

Initial Diagnostic Workup of Acute Leukemia

Guideline From the College of American Pathologists and the American Society of Hematology

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• **Context.**—A complete diagnosis of acute leukemia requires knowledge of clinical information combined with morphologic evaluation, immunophenotyping and karyotype analysis, and often, molecular genetic testing. Although many aspects of the workup for acute leukemia are well accepted, few guidelines have addressed the different aspects of the diagnostic evaluation of samples from patients suspected to have acute leukemia.

Objective.—To develop a guideline for treating physicians and pathologists involved in the diagnostic and prognostic evaluation of new acute leukemia samples, including acute lymphoblastic leukemia, acute myeloid leukemia, and acute leukemias of ambiguous lineage.

Design.—The College of American Pathologists and the American Society of Hematology convened a panel of

experts in hematology and hematopathology to develop recommendations. A systematic evidence review was conducted to address 6 key questions. Recommendations were derived from strength of evidence, feedback received during the public comment period, and expert panel consensus.

Results.—Twenty-seven guideline statements were established, which ranged from recommendations on what clinical and laboratory information should be available as part of the diagnostic and prognostic evaluation of acute leukemia samples to what types of testing should be performed routinely, with recommendations on where such testing should be performed and how the results should be reported.

Conclusions.—The guideline provides a framework for the multiple steps, including laboratory testing, in the evaluation of acute leukemia samples. Some aspects of the guideline, especially molecular genetic testing in acute leukemia, are rapidly changing with new supportive literature, which will require on-going updates for the guideline to remain relevant.

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The laboratory evaluation of patients suspected of having acute leukemia (AL) is complex and has evolved significantly with the incorporation of advanced laboratory techniques. The first broadly accepted classification in modern history was that of the French-American-British (FAB) cooperative group, which was initially based entirely on morphologic features of blast cells on Wright- or Wright-Giemsa-stained bone marrow smears and a variety of cytochemical stains.¹ With the introduction of clinical immunophenotyping assays, particularly flow cytometry immunophenotyping (FCI), the FAB classification was modified to incorporate limited immunophenotypic studies, primarily to distinguish minimally differentiated acute myeloid leukemia (AML) from acute lymphoblastic leukemia (ALL).² Immunophenotyping to distinguish precursor B-cell from precursor T-cell ALL (T-ALL) was not included nor were other immunophenotypic markers used to define FAB disease groups, other than identification of the megakaryocytic lineage in acute megakaryoblastic leukemia.³ Although a few categories of the FAB classification correlated with recurring cytogenetic abnormalities (partic-

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ularly, acute promyelocytic leukemia and acute myelomonocytic leukemia with abnormal eosinophils), the classification did not incorporate genetic studies. In 2001, the 3rd edition⁴ of the World Health Organization (WHO) classification of AL was published and formally introduced the requirement for immunophenotyping and cytogenetic studies for the diagnosis of AL. The 4th edition⁵ of the WHO classification, published in 2008, added additional cytogenetic disease groups for AML and ALL, introduced the category of mixed-phenotype acute leukemia (MPAL), and included provisional entities of AML that were based on gene mutation studies. Since 2008, many other mutations have been described in all types of AL, and epigenetic changes, including protein and microRNA (miRNA) expression and global and gene-specific methylation, have been reported to be common and prognostically relevant in AL.^{6,7} The 2016 WHO classification⁸ of AL continued to define some disease entities by a combination of morphologic, immunophenotypic, and genetic (including molecular genetic) changes, but some gene mutations and cytogenetic abnormalities, although not disease defining, offer significant prognostic information. These genetic and epigenetic changes in AL may be detected by individual, often polymerase chain reaction (PCR) or reverse-transcriptase PCR-based, assays; by gene panels using next-generation sequencing (NGS) methods; or by looking at the entire genome of a given sample. The latter approaches are becoming increasingly available because of major advances in molecular genetic testing technology.

Because of the increasing complexity of testing needed to completely diagnose and predict prognosis in cases of AL, the College of American Pathologists (CAP) and the American Society of Hematology (ASH) formed an expert panel to review the relevant literature and to establish a guideline for appropriate laboratory testing and for the clinical information necessary for the initial diagnosis of AL, including AML, ALL, and ALs of ambiguous lineage. Six key questions were initially developed, with literature searches performed based on the initial questions. A draft guideline was developed by the expert panel and was modified based on comments received during an open-comment period. This article describes the process used for the development of the AL guideline statements, the strength of evidence for each statement, and the rationale for the specific recommendations.

MATERIALS AND METHODS

This guideline was developed using an evidence-based methodology intended to meet recommendations for a report from the Institute of Medicine.⁹ This guideline is based on the results of a systematic review (SR) of available evidence. A detailed description of the methods and SR (including the quality assessment and complete analysis of the evidence) used to create this guideline can be found in the supplemental digital content, which also includes 24 tables and 2 figure files, at www.archivesofpathology.org in the October 2017 table of contents.

Panel Composition

The CAP Pathology and Laboratory Quality Center (the Center) and the ASH members included 7 pathologists, one hematologist, one hematologist/oncologist, and one methodologist consultant. These panel members served as the expert panel (EP) for the systematic evidence review and development of the guideline statements. An advisory panel including one patient advocate, one cytogeneticist, 3 hematologists/oncologists (including one pediatric hematologist/oncologist), one medical oncologist, and 2 hemato-

pathologists assisted the EP in determining the project scope and reviewing and providing guidance on the draft recommendations and manuscript development.

