakaryoblastic leukemia, all of which would be considered as AML-M7 in the FAB classification. One type, acute megakaryoblastic leukemia with t(1;22) represents what appears to be a unique disease type that occurs in infants. While this disease is not included as a specific disease group in the AMLs with recurrent genetic abnormalities of the WHO classification, this de novo leukemia should be considered as a rare, but comparable entity. Acute megakaryoblastic leukemia of infants with the t(1;22)(p13;q13) results in an OTT/MAL fusion product. Although rare, this disease most commonly occurs before six months of age with a female predominance, and the presence of bone marrow dysmegakaryopoiesis. Many infants with AML harboring this fusion gene are long-term survivors.

Recurring Cytogenetic Abnormalities in AML with Multilineage Dysplasia

AML with t(6;9)
Balanced translocations are relatively uncommon in AML with multilineage dysplasia, but a few recurrent balanced translocations occur with this disease group. Acute myeloid leukemias with t(6;9)(p23;q34) is a rare, but distinct entity associated with erythroid hyperplasia, dysplasia and bone marrow basophilia. The t(6;9)(p23;q34) results in a fusion of *DEK* gene on chromosome 6 with the *CAN* (aka *NUP214*) gene on chromosome 9. Most reported cases would meet WHO criteria of acute myeloid leukemia with over 20% blast cells. While erythroid dysplasia appears to be the most common, a recent report has also noted at least some granulocyte and megakaryocyte dysplasia with this abnormality. Bone marrow basophils are also often increased, a finding that is extremely unusual in other AML types other than rare t(9;22) AMLs or myeloid blast crisis of chronic myelogenous leukemia. The blasts are myeloperoxidase positive by cytochemistry, express myeloid-associated markers, such as CD13 and CD33, and are often TdT and CD117 positive. Early reports suggested that this abnormality was more frequently associated with a lack of CD34 expression, but a more recent study has not confirmed this finding. While this leukemia has a generally poor prognosis in the literature, some patients appear to do well with aggressive therapy that included hematopoietic stem cell transplantation.

AML/MDS with t(3;5)

Myelodysplasia and acute myeloid leukemia with t(3;5)(q25;q35) appear to represent a distinct subset of myelodysplasia-associated diseases. Unlike most cases of AML with multilineage dysplasia and myelodysplasia, this disease tends to occur in young adults. This cytogenetic abnormality may occur with a spectrum of blast cell counts, ranging from refractory cytopenia with multilineage dysplasia and less than 5% blast cells to overt acute leukemia at presentation. The disease occurs most frequently in the third and fourth decades of life and may show a male predominance. In addition to the presence of multilineage dysplasia, there is often an erythroid hyperplasia that may fulfill criteria for acute erythroid leukemia. Auer rods are frequently seen with the abnormality, even in the cases with lower blast cell counts. Thrombocytosis is not a feature of this disease. The morphologic and immunophenotypic features of these cases are otherwise nonspecific. The t(3;5) results in a fusion of the *nucleophosmin* (*NPM*) gene and the *myeloid leukemia factor 1* (*MLF1*) genes. While early studies of this leukemia type suggested a poor prognosis, more recent studies suggest that a more favorable prognosis with hematopoietic stem cell transplantation.

AML/MDS with inv(3)

Myelodysplasia and AML with multilineage dysplasia associated with an inversion of chromosome 3 at q21q26 or translocations involving this chromosome region are distinct from the t(3;5) AMLs discussed above. The inv(3) AMLs most commonly involve the *ecotropic virus insertion site 1* (*EVI1*) gene, which may also be disrupted with a t(3;3)(q21;q26) or with other translocations. This disease is associated with multilineage dysplasia and thrombocytosis. Giant platelets may be seen in the peripheral blood which may not be counted by automated methods. The most distinctive bone marrow feature is the presence of small, unilobated or bilobated megakaryocytes. The megakaryocytes are similar to those seen in the 5q-minus syndrome of myelodysplasia, but they are accompanied by the presence of multilineage dysplasia and an increase in blast cells in inv(3) AML, features not seen with 5q-minus syndrome. This disease is usually associated with a poor prognosis.

Others Genetic Abnormalities in AML

A wide number of other genetic abnormalities also occur in acute myeloid leukemia, but the significance of many is not yet well understood. These include both balanced translocations as well as gene mutations. The significance of point mutations, as well as balanced translocations, involving the *MLL* gene is discussed above. In recent years, it has become clear that mutations of the *FLT3* and NPM1 genes are the most common genetic abnormalities in AML. *FLT3* mutations occur in 20-28% of adult AMLs and 11.5% of all de novo pediatric AMLs. They occur with almost any FAB type of AML and in association with any of the recurring cytogenetic abnormalities, but are most common in acute promyelocytic leukemia and in AMLs with a normal karyotype. *FLT3*-positive AML in children is associated with older age (median age of 13.4 years), and in many studies is associated with a worse outcome than *FLT3*-negative AML, although the latter finding remains controversial. These mutations are associated with decreased disease free survival in adult AML. Because of the relatively high frequency

and clinical significance of these mutations in AML, *FLT3* mutation testing has become common in many institutions.

Recently, mutations of the *NPM1* gene, which is distinct from the *MLF1/NPM1* fusion of t(3;5) described above, have been reported as the most common molecular genetic abnormality in AML.[164-168] Mutation of this gene occurred in 27.5% of cases in one study, and in almost half of patients with a normal karyotype. *NPM1* mutations in AML appear to be more common in de novo AML and are unusual in acute promyelocytic leukemia and t(6;9) AML. AML with this mutation is more common in females and is associated with high white blood cell counts and platelet counts as well as high bone marrow blast cell counts. *FLT3* mutations are also common in these patients, but patients with *NPM1* mutations that are not associated with FLT3 mutations have an improved response to therapy, while patients with wild type *NPM1* and mutated *FLT3* have the worse prognosis.

Mutations of the *CEBPA* gene occur in 7 to 11% of acute myeloid leukemia. Acute myeloid leukemias with *CEPBA* mutations are associated with intermediate risk cytogenetics and are more commonly associated with FAB M1 or M2 morphology (although virtually all FAB types are reported). Detection of a *CEPBA* mutation is generally associated with a favorable prognosis, although patients with both *FLT3* and *CEPBA* mutations have an intermediate prognosis.

Acute myeloid leukemia represents a number of disease entities, and a combined morphologic, immunophenotypic and genetic approach is the best means of accurately diagnosing specific disease types. While new discoveries make diagnosis more complicated, they also allow for identification of new targets for therapy and provide new hope for improved outcome in many AML patients.

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