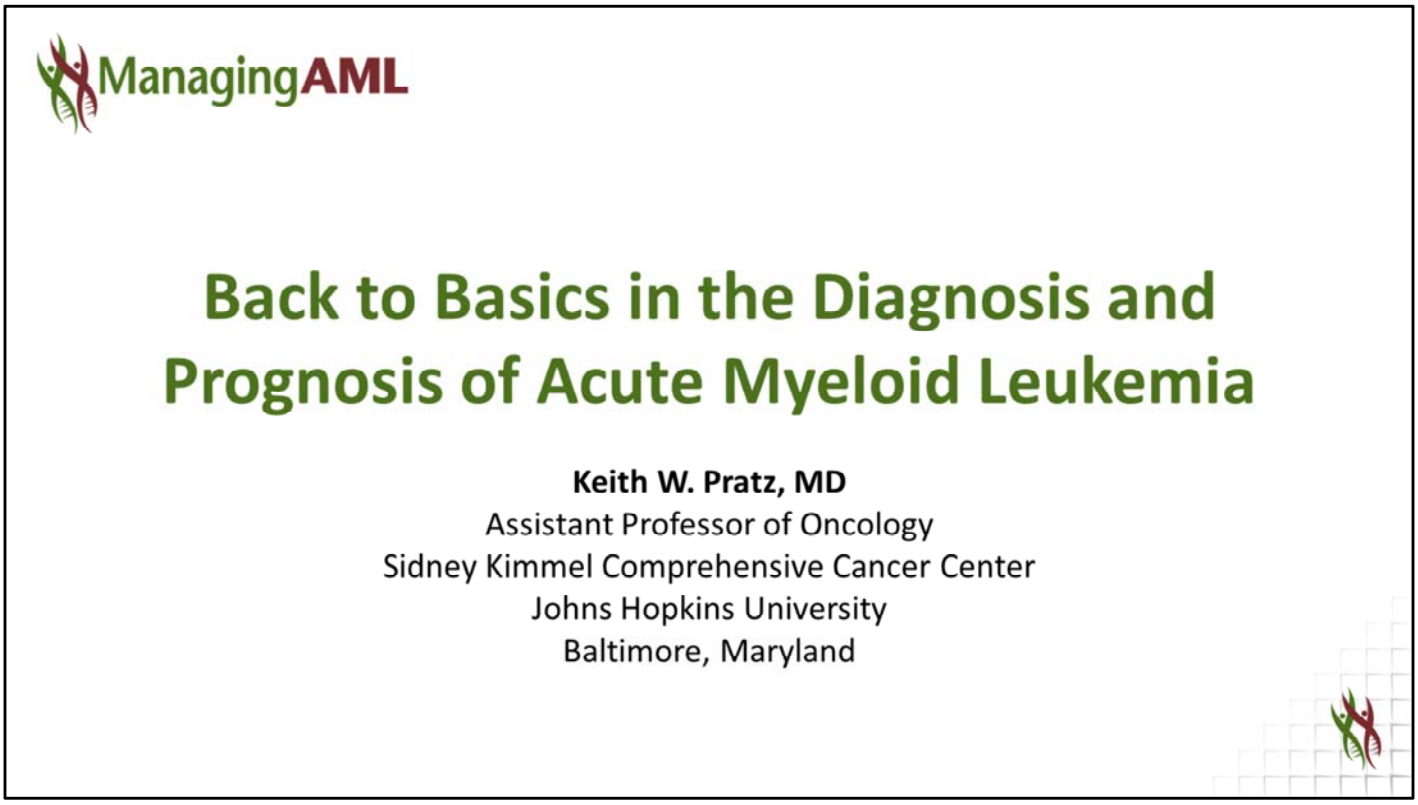
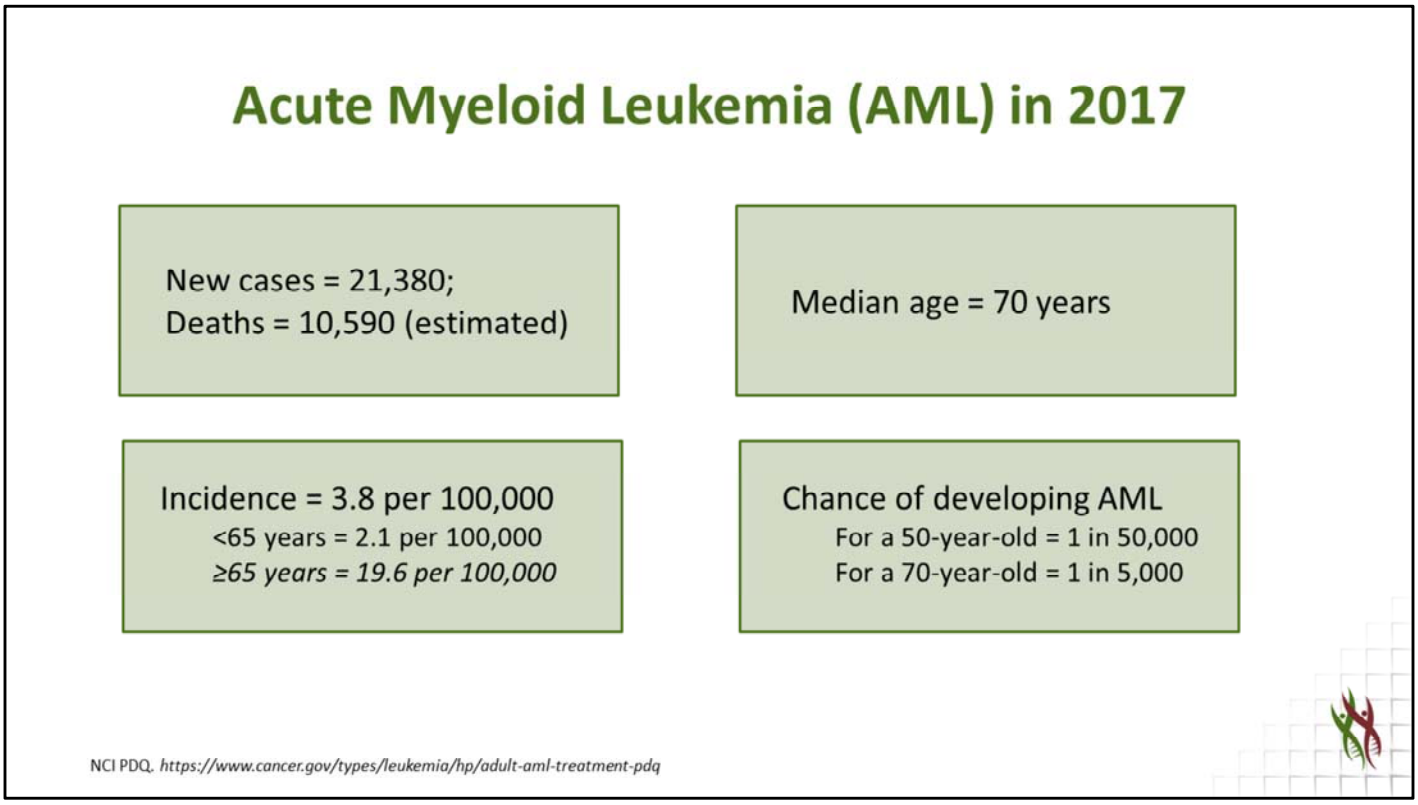
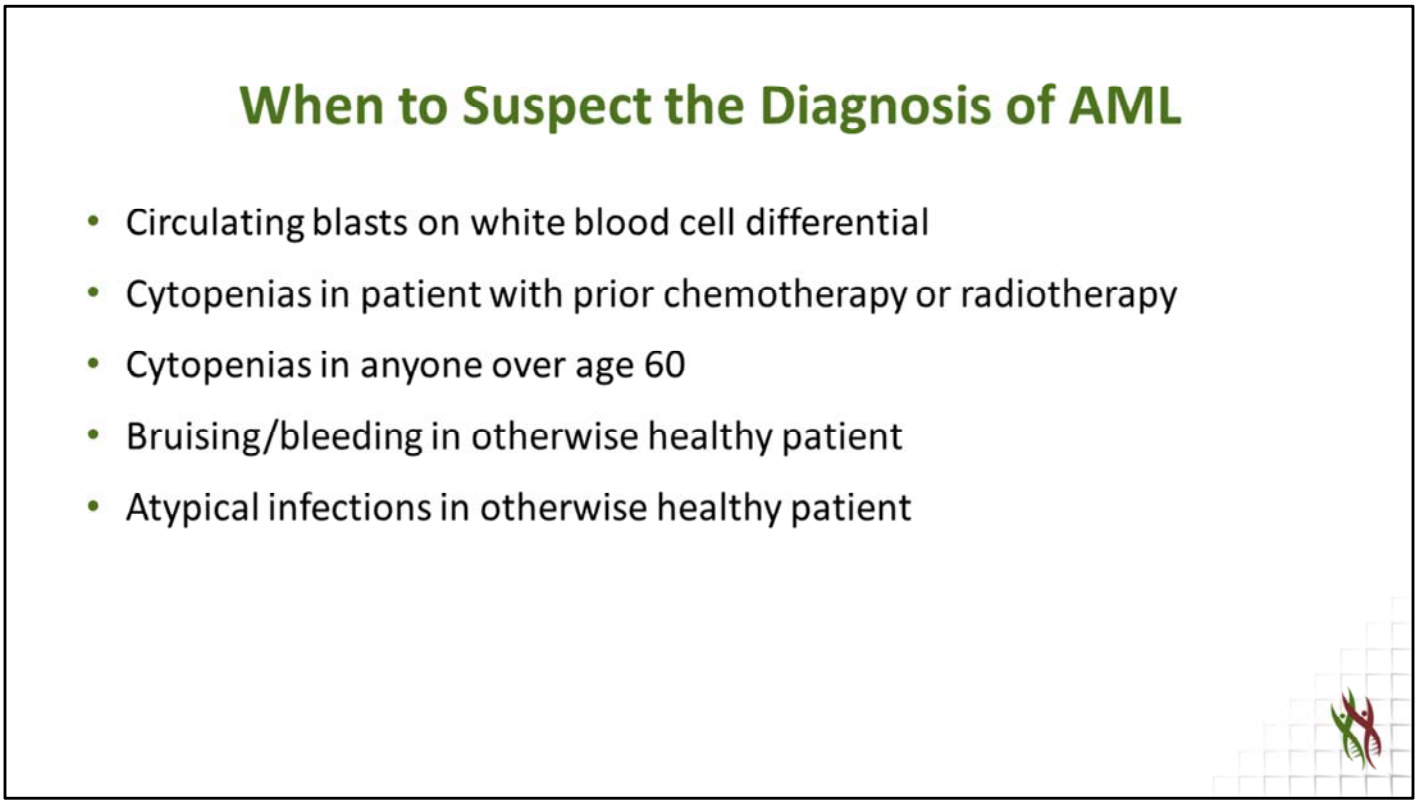
Back to Basics in the Diagnosis and Prognosis of Acute Myeloid Leukemia