Conflict of Interest Policy

In accordance with the CAP conflict of interest policy (in effect April 2010), members of the expert panel disclosed all financial interests of possible relevance to the guideline, from 12 months before appointment through publication of the guideline. Individuals were instructed to disclose any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Disclosures were collected by the CAP staff before beginning the SR and were updated continuously throughout the project at each virtual and face-to-face meeting. A separate oversight group (consisting of staff and members of the CAP and ASH) reviewed the disclosures and agreed that most of the expert panel had no conflicts of interest. Complete disclosures of the expert panel members are listed in the Appendix. Disclosures of interest judged by the oversight group to be conflicts are as follows: D.A.A., consultancy and board/advisory board with Celgene Corporation (Summit, New Jersey), board/advisory board of DAVA Oncology (Dallas, Texas), Bristol-Myers Squibb (New York, New York), Novartis (Deerfield, Illinois), and Agios Pharmaceuticals (Cambridge, Massachusetts); M.J.B., grants received from Amgen Inc (Thousand Oaks, California), Beckman Coulter (Brea, California), Becton, Dickinson and Company (San Jose, California), Bristol-Myers Squibb (New York, New York), Genzyme Corporation (Cambridge, Massachusetts), Medimmune (Gaithersburg, Maryland), and Micromet (Rockville, Maryland); K.F., consultancy with Celgene Corporation (Summit, New Jersey); R.P.H., consultancies with Cancer and Leukemia Group B, Genzyme Corporation (Cambridge, Massachusetts), and Incyte Corporation (Wilmington, Delaware); S.A.W., consultancy with Genzyme Corporation (Cambridge, Massachusetts), board/advisory board with, and grants received from, Seattle Genetics, Inc (Bothell, Washington), and GlaxoSmithKline plc (Brentford, United Kingdom). Most of the EP (6 of 11 members) was assessed as having no relevant conflicts of interest. The CAP and ASH provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist. Please see the SDC for full details on the conflict of interest policy.

Objective

The objective of the guideline is to recommend laboratory testing for the initial workup for proper diagnosis, determination of prognostic factors, and possible future monitoring of ALs, including AML, ALL and ALs of ambiguous lineage, in children and adults.

The key questions were as follows:

1. What clinical and laboratory information should be available during the initial diagnostic evaluation of a patient with AL?
2. What specimens and sample types should be evaluated during the initial workup of a patient with AL?
3. At the time of diagnosis, what tests are required for all patients for the initial evaluation of an AL?
4. Which tests should be performed on only a subset of patients, including in response to results from initial tests and morphology?
5. Where should laboratory testing be performed?
6. How should test results and the diagnosis be correlated and reported?

Literature Search and Selection

A systematic literature search was completed on October 4, 2011, for relevant evidence using OvidSP (Ovid Technologies, New York, New York), PubMed (US National Library of Medicine, Bethesda, Maryland), and Science Direct (Elsevier, Amsterdam, the Nether-

Table 1. Grades for Strength of Evidence^a

Designation	Description	Quality of Evidence
Convincing	High confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect.	High-quality to intermediate-quality evidence.
Adequate	Moderate confidence that available evidence reflects true effect. Further research is likely to have an important effect on the confidence in estimate of effect and may change the estimate.	Intermediate-quality to low-quality of evidence.
Inadequate	Little confidence that available evidence reflects true effect. Further research is very likely to have an important effect on the confidence in the estimate of effect and is likely to change the estimate.	Low or insufficient evidence, and expert panel used formal consensus process to reach recommendation.
Insufficient	Evidence is insufficient to discern net effect. Any estimate of effect is very uncertain.	Insufficient evidence, and expert panel used formal consensus process to reach recommendation.

^a Adapted from Balshem H, Helfand M, Schunemann HJ, et al.⁴³⁰ GRADE guidelines, 3: rating the quality of evidence. *J Clin Epidemiol.* 2011;64(4):401–406; copyright 2011, with permission from Elsevier.

lands) to identify literature published from January 2005 through September 2011. A literature refresh was completed on April 24, 2013, and again on August 11, 2015, to identify recently published material. Database searches were supplemented with expert panel recommendations and the references from those supplemental articles were reviewed to ensure all relevant publications were included.

Selection at all 3 levels of the SR was based on predetermined inclusion/exclusion criteria for the outcomes of interest. Detailed information about the literature search and selection can be found in the supplemental data.

Quality Assessment

An assessment of the quality of the evidence was performed for all retained studies after application of the inclusion and exclusion criteria by the methodologist (see Supplemental Table 6). Using that method, studies deemed to be of low quality would not be excluded from the SR but would be retained and their methodological strengths and weaknesses discussed where relevant. Studies would be assessed by confirming the presence of items related to both internal and external validity, which are all associated with methodological rigor and a decrease in the risk of bias. The quality assessment of the studies was performed by determining the risk of bias by assessing key indicators based on study design against known criteria. Only studies obtained from our SR were assessed for quality by these methods and any additional articles brought in to support the background and to contextualize the findings were not. Each study was assessed individually (refer to the SDC for individual assessments and results by guideline statement) and then summarized by study type. A summary of the overall quality of the evidence was given considering the evidence in totality.

A rating for the strength of evidence is given for guideline statements for which quality was assessed (ie, only studies obtained from our SR). Ultimately, the designation (rating) of the strength of evidence is a judgment by the expert panel of their level of confidence that the evidence from the studies informing the recommendations reflects a true effect. Table 1 describes the grades for strength of evidence. (Refer to the SDC for a detailed discussion of the quality assessment.)

Assessing the Strength of Recommendations

Development of recommendations required that the EP review the identified evidence and make a series of key judgments, including the balance of benefits and harms. Grades for strength of recommendations were developed by the CAP Pathology and Laboratory Quality Center and are described in Table 2.

Guideline Revision

This guideline will be reviewed every 4 years, or earlier in the event of the publication of substantive and high-quality evidence that could potentially alter the original guideline recommendations. If necessary, the entire EP will reconvene to discuss potential changes. When appropriate, the EP will recommend revision of the guideline to the CAP and ASH for review and approval.

Disclaimer

Practice guidelines and consensus statements reflect the best available evidence and expert consensus supported in practice. They are intended to assist physicians and patients in clinical decision-making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time a practice guideline or

Table 2. Grades for Strength of Recommendations^a

Designation	Recommendation	Rationale
Strong recommendation	Recommend for, or against, a particular practice. (Can include “must” or “should.”)	Supported by convincing (high) or adequate (intermediate) quality of evidence and clear benefit that outweighs any harms.
Recommendation	Recommend for, or against, a particular practice. (Can include “should” or “may.”)	Some limitations in quality of evidence (adequate [intermediate] or inadequate [low]), balance of benefits and harms, values, or costs, but panel concluded that there is sufficient evidence and/or benefit to inform a recommendation.
Expert consensus opinion	Recommend for, or against, a particular practice. (Can include “should” or “may.”)	Serious limitations in quality of evidence (inadequate [low] or insufficient), balance of benefits and harms, values or costs, but panel consensus was that a statement was necessary.
No recommendation	No recommendation for, or against, a practice.	Insufficient evidence or agreement of the balance of benefits and harms, values, or costs to provide a recommendation.

^a Derived from Andrews et al.⁴³¹ 2013.

consensus statement is developed and when it is published or read. Guidelines and statements are not continually updated and may not reflect the most recent evidence. Guidelines and statements address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines and statements cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for the patient. Accordingly, adherence to any practice guideline or consensus statement is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. The CAP and ASH makes no warranty, express or implied, regarding guidelines and statements and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. The CAP and ASH assumes no responsibility for any injury or damage to persons or property arising out of, or related to any, use of this statement or for any errors or omissions.

RESULTS

Of the 4901 unique studies identified in the SR, 174 published, peer-reviewed articles were included, which underwent data extraction and qualitative analysis. Among the extracted documents, 55 articles/documents did not meet any inclusion criteria and were excluded from the SR but retained for discussion purposes.

The EP met 23 times through teleconference webinars from June 8, 2011, through August 16, 2016. Additional work was completed via email. The panel met in person July 19, 2013, to review evidence to date and draft recommendations.

A public comment period was held from August 10 through August 31, 2015, on the ASH Web site. Twenty-nine draft recommendations and 2 demographic questions were posted for peer review.

Agree and *disagree* responses were captured for every proposed recommendation. The Web site also received 789 written comments. Twenty-six draft recommendations achieved more than 90% agreement, 2 draft statements achieved more than 80% to 90% agreement, and 1 received more than 70% to 80% agreement. Each EP member was assigned 3 draft statements for which they had to review the public comments and present them to the entire panel for group discussion. After consideration of the comments, 2 draft recommendations were maintained with the original language, 25 were revised, and 2 draft recommendations were combined into other statements, which resulted in 27 final recommendations.

The panel convened again September 14, 2015, to review the comments received and revise the recommendations. Resolution of all changes was obtained by unanimous consensus of the panel members using a nominal group technique (rounds of subsequent teleconference webinars and email discussions). Final EP recommendations were approved by a formal vote. The panel considered laboratory efficiency and feasibility throughout the entire process, although neither cost nor cost-effectiveness analyses were performed.

An independent review panel, masked to the EP and vetted through the conflict of interest process, provided a review of the guideline and recommended the guideline for approval by the CAP Council on Scientific Affairs and the ASH Executive Committee. The final recommendations (guideline statements) are summarized in Table 3.

Guideline Statements

Statement 1.—Strong Recommendation.—The treating clinician should provide relevant clinical data or ensure that they are readily accessible by the pathologist.

Note.—These data include, but are not limited to, the patient's age; sex; ethnicity; history of any hematologic disorder or known predisposing conditions or syndromes; any prior malignancy; exposure to cytotoxic therapy, immunotherapy, radiotherapy, or other possibly toxic substances; and any additional clinical findings of diagnostic or prognostic importance. The treating clinician should also include any history of possibly confounding factors, such as recent growth factor therapy, transfusions, or other medications that might obscure or mimic the features of AL. The treating clinician should also obtain and provide information regarding any family history of any hematologic disorder or other malignancies.

The strength of evidence was *convincing* to support this guideline statement.

Twenty-eight studies, comprising 2 nonrandomized clinical trials (NRCTs)^{10,11} and 26 prospective cohort studies (PCs)^{12–37} support including data on age. Most of the PCs had a risk of bias determination of low to moderate, except for 3 studies determined to be low^{12,24,30} and 6 determined to be moderate.^{14,22,26,29,32,33} None of those studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 7 in the SDC for the quality-assessment results for the studies included for statement 1. For the quality assessment and summaries of study data for family history, ethnicity, and performance status, for which there were fewer studies identified in our SR, refer to Supplemental Tables 1 and 2.

Although it may seem that inspection of blood and marrow samples is sufficient to make a diagnosis of AL, in fact, clinical information is often essential for the correct diagnosis, classification, and/or determination of prognosis. For example, a patient with a high white blood cell (WBC) count and 20% blasts in a peripheral blood (PB) leukocyte differential might be erroneously diagnosed as having AL if the pathologist is not aware that the blood was from a 1-week old baby with Down syndrome, in which case, the more likely diagnosis is transient abnormal myelopoiesis rather than AL.^{38–40} Further, if the clinician and/or pathologist are not aware that a newly diagnosed patient with AML has a strong family history of leukemia or other hematologic abnormalities, appropriate genetic testing may not be performed to confirm a myeloid neoplasm with a germline predisposition, which, if present, is important not only for genetic counseling of the patient's family but also for the selection of family members as potential donors for hematopoietic stem cell therapy for the patient.^{8,41} Although somewhat unusual, these examples illustrate the importance of a detailed clinical history, including information regarding possible predisposing factors, such as a family history of hematopoietic neoplasms or other hematologic abnormalities, exposure to cytotoxic therapies or other leukemogenic toxins, and exposure to any medications or known factors that might mimic the clinical and morphologic features of AL.

The most routine and basic clinical information—the patient's age and sex—are important because of their effect on prognosis in AL. In our SR of the literature, age emerged as a statistically significant prognostic factor in AL.^{10–37} In