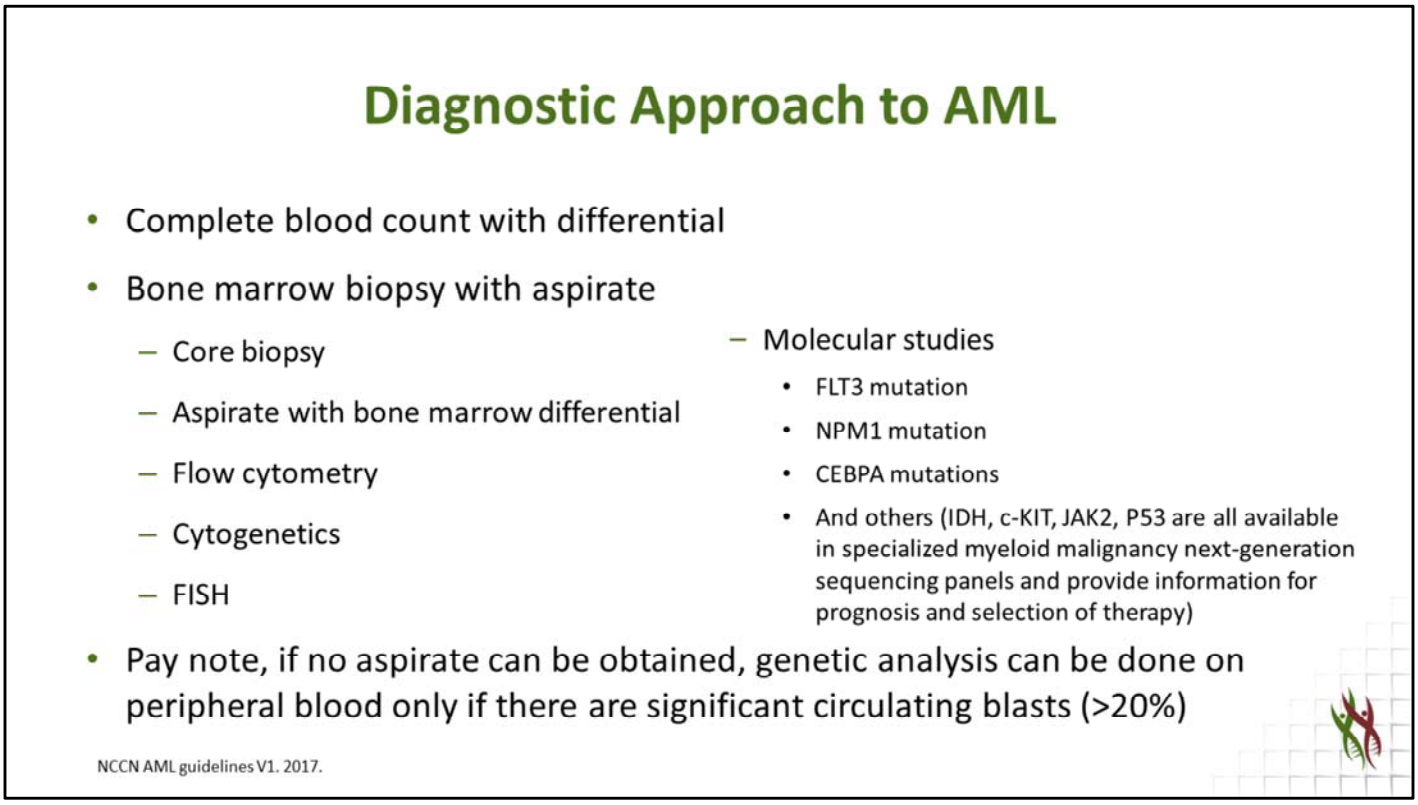
Welcome to *Managing AML*. I am Keith Pratz. In today's presentation, I will be reviewing the diagnosis and prognosis of acute myeloid leukemia, or AML. I hope to improve your comprehension of real-world issues associated with diagnosis, and increase awareness of diagnostic criteria and prognostic factors under investigation. In this video, I will provide you with the information and tools necessary to identify patients suspected of AML and summarize diagnostic testing strategies that should be implemented, and understand revisions to the World Health Organization's criteria for diagnosing and prognosticating AML and their impact on clinical practice. So, let's begin.



Acute myeloid leukemia in 2017 in the United States, occurs in approximately 21,000 adult patients, and of those, approximately 10,000 will die each year. The median age of patients with acute myeloid leukemia is 70 years old. As you can see, the incidence in over age 65 patients is quite high with approximately 20 cases per 100,000 individuals. The chance of developing acute myeloid leukemia in a 50-year-old is 1 in 50,000 whereas in a 70-year-old it is much higher, 1 in 5,000.