Table 3. Guideline Statements and Strengths of Recommendations

Guideline Statement	Strength of Recommendation
<p>1. The treating clinician should provide relevant clinical data or ensure that this is readily accessible by the pathologist. Note.—These data include, but are not limited to, the patient's age, sex, and ethnicity; history of any hematologic disorder or known predisposing conditions or syndromes; any prior malignancy; exposure to cytotoxic therapy, immunotherapy, radiotherapy, or other possibly toxic substances; and any additional clinical findings of diagnostic or prognostic importance. The treating clinician should also include any history of possibly confounding factors, such as recent growth factor therapy, transfusions or other medications that might obscure or mimic the features of acute leukemia. The treating clinician should also obtain and provide information regarding any family history of any hematologic disorders or other malignancies.</p>	Strong recommendation
<p>2. The treating clinician should provide relevant physical examination and imaging findings or ensure that those results are readily accessible by the pathologist. Note.—This includes, but is not limited to, neurologic exam findings and the presence of tumor masses (eg, mediastinal), other tissue lesions (eg, cutaneous), and/or organomegaly.</p>	Recommendation
<p>3. The pathologist should review recent or concurrent complete blood cell (CBC) counts and leukocyte differentials and evaluate a peripheral blood smear.</p>	Strong recommendation
<p>4. The treating clinician or pathologist should obtain a fresh bone marrow aspirate for all patients suspected of acute leukemia, a portion of which, should be used to make bone marrow aspirate smears for morphologic evaluation. If performed, the pathologist should evaluate an adequate bone marrow trephine core biopsy, bone marrow trephine touch preparations, and/or marrow clots, in conjunction with the bone marrow aspirates. Note.—If bone marrow aspirate material is inadequate or if there is compelling clinical reason to avoid bone marrow examination, peripheral blood may be used for diagnosis and ancillary studies if sufficient numbers of blasts are present. If a bone marrow aspirate is unobtainable, touch imprint preparations of a core biopsy should be prepared and evaluated, and an additional core biopsy may be submitted unfixed in tissue culture medium for disaggregation for flow and genetic studies. Optimally, the same physician should interpret the bone marrow aspirate smears and the core biopsy specimens, or the interpretations of those specimens should be correlated if performed by different physicians.</p>	Strong recommendation
<p>5. In addition to morphologic assessment (blood and bone marrow), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (ie, karyotype), appropriate molecular genetic and/or fluorescent in situ hybridization (FISH) testing, and flow cytometric immunophenotyping (FCI). The flow cytometry panel should be sufficient to distinguish acute myeloid leukemia (including acute promyelocytic leukemia), T-cell acute lymphoblastic leukemia (T-ALL) (including early T-cell precursor leukemias), B-cell precursor ALL (B-ALL), and acute leukemia of ambiguous lineage on all patients diagnosed with acute leukemia. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis. Note.—If sufficient bone marrow aspirate or peripheral blood material is not available for FCI, immunohistochemical studies may be used as an alternative method for performing limited immunophenotyping. In addition, a second bone marrow core biopsy can be obtained and submitted, unfixed in tissue culture media, for disaggregation for genetic studies and flow cytometry.</p>	Strong recommendation
<p>6. For patients with suspected or confirmed acute leukemia, the pathologist may request and evaluate cytochemical studies to assist in the diagnosis and classification of acute myeloid leukemia (AML).</p>	Expert consensus opinion
<p>7. The treating clinician or pathologist may use cryopreserved cells or nucleic acid, formalin fixed, nondecalified paraffin-embedded (FFPE) tissue, or unstained marrow aspirate or peripheral blood smears obtained and prepared from peripheral blood, bone marrow aspirate or other involved tissues for molecular or genetic studies in which the use of such material has been validated. Such specimens must be properly identified and stored under appropriate conditions in a laboratory that is in compliance with regulatory and/or accreditation requirements.</p>	Recommendation
<p>8. For patients with acute lymphoblastic leukemia (ALL) receiving intrathecal therapy, the treating clinician should obtain a cerebrospinal fluid (CSF) sample. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist.</p>	Strong recommendation
<p>9. For patients with acute leukemia other than those with ALL who are receiving intrathecal therapy, the treating clinician may, under certain circumstances, obtain a cerebrospinal fluid (CSF) sample when there is no clinical contraindication. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist.</p>	Expert consensus opinion
<p>10. For patients with suspected or confirmed acute leukemia, the pathologist may use flow cytometry in the evaluation of CSF.</p>	Recommendation
<p>11. For patients who present with extramedullary disease without bone marrow or blood involvement, the pathologist should evaluate a tissue biopsy and process it for morphologic, immunophenotypic, cytogenetic, and molecular genetic studies, as recommended for the bone marrow. Note.—Additional biopsies may be indicated to obtain fresh material for ancillary testing.</p>	Strong recommendation