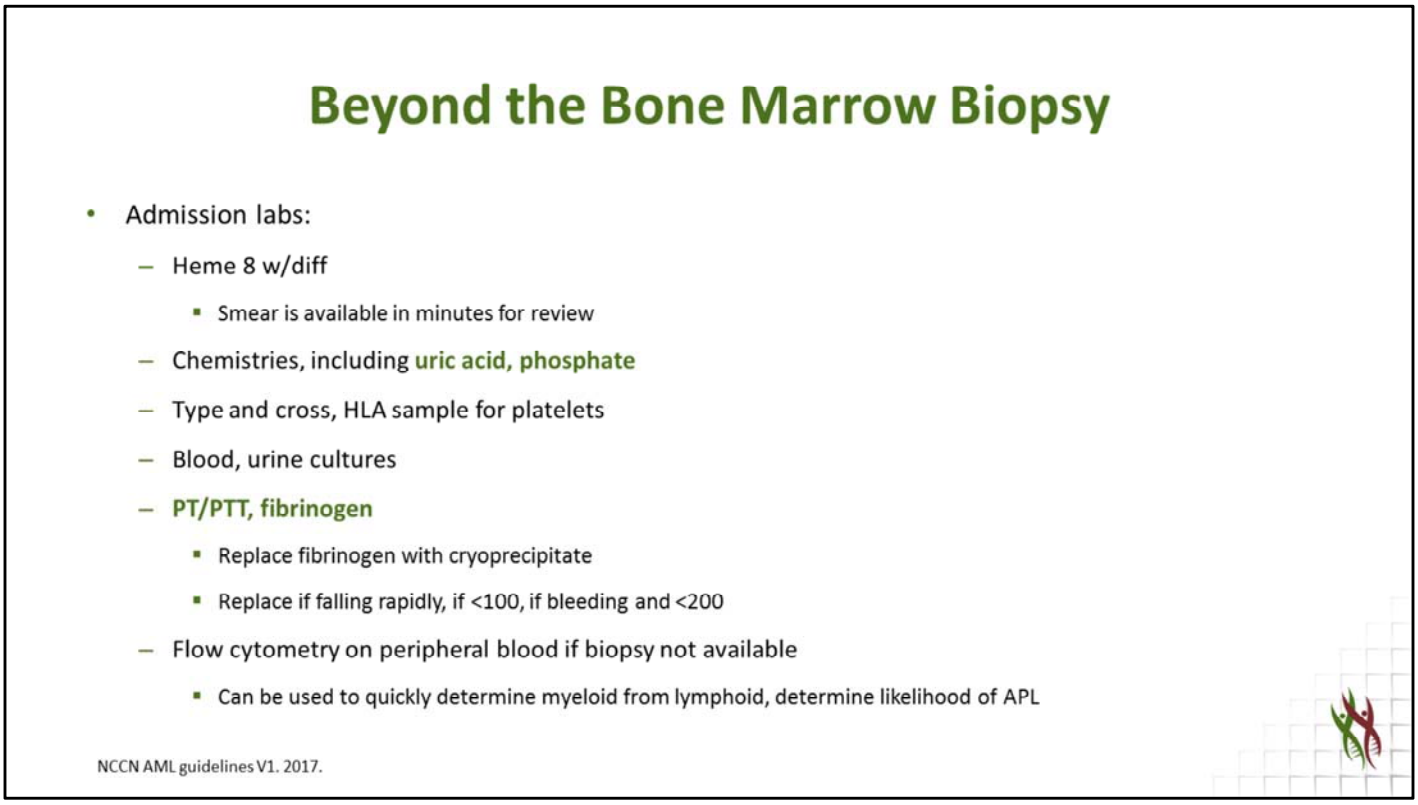


When to suspect the diagnosis of acute myeloid leukemia is when there is the presence of circulating blasts in the white blood cell differential; when you may see cytopenias in a patient with prior chemotherapy or radiation therapy; when there are cytopenias seen in anyone over age 60, there should be suspicion of a bone marrow problem; likewise, even in a younger adult, patients who present with out-of-the-blue bruising, bleeding, or otherwise atypical infections, consideration should be given for the diagnosis of acute myeloid leukemia.

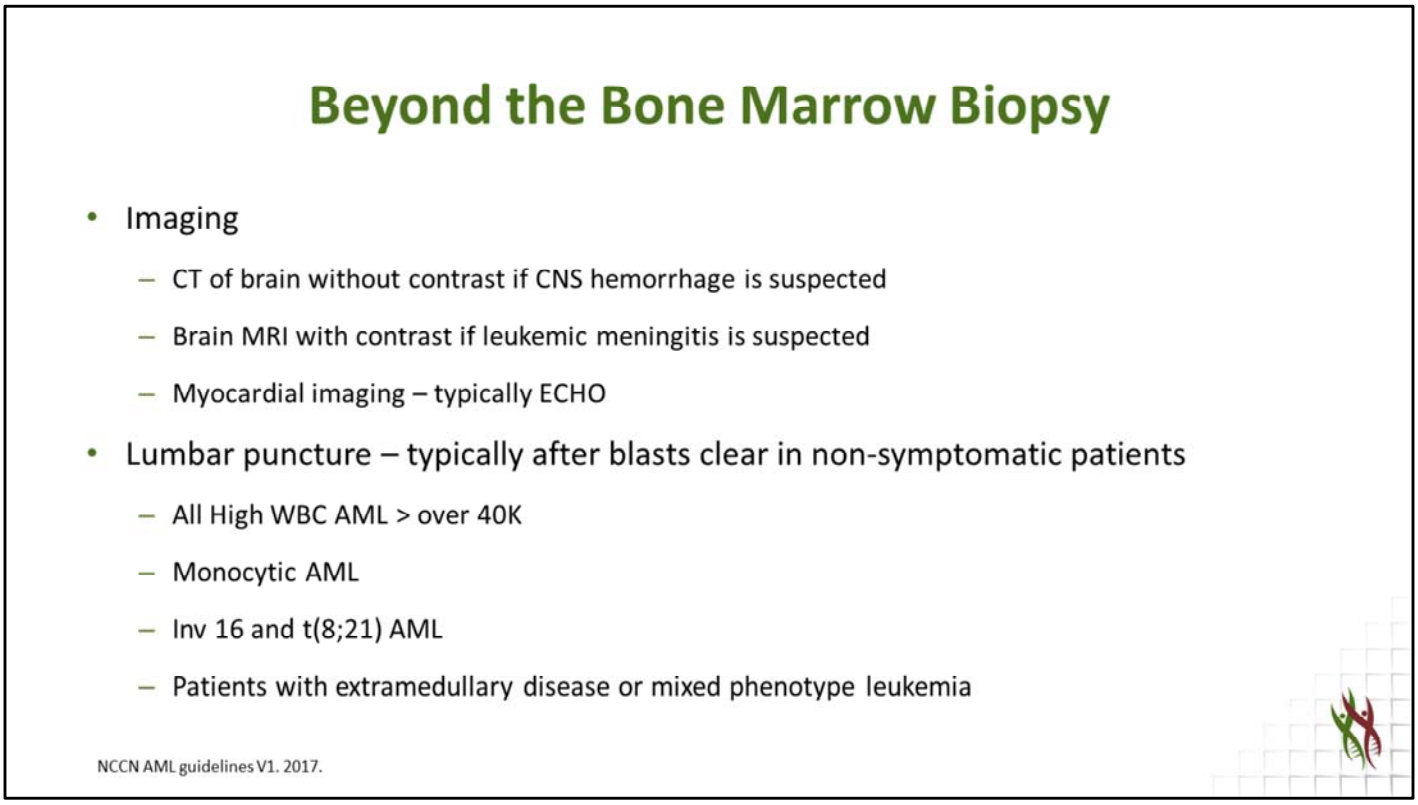


The diagnostic approach to a patient who has a suspicion of acute myeloid leukemia is first done with the complete blood count and differential. Often thereafter, a blood smear will be done to look for the presence of blasts. In those cases where it is readily identified, the patient should then proceed to a bone marrow biopsy with aspirate. There are several important features of the bone marrow biopsy which are critical for the diagnosis. The first of which will be the core biopsy itself, which will give you the relative percentage of cells that have blast-like features. We always get an aspirate and a bone marrow differential, which also quantifies the number of atypical cells in the bone marrow. Flow cytometry is the current state-of-the-art way of making the diagnosis of myeloid leukemia versus lymphoid leukemia. It also can allow us to prognosticate in certain situations. Beyond that, the genetics of the leukemia are critical to establish not only the appropriate treatment, but the risk and response likelihood of a patient undergoing treatments. So, there are FISH (fluorescence in situ hybridization) studies looking for specific translocations and deletions specific for acute myeloid leukemia. There are enlarging numbers of molecular studies now that are critical in the diagnosis of acute myeloid leukemia. The ones that are listed in this slide are mutations in the receptor tyrosine kinase FLT3 that represent approximately 30% of patients with acute myeloid leukemia. There are several therapeutic strategies to take advantage of this mutation, which may confer some improvement in survival, so it is a critical feature to know at the time of diagnosis. Others include mutations in the nucleophosmin gene or NPM1. These occur in approximately 40% of adults with acute myeloid leukemia and can markedly change the risk of a patient going through treatment