Table 3. Continued

Guideline Statement	Strength of Recommendation
12. For patients with suspected or confirmed acute leukemia, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of minimal residual disease (MRD).	Strong recommendation
13. For pediatric patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(12;21)(p13.2;q22.1); <i>ETV6-RUNX1</i> , t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i> , <i>KMT2A (MLL)</i> translocation, <i>iAMP21</i> , and trisomy 4 and 10 is performed.	Strong recommendation
14. For adult patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i> is performed. In addition, testing for <i>KMT2A (MLL)</i> translocations may be performed.	Strong recommendation for testing for t(9;22)(q34.1;q11.2) and <i>BCR-ABL1</i> ; Recommendation for testing for <i>KMT2A (MLL)</i> translocations
15. For patients with suspected or confirmed ALL, the pathologist or treating clinician may order appropriate mutational analysis for selected genes that influence diagnosis, prognosis, and/or therapeutic management, which includes, but is not limited to, <i>PAX5</i> , <i>JAK1</i> , <i>JAK2</i> , and/or <i>IKZF1</i> for B-ALL and <i>NOTCH1</i> and/or <i>FBXW7</i> for T-ALL. Testing for overexpression of <i>CRLF2</i> may also be performed for B-ALL.	Recommendation
16. For pediatric and adult patients with suspected or confirmed acute myeloid leukemia (AML) of any type, the pathologist or treating clinician should ensure that testing for <i>FLT3-ITD</i> is performed. The pathologist or treating clinician may order mutational analysis that includes, but is not limited to, <i>IDH1</i> , <i>IDH2</i> , <i>TET2</i> , <i>WT1</i> , <i>DNMT3A</i> , and/or <i>TP53</i> for prognostic and/or therapeutic purposes.	Strong recommendation for testing for <i>FLT3-ITD</i> Recommendation for testing for other mutational analysis
17. For adult patients with confirmed core-binding factor (CBF) AML (AML with t(8;21)(q22;q22.1); <i>RUNX1-RUNX1T1</i> or inv(16)(p13.1q22) / t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>), the pathologist or treating clinician should ensure that appropriate mutational analysis for <i>KIT</i> is performed. For pediatric patients with confirmed CBF-AML; <i>RUNX1-RUNX1T1</i> or inv(16)(p13.1q22) / t(16;16)(p13.1;q22); <i>CBFB-MYH11</i> —the pathologist or treating clinician may ensure that appropriate mutational analysis for <i>KIT</i> is performed.	Strong recommendation for testing for <i>KIT</i> mutation in adult patients with CBF-AML Expert consensus opinion for testing for <i>KIT</i> mutation in pediatric patients with CBF-AML
18. For patients with suspected acute promyelocytic leukemia (APL), the pathologist or treating physician should also ensure that rapid detection of <i>PML-RARA</i> is performed. The treating physician should also order appropriate coagulation studies to evaluate for disseminated intravascular coagulation (DIC).	Strong recommendation
19. For patients other than those with confirmed core binding factor AML, APL, or AML with myelodysplasia-related cytogenetic abnormalities, the pathologist or treating clinician should also ensure that mutational analysis for <i>NPM1</i> , <i>CEBPA</i> , and <i>RUNX1</i> is also performed.	Strong recommendation
20. For patients with confirmed acute leukemia, no recommendation is made for or against the use of global/gene-specific methylation, microRNA (miRNA) expression, or gene expression analysis for diagnosis or prognosis.	No recommendation
21. For patients with confirmed mixed phenotype acute leukemia (MPAL), the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i> , and <i>KMT2A (MLL)</i> translocations is performed.	Strong recommendation
22. All laboratory testing performed for the initial workup and diagnosis of a patient with acute leukemia must be performed in a laboratory that is in compliance with regulatory and/or accreditation requirements.	Strong recommendation
23. If after examination of a peripheral blood smear, it is determined that the patient will require immediate referral to another institution with expertise in the management of acute leukemia for treatment, the initial institution should, whenever possible, defer invasive procedures, including bone marrow aspiration and biopsies, to the treatment center to avoid duplicate procedures, associated patient discomfort, and additional costs.	Strong recommendation
24. If a patient is referred to another institution for treatment, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogenetic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded when they become available.	Strong recommendation
25. In the initial report, the pathologist should include laboratory, morphologic, immunophenotypic, and, if performed, cytochemical data, on which the diagnosis is based, along with a list of any pending tests. The pathologist should issue addenda/amended reports when the results of additional tests become available.	Strong recommendation
26. The pathologist and treating clinician should coordinate and ensure that all tests performed for classification, management, predicting prognosis, and disease monitoring are entered into the patient's medical records. Note.—This information should include the sample source, adequacy, and collection information, as applicable.	Strong recommendation
27. Treating physicians and pathologists should use the current World Health Organization (WHO) terminology for the final diagnosis and classification of acute leukemia.	Strong recommendation

ALL children 1 to 9 years old generally had a more-favorable outcome than those younger than 1 or older than 10 years,^{17,22,27,34,36} whereas, in AML, patients 60 years old or older have worse outcomes compared to younger patients.^{10,16,23,26,28,34,37}

Published evidence revealed that sex was also prognostically important. In childhood ALL, males tended to have a worse overall prognosis than females did,^{19,42} although that difference was not as clear in adult ALL.⁴³ In AML, males fared worse than females did.^{44,45}

Although familial acute leukemia is generally regarded as rare, an inherited predisposition to hematopoietic neoplasms—including AL—is likely more common than appreciated and can only be recognized by detailed clinical information.⁴¹ Because of the importance of hematopoietic neoplasms with germline predisposition for genetic counseling and for the detection of family members as potential donors for hematopoietic stem cell therapy, verified cases of inherited-predisposition syndromes should be documented in the patient's medical record and in the pathology report. A number of predisposing syndromes with germline mutations have been described and included in the most recent revision of the WHO *Classification of Tumours of Haematopoietic and Lymphoid Tissues*⁸; the most well-known of which include AML with germline *CEBPA* mutation,^{46,47} myeloid or lymphoid neoplasms with germline *RUNX1* mutation,^{48,51} myeloid or lymphoid neoplasms with germline *ANKRD26* mutation,^{52,53} and myeloid neoplasms with *GATA2* mutation,^{54,58} among others. In addition, AL arising in patients with a background of inherited bone marrow (BM) failure syndromes, such as dyskeratosis congenita and other telomerase biology diseases, and Fanconi anemia, should be recognized in the medical record.^{41,59,60} Family histories that include a first-degree or second-degree relative with AML, ALL, myelodysplastic syndrome (MDS), persistent thrombocytopenia, clinical bleeding propensity, immunodeficiency, or a hematologic malignancy at a young age are important clues for AL with germline predisposition.^{41,61,62}

Therapy-related myeloid neoplasms, including therapy-related AML (t-AML), are late complications of cytotoxic chemotherapy and/or radiotherapy administered for a prior neoplastic or nonneoplastic disorder.^{63,67} Currently, t-AML comprises nearly 15% to 20% of all cases of AML, but the incidence is rising as more patients survive treatment for their initial cancers.⁶⁸ Therapy-related lymphoblastic leukemia has been reported but is much less common than t-AML.^{69,70}

In the WHO classification system, therapy-related myeloid neoplasms are recognized as a distinct category for patients who have a history of prior exposure to alkylating agents, topoisomerase II inhibitors, antimetabolites, anti-tubulin agents, and/or ionizing radiation.⁸

Information regarding the specific therapy implicated in the pathogenesis of therapy-related myeloid neoplasms is important because of the correlations between the clinical, morphological, genetic findings, and prognosis with the prior therapeutic regimen.^{65,67,68,71–73}

Although most cases of t-AML are thought to be related to mutational events induced by prior cytotoxic therapy, the exact mechanisms and pathways involved are not clear. Most patients treated with cytotoxic therapies do not develop t-AML, suggesting there may be underlying predisposing genetic factors.^{74,75} Thus, the history of previous cytotoxic therapy in a patient with t-AML or therapy-related lymphoblastic leukemia is important for diagnosis and classification but also, perhaps, for identification of an inherited predisposition to drug-induced cancer.⁷⁶