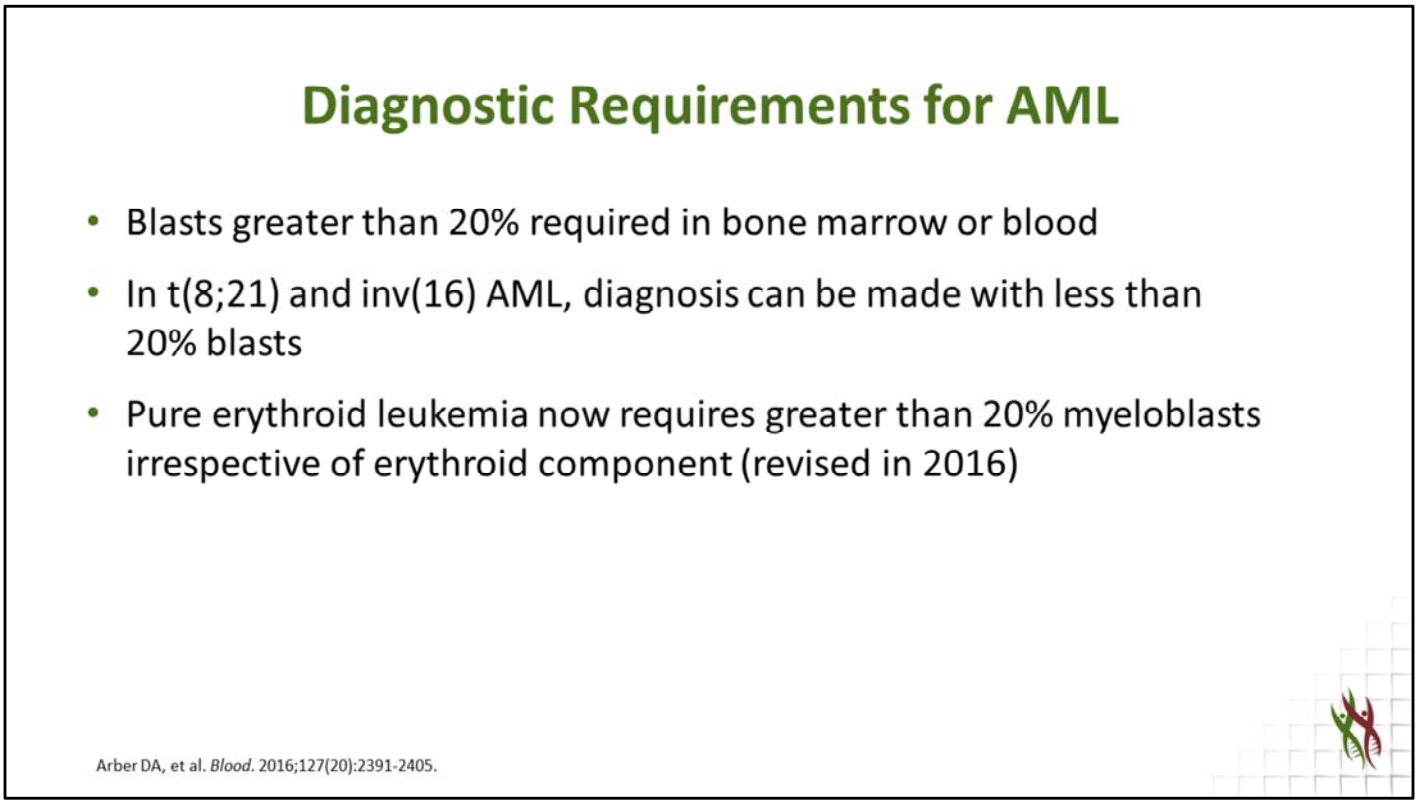
and the likelihood of ultimately relapsing or not. The other gene that is clearly associated with a favorable outcome are mutations at C/EBP alpha. We will talk a little bit later about some nuance about those mutations, but these clearly do represent an important diagnostic subtype of leukemia which only has a very favorable outcome. Other mutations include point mutations in the genes such as IDH, c-KIT, JAK2, and p53 are being picked up in the molecular genetics assessments of leukemias now. Several companies now are doing a large number of mutational screening analyses at the time of diagnosis and some of these genes do represent therapeutic targets. It may become more and more important to know the status of the leukemia on mutations associated at the time of diagnosis. Of note, diagnosis of acute myeloid leukemia is something we can obtain on a core biopsy, but if no aspirate is obtained, genetic analysis is very difficult to do. In patients who have significant circulating leukemia, those with greater than 20% blasts, most of these genetic tests can be done on peripheral blood. We still favor the bone marrow aspirate as the primary source for the genetic testing.



Beyond the bone marrow biopsy, we should be looking toward the chemistries, such as uric acid and phosphorus, to determine whether the patient is undergoing tumor lysis at the time of diagnosis. These things are treatable and important to keep in a good range at the time you do institute therapy. Other things include looking at coagulation cascade factors to look for DIC (disseminated intravascular coagulation). It is not uncommon for acute myeloid leukemia to present in certain circumstances with disseminated intravascular coagulation that can be associated with not only bleeding but clotting disorders. Patients with fibrinogens under 100, we'll often replace it with cryoprecipitate. As I mentioned earlier, not only can the genetics be done on peripheral blood, but flow cytometry often can be done on peripheral blood. However, the diagnosis of acute myeloid leukemia can only be made in patients who had 20% or greater blasts either in bone marrow or blood in most circumstances.



Imaging is not a routine requirement at the time of diagnosing acute myeloid leukemia, but in patients who do have neurologic symptoms, CT imaging of the brain at the time of diagnosis to assess for hemorrhage is important because in the event that there is hemorrhage identified, the goal thresholds for replacement of platelets and other blood products certainly change. Patients sometimes come in with confusion. There is suspicion of involvement of the brain itself with the leukemia. In those circumstances, MRI of the brain with contrast can help determine evidence of that. Lastly, imaging of the heart prior to any chemotherapy is important to document normal ejection fraction and allow for appropriate dosing of anthracycline in those cases. Lastly, patients with acute myeloid leukemia tend to have it spreading in the spinal fluid. It is more common in patients with presentations with high white blood cell count. It is more common in patients with monocytic acute myeloid leukemia. It is more common in patients with inversion 16 and translocation 8;21 acute myeloid leukemia. It is more common in patients who have extramedullary disease or granulocytic sarcoma at the time of diagnosis. In all of these circumstances, we do recommend patients undergo lumbar puncture with intrathecal cytarabine to screen for involvement of CNS leukemia and treat any disease that might be present.



As I mentioned before, the diagnostic requirement for acute myeloid leukemia, in most cases require greater than 20% blasts in the blood or bone marrow sampling. There are at least two exceptions. Those are the genetically-defined subtypes which include translocations of chromosome 8 and 21, and inversion 16 leukemias. Those diagnoses can be made in the presence of fewer than 20% blasts and do occasionally get picked up this way. Those leukemias are treated as the standard AML even when they do have less than 20% blasts. There is one change to the WHO (World Health Organization) criteria with the revisions in 2016 which I will briefly mention here. In previous iterations of the WHO criteria, pure erythroid leukemia had two subtypes, one of which required assessment of the erythroid component of the bone marrow. The current revision removes that requirement and aligns it with the other diagnosis of acute myeloid leukemias which require 20% myeloblasts in the bone marrow.

AML is the Diagnosis – Now What?

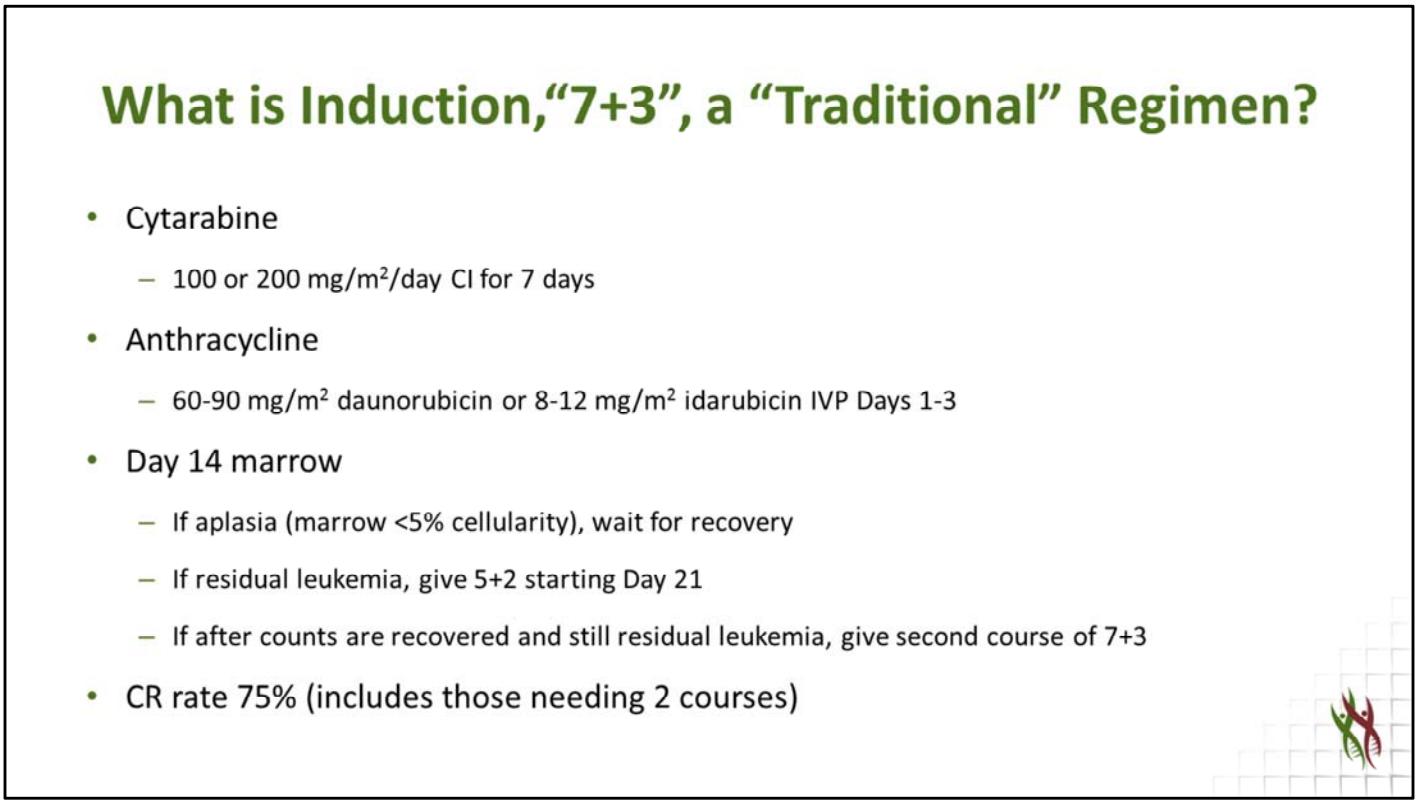
- Are they treatable with intense induction therapy?
  - Performance status and age predict for mortality with treatment
    - 30-day mortality with induction

Mortality within 30 days of initiation of induction

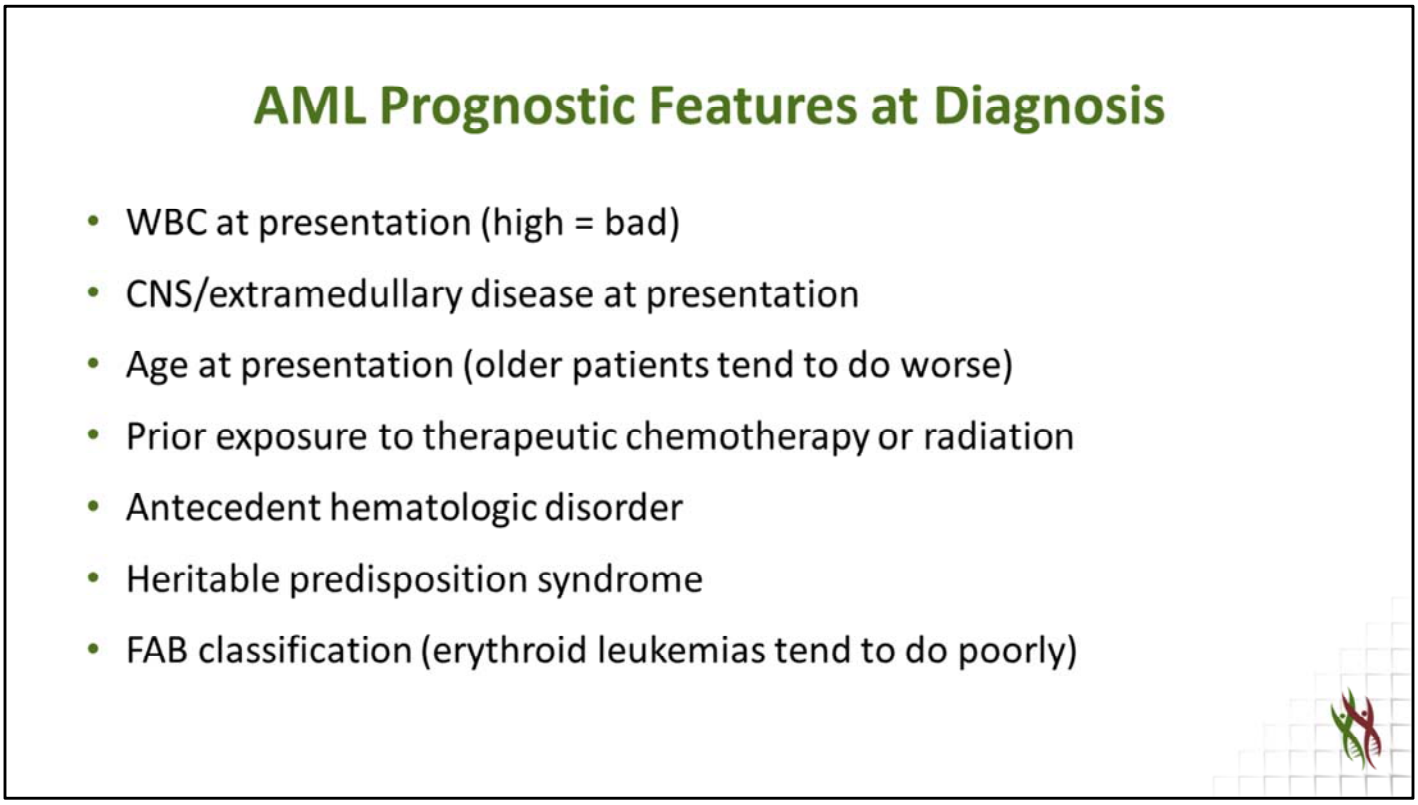
	<56 Years Old	56-65 Years Old	66-75 Years Old	>75 Years Old
PS 0	2%	11%	12%	14%
PS 1	3%	5%	16%	18%
PS 2	2%	18%	31%	50%
PS 3	0%	29%	47%	82%

Appelbaum FR, et al. *Blood.* 2006;107(9):3481-3485.

Now that we have defined patients who have the diagnosis of acute myeloid leukemia, in particular, a large fraction of these patients are over age 65. The question will arise, are they treatable and are they treatable with intense chemotherapy? At the time of diagnosis, there are a few factors that influence the likelihood of safety with treatment with intense therapies, and those involve the age of the patient at the time of diagnosis and the performance status at the time of diagnosis. In the over age 75 group of patients, those with performance status even at level 2 or ECOG performance level 2, the 30-day mortality with induction chemotherapy is approximately 50%. It is quite difficult to treat older patients, and even in the age 60 to 75 range, performance status 2 patients have a 30-day mortality of 30% or more. Alternative strategies are becoming more and more common in that age group that we define as high risk for induction mortality.



Induction is typically a combination of chemotherapy with cytarabine and an anthracycline. We refer to it as 7+3 or traditional chemotherapy induction. Standard cytarabine dosing is either100 or 200 mg/m^2 per day as a continuous infusion for 7 days. The anthracycline dosing is a range of doses, but commonly given as 60 mg/m^2 of daunorubicin as an IV push in day 1, 2, and 3 of treatment. Once this therapy is in, on day 14 of the treatment, a bone marrow aspirate will be obtained looking for aplasia. In those cases where there are fewer than 5% cells in the bone marrow, we wait for recovery. If we see a significant amount of residual leukemia, patients will often be given 5 more days of the cytarabine and two more doses of anthracycline, and then, we will wait for recovery at that point. In the typical under age 60 group of patients, the complete remission rates are approximately 75%. That includes patients who require a second course of chemotherapy. This is not the case in all adults, and ultimately, the over age 60 patients and the patients with more complicated genetics do worse over time.



Other features that influence prognosis in acute myeloid leukemia include patients with a high white blood cell count at presentation. It is often associated with genetic changes in the FLT3 gene; patients who have central nervous system involvement or extramedullary disease at presentation often have a worse prognosis overall. Age at time of presentation is clearly a predictor of outcomes, as older patients typically present with more complicated genetics and less frequent good-risk leukemias. Prior therapy with chemotherapy or radiation therapy is associated with inferior outcome. Patients who have antecedent hematologic disorder, such as patients who present with acute myeloid leukemia after polycythemia vera or other myeloproliferative disorders or myelodysplastic syndrome, clearly have an inferior outcome. There are fairly uncommon but well-described predisposition syndromes such as Li-Fraumeni syndrome which is a heritable mutation in p53 gene. We clearly have a more difficult time curing these leukemias with chemotherapy alone. Then, going back into the older classification system, the FAB classification system that is still reflected in the WHO criteria, but some of the more differentiated leukemias, such as erythroid leukemias and megakaryocytic leukemias, tend to do quite poorly with chemotherapy alone.