As noted in the preceding section, a history of radiation therapy—either alone or in combination with chemotherapy for prior neoplastic or nonneoplastic conditions—is recognized as being associated with an increased risk for AL, particularly AML. Radiation exposure for individuals near natural disasters or atomic bomb explosions is also

associated with an increased risk of leukemia,^{77,78} and reportedly, radiation exposure after diagnostic procedures, including computed tomography scans in children, increases the risk for leukemia.^{79,80}

Patients receiving hematopoietic stem cell therapy are at increased risk for development of MDS and AML. Such patients usually receive a combination of chemotherapy and radiation therapy, and the rate of developing MDS or AML ranges from 2% to 7.6% in the studies reviewed.^{81–83} The incidence appears to be increased in older patients and in patients who received total-body radiation.⁷⁸

Exposure to certain chemicals is associated with an increased risk of development of AL, particularly AML. Benzene exposure, especially at high levels, is associated with an increased risk of AML.^{84,85} Other exposures are more controversial. Embalmers and funeral-home workers exposed to formaldehyde are reported to have an increased mortality rate from AML,⁸⁶ but a more recent meta-analysis found no such increase in risk of leukemia for workers exposed to formaldehyde when results were adjusted for smoking history.⁸⁷

The use of recombinant granulocytic growth factors, such as granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor, may transiently increase blasts in the blood and/or BM, which, in some cases, may account for 20% or more of the cells and lead to an erroneous diagnosis of AML. The increase in blasts may persist up to 5 weeks after cessation of growth factor therapy.⁸⁸ The pathologist should be aware of any growth factor or other cytokine therapy and its time of administration relative to the BM or blood sample in question to ascertain whether an elevated blast count is due to AML or could be attributable to a transient growth factor effect.

In addition, vitamin B12 or folate deficiency can markedly alter the BM appearance because of a proliferation of immature erythroblasts, potentially mimicking acute erythroid leukemia. It is critical to exclude vitamin B12 or folate deficiency before making a diagnosis of AL if the BM shows numerous blasts with erythroid features.^{89,90}

Public Comment Response to Statement 1.—There were 200 respondents to statement 1, of whom, 97% (n = 194) agreed, and 3% (n = 6) disagreed with the statement. The reason for the 3% disagreement was not clear. There were 42 written responses; most of which suggested that the statement include even more-specific and more-detailed information and that the data should reside in the patient's electronic medical record, where it would be accessible to the pathologists and treating physicians. The comments were considered in the final draft of statement 1 in this document.

There is strong evidence, based on our SR, as well as in literature gathered outside of our SR, to support statement 1. The clinical history is the starting point for the workup of AL and provides information that may be necessary for diagnosis, classification, and prognosis. In addition to the literature, the statement was enthusiastically supported—almost unanimously—by the respondents during the open comment period. Refer to Table 4 for study data on age.

Statement 2.—Recommendation.—The treating clinician should provide relevant physical examination and imaging findings or ensure that those results are readily accessible by the pathologist.

Note.—This includes, but is not limited to, neurologic exam findings and the presence of tumor masses (eg, mediastinal), other tissue lesions (eg, cutaneous), and/or organomegaly.

The strength of evidence was *convincing* to support this recommendation.

This recommendation was supported by 4 PCSs.^{19,20,22,91} One study was deemed to have a low risk of bias,⁹¹ 2 were deemed to have a low to moderate risk,^{19,20} and one was deemed to have a moderate risk of bias.²² None of those studies were found to have methodological flaws that would raise concerns about the studies' findings.

This recommendation was based on evidence from our SR as well as from expert consensus opinion. Evidence was available from the SR only for the relevance of central nervous system (CNS) involvement—which typically manifests as cranial nerve abnormalities or meningeal symptoms—for the outcome of AL (refer to Supplemental Table 3); based on expert opinion, other studies, which did not meet the criteria for SR, informed the recommendation for tumor masses, cutaneous lesions, and organomegaly.

Our SR identified 4 PCSs relevant to CNS status at presentation and outcomes in lymphoblastic leukemia.^{19,20,22,91} In a study of 4959 noninfant patients with B-precursor ALL treated between 1986 and 1999 by various protocols, patients having CNS disease at presentation were associated with poor outcomes in a multivariate analysis (hazard ratio [HR], 1.34; $P = .04$).¹⁹ A Nordic study of 2668 children with ALL, who were treated in 2 successive trials between 1992 and 2007, showed a significantly increased risk of treatment failure ($P < .01$ in both study cohorts) if CNS disease (defined as $\geq 5 \times 10^3/\text{mm}^3$ leukemic cells in the diagnostic spinal tap) was present at presentation.⁹¹ A study of 546 consecutive, pediatric patients from St Jude Children's Research Hospital (Memphis, Tennessee) treated from 1984 to 1991 with 2 protocols showed generally worse event-free survival (EFS) and overall survival (OS) when CNS disease was present at presentation (study 11: EFS, $P < .001$, OS, $P = .01$; study 12: EFS, $P = .03$, OS, $P = .22$).²⁰ In subsequent studies, improvements in identifying those at high risk of CNS relapse and with reinduction treatment as an integral component of overall therapy, differences in EFS and OS were lessened between those with and those without CNS involvement.²⁰ A Brazilian study of 229 consecutive children with ALL treated with a single protocol, for whom 220 were evaluable for CNS status at presentation, reported a statistically significant difference ($P < .001$) in 5-year EFS between children with ($n = 9$; 5-year EFS mean [SD], $79.4\% \pm 3.1$) and without ($n = 211$; 5-year EFS mean [SD], $40\% \pm 17.4$) CNS involvement.²² Refer to Supplemental Table 8 for the quality-assessment results of included studies for statement 2.

Acute leukemia may involve extramedullary sites, such as mediastinum (thymus and lymph nodes) and skin at presentation; those sites may be involved before, or concurrent with, BM and PB cells and may be sites of disease relapse. In ALL, an anterior mediastinal mass is present in 8% to 10% of childhood cases and in 15% of adult cases.⁹² Mediastinal enlargement visualized on imaging studies may point to T-lymphoblastic lymphoma/leukemia,⁹³ whereas mediastinal adenopathy was associated with inferior survival outcome in pediatric ALL ($P = .01$) in one PCS.⁹⁴ Myeloid sarcoma and leukemia cutis are 2 manifestations more commonly associated with AML and usually require a tissue biopsy for diagnosis⁹⁵; lymphoblastic leukemias can also involve the skin.^{96–98} Enlargement of liver and spleen are most common sites of extramedullary involvement in ALL with marked organomegaly being more frequent in children and uncommon in adults.⁹² In AML,

palpable splenomegaly or hepatomegaly occurs in about one-quarter of patients.⁹⁹ Knowledge of involvement at those sites can assist the pathologist in making an accurate diagnosis, offering recommendations for sampling other sites, and performing ancillary testing.