Risk Status of AML Based on Genetics

NCCN Guidelines Version 1.2017 Acute Myeloid Leukemia

RISK STATUS BASED ON VALIDATED CYTOGENETICS AND MOLECULAR ABNORMALITIES[1]

RISK STATUS	CYTOGENETICS	MOLECULAR ABNORMALITIES
Favorable-risk	Core binding factor: inv(16)[2,3,4] or t(16;16)[2,3,4] or t(8;21)[2,4] or t(15;17)[4]	Normal cytogenetics: NPM1 mutation in the absence of FLT3-ITD or isolated biallelic (double) CEBPA mutation
Intermediate-risk	Normal cytogenetics +8 alone t(9;11) Other non-defined	Core binding factor with KIT mutation[2]
Poor-risk	Complex (≥3 clonal chromosomal abnormalities) Monosomal karyotype -5, 5q-, -7, 7q- 11q23 - non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)[5]	Normal cytogenetics: with FLT3-ITD mutation[6] TP53 mutation

[1]The molecular abnormalities included in this table reflect those for which validated assays are available in standardized commercial laboratories. Given the rapidly evolving field, risk stratification should be modified based on continuous evaluation of research data. Other novel genetic mutations have been identified that may have prognostic significance.
[2]Emerging data indicate that the presence of KIT mutations in patients with t(8;21), and to a lesser extent inv(16), confers a higher risk of relapse. These patients are considered intermediate risk and should be considered for HCT or clinical trials, if available. Recent data suggest that certain KIT mutations may be more or less adverse in prognosis. See Discussion.
[3]Paschka P, et al. Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML study group (AMLSG). Blood 2013;121:170-177.
[4]Other cytogenetic abnormalities in addition to these findings do not alter better risk status.
[5]For Philadelphia+ AML t(9;22), manage as myeloid blast crisis in CML, with addition of tyrosine kinase inhibitors.
[6]FLT3-ITD mutations are considered to confer a significantly poorer outcome in patients with normal karyotype, and these patients should be considered for clinical trials where available. There is controversy as to whether FLT3-TKD mutations carry an equally poor prognosis.

The current state-of-the-art for risk stratification for acute myeloid leukemia is described in this slide. Favorable-risk leukemias are only associated with specific karyotypic abnormalities. The inversion 16, translocation 16;16, and translocation 8;21 represent most common favorable cytogenetic abnormalities in acute myeloid leukemia. Translocation 15;17 is the pathognomonic translocation associated with acute promyelocytic leukemia. I will not be discussing that today, as it is typically treated in a much different manner. Beyond the cytogenetics, now, we are defining patients with normal karyotypes with specific mutations that are conferring favorable risks. The most common one being patients who present with normal karyotype and NPM1 mutation in the absence of a FLT3/ITD mutation. Those patients do reasonably well with standard chemotherapy in consolidation and we would not deem them as high risk at the time of diagnosis. The other molecular subtype that seems to confer a favorable risk are those patients who have what we call biallelic, or double mutation, in the gene C/EBP alpha. This is somewhat uncommon but seems to be associated with quite a favorable outcome in patients treated with standard chemotherapy followed by consolidation. The intermediate-risk acute myeloid leukemia is associated with generally normal karyotype, but there are a few translocations and additions that are associated with this risk category. Patients who present with translocation 8;21 or inversion 16, who have also been found to have a c-KIT mutation fall into this immediate-risk category, and virtually, everything beyond that, we will define as poor risk. These include patients with complex karyotypic abnormalities; patients with monosomies in chromosome 5, chromosome 7; patients with translocations 11 not involving the 9th chromosome; those typically have poor outcome. And then others, including inversion 3 and blast crisis CML tend to have poor risk with standard chemotherapy. Then, in the molecular abnormalities category in patients who present with normal karyotypes, those who present with a FLT3/ITD gene mutation are at poor risk. Those who are found to have p53 mutation are also difficult to cure with chemotherapy alone.

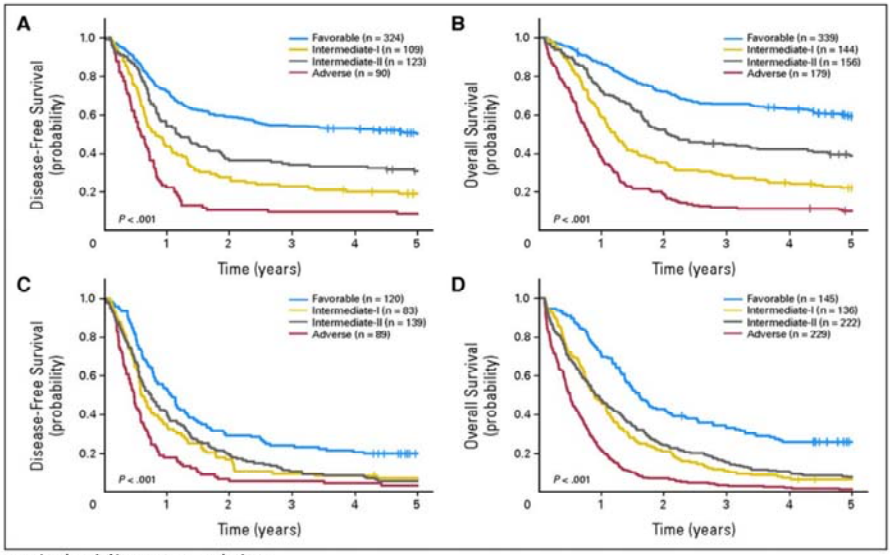
New Recommended Standardized Reporting for Correlation of Cytogenetic and Molecular Genetic Data with Clinical Data in AML

Genetic Group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1* inv(16)(p13.1q22)/ t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD (normal karyotype) Mutated *CEBPA* (normal karyotype)
Intermediate-I	Mutated *NPM1* and *FLT3*-ITD (normal karyotype) Wild type *NPM1* and *FLT3*-ITD (normal karyotype) Wild type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*; Cytogenetic abnormality not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* t(6;9)(p23;q34); *DEK-NUP214*; other t(v;11q23); *MLL* rearranged -5 or del(5q); -7; abn(17p); complex karyotype

Döhner H, et al. *Blood*. 2010;115(3):453-474.

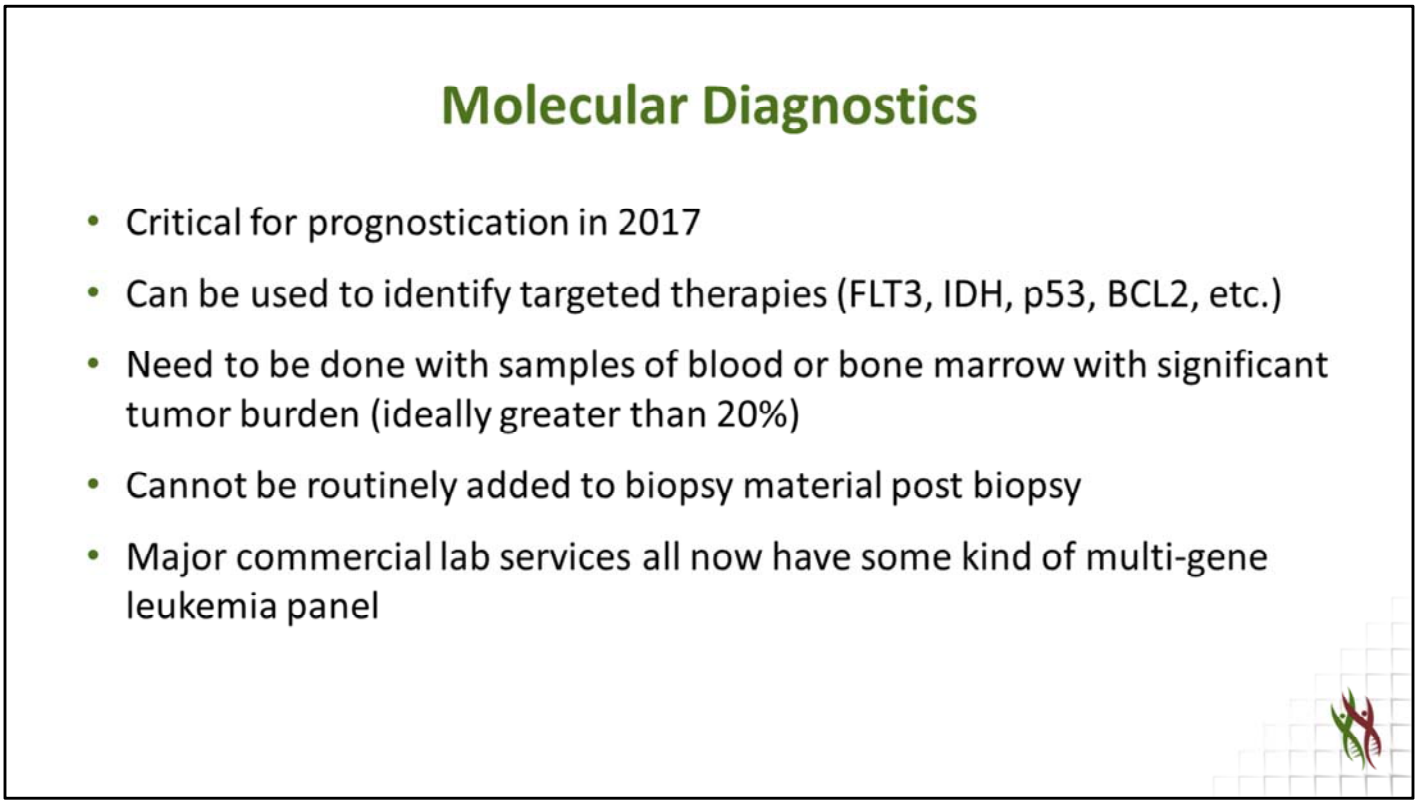
More recent parsing of the molecular abnormalities data by groups in Europe have started to try to parse out some of the intermediate patients to better define their outcomes. In one commonly used risk stratification system through the European LeukemiaNet group, there are two subtypes of intermediate, one of which involves patients with various degrees of FLT3 mutations. Patients without FLT3 mutations and without an NPM1 mutation also fall in this intermediate classification.

Outcome of Patients with Primary AML Classified into the Four European LeukemiaNet Genetic Groups*



*According to the European LeukemiaNet recommendations
Mrózek K, et al. *J Clin Oncol*. 2012;30:4515-4523.

As you can see in this slide set, favorable-risk patients in ELN risk stratification system with chemotherapy alone have 5-year disease-free survival of greater than 60%, whereas those with adverse prognostic features have 5-year survival of 10% or less. The intermediate classifications fall in between. One should note the intermediate-2 patients do somewhat better than the intermediate-1 patients in this risk-stratification system, which is somewhat counterintuitive, but looking closely at the colors of these figures you can pick that up.



So, molecular diagnostics in the year 2017 are critically important for risk prognostication. Likewise, they can be used to identify specific targeted therapies and there are several in development for targeting FLT3 mutations, targeting IDH mutations, targeting patients with mutated or deleted p53 genes and others, including BCL2 targeted agents which seem to be quite active in the IDH mutation group of patients. Basing therapeutic decisions on the genetics can only be done when we have a quality sample of the tumor. This is best found in the bone marrow and it is quite important to understand what you are assessing with the molecular tests, as molecular diagnostics are only able to identify these mutations when there is significant amount of tumor in the sample sent. Unfortunately, many of these tests cannot be routinely added to biopsy material post biopsies. It is important to ask for these tests to be done at the time of diagnosis. As I mentioned earlier, most of the commercial lab services, and referral services do have some kind of multi-gene leukemia panel now. It is not a consensus guideline, but it is becoming more and more common to have these multi-gene panels done to look for not only prognostic information, but potential therapeutic information. As these panels include more and more genes, the costs of the panels are becoming less than asking for individual genes themselves.

World Health Organization Criteria for AML 2016 Revisions in Red

- AML with recurrent genetic abnormalities
  - AML with t(8; 21)(q22;q22); (AML/ETO)
  - AML with inv(16)(p13q22) or t(16;16)(p13; q22); (CBFβ/MYH11)
  - APL (AML with t(15;17)(q22; q12); (PML/retinoic acid receptor alpha [RARA]) and variants)
  - AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
  - AML with t(6;9)(p23;q34.1);DEK-NUP214
  - AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
  - AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1
  - AML with mutated NPM1
  - AML with biallelic mutations of CEBPA
- AML with myelodysplasia-related changes

Arber DA, et al. *Blood*. 2016;127(20):2391-2405.

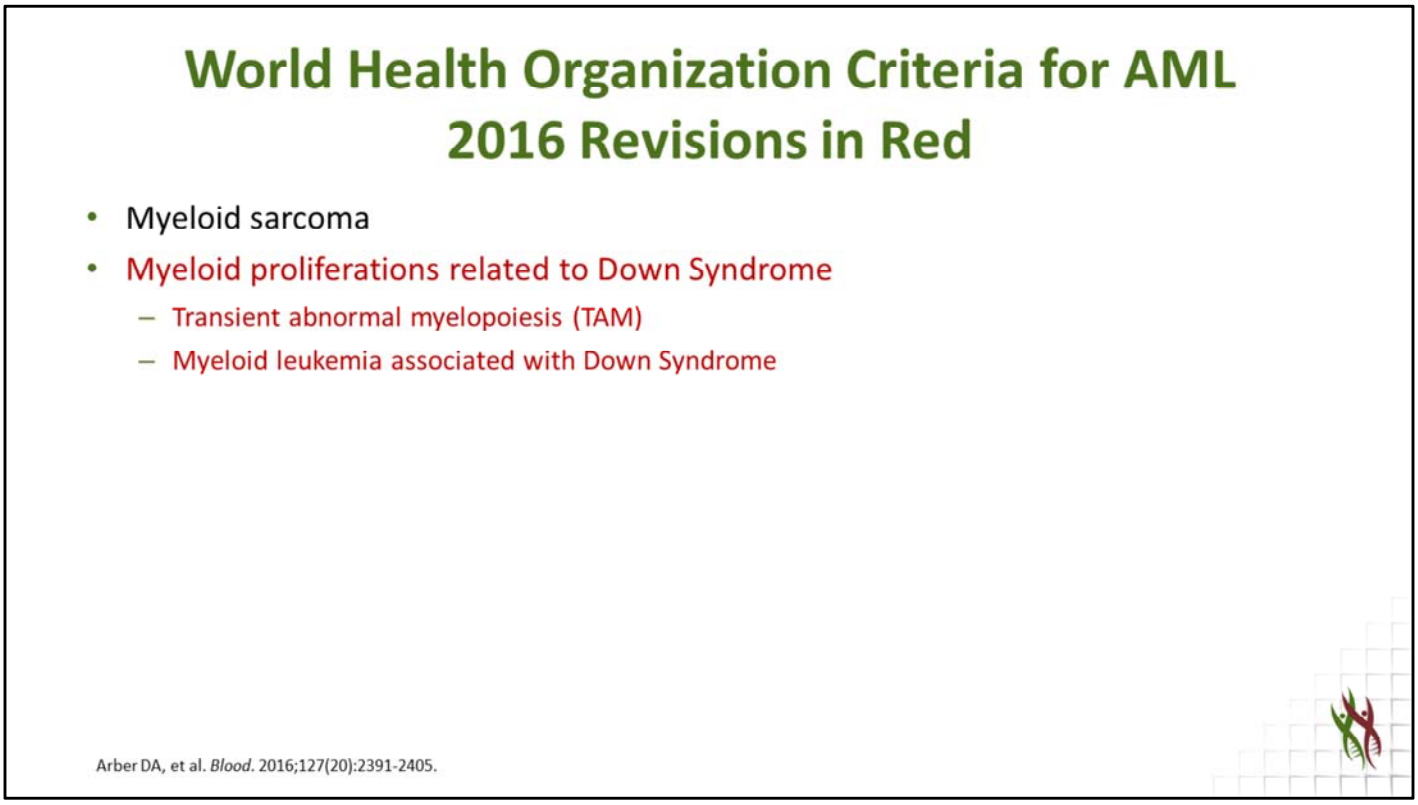
Reflected in this slide are the changes to the World Health Organization criteria for acute myeloid leukemia in 2016. I am pointing out here those revisions which include specific subtyping based on recurrent genetic abnormalities, at least two of these rely only on molecular testing, including those with NPM1 mutation and those with biallelic mutations of C/EBP alpha.

World Health Organization Criteria for AML 2016 Revisions in Red

- Therapy-related myeloid neoplasms
- AML not otherwise categorized.
  - Acute myeloblastic leukemia, minimally differentiated (FAB Classification M0)
  - Acute myeloblastic leukemia without maturation (FAB Classification M1)
  - Acute myeloblastic leukemia with maturation (FAB Classification M2)
  - Acute myelomonocytic leukemia (AMML) (FAB Classification M4)
  - Acute monoblastic leukemia and acute monocytic leukemia (FAB classifications M5a and M5b)
  - Pure erythroid leukemia
  - Acute megakaryoblastic leukemia (FAB Classification M7)
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis

Arber DA, et al. *Blood*. 2016;127(20):2391-2405.