The EP noted that the physical examination and imaging information was typically obtained in the course of routine workup and making such information available should pose no additional burden on the clinician submitting the samples.

Public Comment Response to Statement 2.—There were 195 respondents, of whom, 97.44% ($n = 190$) agreed and 2.56% ($n = 5$) disagreed with statement 2. There were 21 written comments, including a number that suggested adding imaging studies and that comments regarding mediastinal disease and cutaneous manifestations be specifically mentioned. Others commented that the data should be available through the electronic medical record. These comments were taken into consideration in the final draft of statement 2 presented in this article.

Statement 3.—*Strong Recommendation.*—The pathologist should review recent or concurrent complete blood cell (CBC) counts and leukocyte differentials and evaluate a PB smear.

The strength of evidence was *convincing* to support this guideline statement.

This statement is supported by 51 studies,* comprising 2 randomized, controlled trials (RCTs)^{113,117}; 5 NRCTs^{11,106,111,112,131}; and 44 PCSs.[†] For the 2 RCTs, the trial by Lange et al¹¹³ was deemed to have a very low risk of bias, and the trial by Schneider et al¹¹⁷ was deemed to have a high risk of bias. For the 5 NRCTs, 4 reported a risk of bias of low to moderate,^{11,111,112,131} and one reported a moderate risk of bias.¹⁰⁶ For the 44 PCSs, 7 of the studies were deemed to have a low risk of bias,[‡] 30 were deemed to have a low to moderate risk of bias,[§] and 7 were deemed to have a moderate risk of bias.^{**} None of these studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 9 for the quality-assessment results of studies included for statement 3.

The diagnosis of AL is usually first suspected when a patient presents with symptoms related to an abnormal CBC, such as fatigue or weakness because of low hemoglobin, bleeding, or bruising from thrombocytopenia, or fever caused by an infection related to neutropenia. Thus, an abnormal CBC is frequently the starting point for the workup and evaluation for AL. The CBC may also identify hematologic abnormalities, such as a dangerously low hemoglobin level or platelet count, for which therapeutic measures are immediately indicated. Lastly, according to the guidelines for application of the WHO classification, inspection of the PB smear is critical because it may provide evidence for a diagnosis of AL as well as provide information on features that aid in its classification.¹³²

* References 11–21, 23, 24, 26, 29–31, 71, 91, 100–131.

† References 12–21, 23, 24, 26, 29–31, 71, 91, 100–105, 107–110, 114–116, 118–130.

‡ References 12, 24, 30, 91, 101, 102, 119.

§ References 13, 15–21, 23, 31, 71, 100, 103–105, 107–110, 114–116, 118, 120–122, 124, 126–128.

** References 14, 26, 29, 106, 123, 125, 129.

Table 4. Summary of Study Data on Age

Source, y	Study Design	Influence of Age (<1 y) on Outcome	Influence of Age (>2–10 y) on Outcome
Mendler et al, ¹⁰ 2012	NRCT
Damm et al, ¹¹ 2012	NRCT
Schwind et al, ³⁰ 2010	PCS
Marks et al, ²¹ 2009	PCS
Medeiros et al, ³³ 2010	PCS
Taskesen et al, ¹² 2011	PCS
Wagner et al, ¹⁵ 2010	PCS
Kühnl et al, ¹⁷ 2010	PCS
Lo-Coco et al, ²³ 2008	PCS
Langer et al, ²⁴ 2008	PCS
Gale et al, ²⁶ 2008	PCS
Roman-Gomez et al, ²⁷ 2007	PCS
Dufour et al, ²⁸ 2010	PCS
Santamaria et al, ³¹ 2010	PCS
Tauchi et al, ³⁵ 2008	PCS	Age <6 mo, significant in MVA with risk ratio, 2.063, <i>P</i> = .04; <i>N</i> = 74	...
Röllig et al, ¹⁴ 2010	PCS
Wandt et al, ²⁵ 2008	PCS
Lugthart et al, ¹³ 2010	PCS
Escherich et al, ¹⁸ 2010	PCS	...	EFS, age 1–9 y, 69.1% versus ≥10 y, 55%, <i>P</i> = .001; OS, age 1–9 y, 80.2% versus ≥10 y, 66.9%, <i>P</i> = .001; <i>N</i> = NR
Salzer et al, ¹⁹ 2010	PCS	...	Noninfant B-precursor ALL age >10 y versus 1–9 y HR, 1.64 <i>P</i> < .001; <i>N</i> = 5255