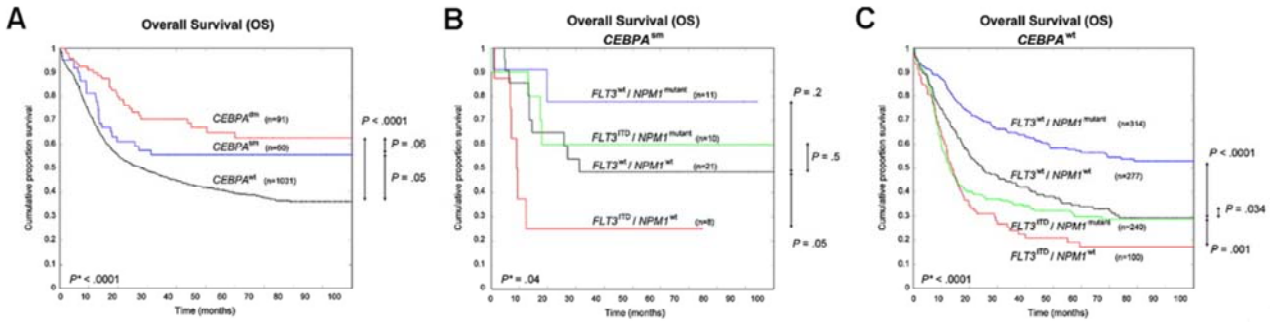
There has been more description of therapy-related myeloid neoplasms currently. One should note that therapy-related myeloid neoplasm does not require greater than 20% blasts to land in this criteria. It only has to be associated with patients who receive cytotoxic chemotherapy or therapeutic radiation prior to development of their myeloid neoplasm. As I mentioned earlier, the pure erythroid leukemia diagnostic criteria has changed in this revision,



and there are two revisions to the myeloid proliferations associated with Down syndrome which is somewhat uncommon in adults but occasionally picked up in the younger adult group.

CEPBA Mutation Prognostication

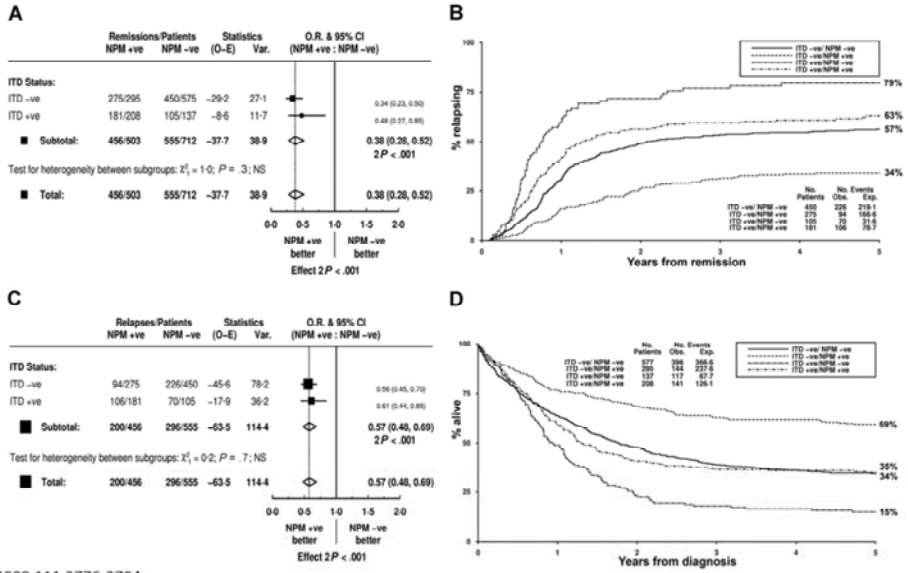
- Retrospective data suggests biallelic mutations confer favorable prognosis
- Single mutation carriers (heterozygous mutations) do not carry the same favorable prognosis



Taskesen E, et al. *Blood*. 2011;117:2469-2475.

I am going to walk through some of the prognostic information gained from two specific genetic mutations. In this slide, we are reviewing the outcomes of patients with mutations of C/EBP alpha. The overall survival in patients with double-mutant C/EBP alpha treated with standard induction chemotherapy is approximately 70% in this group of patients at 5 years. Single-mutant patients still do better than those with no mutations in C/EBP alpha, but it is not quite as good as those with double mutations. Beyond that, there are ways to parse out more than just the influence of C/EBP alpha on the outcomes. Figure B and C here overlook those patients with single-mutant C/EBP alphas and how that influences outcomes with or without NPM1 or without FLT3 mutations.

FLT3 ITD Mutations Have Clear Impact on Outcomes and Impact Varies by NPM1 Mutations



Gale RE, et al. *Blood*. 2008;111:2776-2784.

Patients who have FLT3/ITD mutations have various degrees of responses to therapy and relapse. Those relapse likelihoods are influenced by other mutations found in the leukemic clone. This slide reviews patients with FLT3/ITD mutations, with or without NPM1 mutations.

FLT3 ITD Mutations Have Clear Impact on Outcomes and Impact Varies by NPM1 Mutations

Table 5. Outcome data according to *NPM1* and *FLT3*/ITD mutant status in the total cohort of 1217 patients

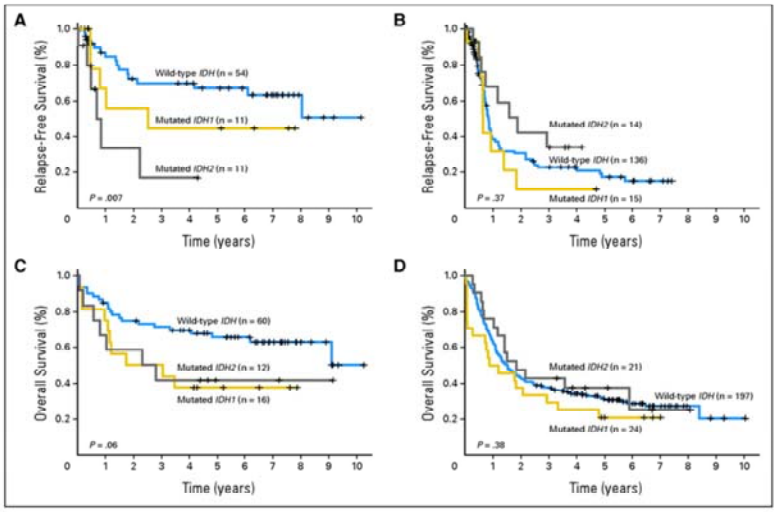
	NPM1⁻, %	*NPM1*⁺, %	*OR (CI)*	*P*	*NPM1*⁻, ITD⁻, %	*NPM1*⁻, ITD⁺, %	*NPM1*⁺, ITD⁻, %	*NPM1*⁺, ITD⁺, %	OR (CI) for *NPM1* stratified by ITD status	*P*	Het.
CR	78	91	0.40 (0.30-0.55)	<.001	78	77	94	87	0.38 (0.28-0.52)	<.001	0.3
RD	15	3	0.28 (0.19-0.41)	<.001	15	17	2	6	0.26 (0.18-0.38)	<.001	0.6
ID	7	6	0.86 (0.54-1.36)	.5	7	7	5	7	0.83 (0.52-1.34)	.4	0.4
OS at 5 yr	31	49	0.67 (0.58-0.77)	<.001	34	15	59	35	0.60 (0.52-0.69)	<.001	0.6
DFS at 5 yr	31	45	0.72 (0.62-0.84)	<.001	34	15	55	31	0.62 (0.53-0.73)	<.001	0.7
RR at 5 yr	61	46	0.69 (0.58-0.82)	<.001	57	79	34	63	0.57 (0.48-0.69)	<.001	0.6

Het. indicates testing for heterogeneity (*P* value for difference in effect of an *NPM1* mutation in *FLT3*/ITD⁻ and ITD⁺ patients).

Gale RE, et al. *Blood*. 2008;111:2776-2784.

As you can see, patients with a FLT3/ITD without NPM1 mutation have approximately a 30% disease-free survival at 3 years, whereas those patients with NPM1 mutation have 40% to 50% survival at that time.

Outcome of Cytogenetically Normal Acute Myeloid Leukemia (CN-AML) According to IDH1 and IDH2



Paschka P, et al. *J Clin Oncol.* 2010;28:3636-3643.

This slide reviews the outcomes of patients with cytogenetically normal acute myeloid leukemia with mutations in IDH1 and IDH2. IDH1 and IDH2 mutations collectively are found in approximately 15% of patients with acute myeloid leukemia; individually they are found in 7%-10% of cases. The overall survival in patients with either IDH1 or IDH2 mutant AML tends to be worse than those without these mutations. Reflected in this slide are the impact of NPM1 mutation found in patients with IDH1 or IDH2 mutations. One should note that patients with IDH1 mutations without NPM1 mutation do quite poorly, and those patients often are recommended to pursue something beyond chemotherapy to improve their outcomes over time.

Key Points

- The management of AML in 2017 is more complicated than just finding more than 20% blasts
- Genetics beyond chromosomes are now an important feature for subtype classification and prognostication
- Most prognostic systems use FLT3, NPM1 and CEPBA to risk adjust

The key points that we should take from this discussion are the management of acute myeloid leukemia in 2017 is more complicated than just finding 20% blasts. Genetics beyond chromosomes are now important feature for subtype classification and prognostication. Most prognostic systems use FLT3, NPM1, and C/EBP alpha to risk adjust. Thank you for viewing this activity.