Table 4. Extended

Influence of Age (>10–20 y) on Outcome	Influence of Age (>20–65 y) on Outcome	Influence of Age (>65 y) on Outcome
...	Age group ≥60 versus <60 y for DFS: HR, 2.19 (95% CI, 1.67–2.88) <i>P</i> < .001; for OS: HR, 2.46 (95% CI, 1.93–3.15) <i>P</i> < .001; for CR: OR, .55 (95% CI, .33–.91) <i>P</i> = .02; N = 175 patients were 18–59 y; 225 were 60–83 y	...
...	In MVA, for OS, age above versus below the median HR, 1.96 (95% CI, 1.34–2.87), <i>P</i> = .001; N = 269 patients were 16–60 y	...
...	In MVA, in all patients for CR, age OR/HR, 0.36 (95% CI, 0.17–0.78) <i>P</i> = .01; N = 187	...
Remission rates were higher in younger patients (98% at ages 15–19 y and 20–29 y; 93% at ages 30–39 y and 40–49 y; and 79% in those 50 y and older) <i>P</i> < .001; N = 311	Remission rates were higher in younger patients (98% at ages 20–29 y; 93% at 30–39 y and 40–49 y; and 79% in 50 y and older) <i>P</i> < .001; N = 1192	...
...	OS: HR for age >60 y, 2.4 (95% CI, 2.1–2.8), <i>P</i> < .01 [HR > 1, worse OS]; N = 1344	...
...	OS for age ≤60 y: HR, 1.02 (95% CI, 1.01–1.03) <i>P</i> < .001	...
...	OS (age above/below median): HR, 1.69 (95% CI, 1.21–2.35) <i>P</i> = .01; N = 275	...
...	HR for OS for age, 10-y increase, 1.5 (95% CI, 1.3–1.8) <i>P</i> < .001; N = 368	...
...	MVA for response to induction therapy: age increase by 1 y, HR, 0.98 (95% CI, 0.96–1.00), <i>P</i> = .03; N = 509	...
...	The OR of CR for age (10-y increase): OR, 0.47 (95% CI, 0.25–0.91), <i>P</i> = .02; N = 172	...
...	In MVA, for CR, age OR, 1.04 (95% CI, 1.02–1.06) <i>P</i> < .001; for relapse-risk OR, 1.01 (95% CI, 1.01–1.02) <i>P</i> < .001; for OS OR, 1.02 (95% CI, 1.01–1.03) <i>P</i> < .001; N = 1425	...
...	In MVA, age >15 y was a significant factor in DFS in the global series (<i>P</i> = .01); N = 100	...
...	Age (10-y increase) has HR, 1.35 (95% CI, 1.20–1.53) <i>P</i> < .001 for OS; HR, 1.18 (95% CI, 1.07–1.31) <i>P</i> = .01 for EFS; N = NR	...
...	...	Age >65 HR for OS, 3.2 (95% CI, 1.7–5.8) <i>P</i> < .001; HR for RFS, 2.7 (95% CI, 1.4–5.2) <i>P</i> = .01; N = 127
...
...	In UVA, age 61–65 versus age >65 was a significant factor for DFS; <i>P</i> = .04; N = 909	Remission rates: In UVA, age 61–65 versus age >65 was a significant factor for remission rates <i>P</i> = .04; N = 909
...	Age ≤60 versus >60 y was a significant factor for CR, EFS, and OS, all <i>P</i> < .001; N = 720	...
...	HR for OS for age (difference of 10 y), 1.23 (95% CI, 1.19–1.27) <i>P</i> < .001; N = 288	...
...
...

Table 4. Continued

Source, y	Study Design	Influence of Age (<1 y) on Outcome	Influence of Age (>2-10 y) on Outcome
Pui et al, ²⁰ 2010	PCS	...	Study 11.—EFS at 10 y: <1 y (N = 11), 45.5 ± 13.7 versus 1-9 y (N = 257), 735.5 ± 2.8 versus >10 y (N = 90) 61.1 ± 5.1; P < .001 OS at 10 y: <1 y, 63.6 ± 13.6 versus 1-9 y, 80.2 ± 2.5 versus <10 y, 67.8 ± 4.9; P = .01 Study 12.—EFS at 10 y: <1 y (N = 8), 25 ± 12.5 versus 1-9 y (N = 128), 69.8 ± 4.1 versus >10 y (N = 52) 48 ± 6.9; P = .001 OS at 10 y: <1 y, 50 ± 15.8 versus 1-9 y, 85.2 ± 3.1 versus >10 y, 67.2 ± 6.5; P = .01 Study 13A.—EFS at 10 y: >1 y (N = 5) 20 ± 12.6 versus 1-9 y (N = 117), 82 ± 3.6 versus >10 y (N = 43), 48.8 ± 7.4; P < .001 OS at 10 y: <1 y, 40 ± 17.9 versus 1-9 y, 86.3 ± 3.2 versus >10 y, 60.5 ± 7.3; P < .001 Study 13B.—EFS at 10 y: <1 y (N = 10), 70.0 ± 13.6; 1-9 y (N = 161), 81.2 ± 3.3; >10 y, 70.9 ± 5.6; P = .08; OS at 10 y: <1 y, 70 ± 13.6, 1-9 y, 89.3 ± 2.6, >10 y (73.5 ± 5.5; P = .004) 5-y EFS for 1-9 y, mean (SD) 80.2% (3.6) versus 70.5% (6.1) in >9 y; P = .02; N = 168
Scrideli et al, ²² 2009	PCS
Groschel et al, ¹⁶ 2010	PCS
Damm et al, ²⁹ 2010	PCS
Seifert et al, ³² 2009	PCS
Roman-Gomez et al, ³⁴ 2009	PCS
Moorman et al, ³⁶ 2012	PCS
Schneider et al, ³⁷ 2012	PCS

Abbreviations: ALL, acute lymphoblastic leukemia; CR, complete remission; DFS, disease free survival; EFS, event free survival; HR, hazard ratio; MVA, multivariate analysis; . . . , not available; NR, not reported; NRCT, nonrandomized clinical trial; OR, odds ratio; OS, overall survival; PCS, prospective cohort study; RFS, relapse-free survival; UVA, univariate analysis.

The literature strongly supports the importance of some CBC parameters as independently significant prognostic indicators in AL. In AML, numerous studies show a significant effect of the WBC count on response to therapy, OS, and/or EFS ($P \leq .05$) in all studies cited.^{††} Similar results are reported in ALL.^{‡‡} In some reports of AL, the platelet count,^{§§} hemoglobin level,^{30,116,117} and the percentage of blasts^{14,103} were also independent, significant factors in OS and EFS.

Public Comment Response to Statement 3.—There were 195 respondents, of whom, 96.41% (n = 188) agreed, and 3.59% (n = 7) disagreed with the statement. The reasons for the disagreement responses were not stated. The 31 written comments submitted generally offered strong endorsement for the statement. Some emphasized that the pathologist should review the specimen personally and not rely on

reported data; a few indicated the specimen review should be performed by the treating clinician. These comments were taken into consideration for the final draft of statement 3 that is presented in this article.

The knowledge gained from evaluation of the CBC and review of the PB provides information that is important for diagnostic, prognostic, and classification purposes. The evidence obtained from our SR strongly supports this statement, and opinions gathered during the open comment period were also supportive.

Refer to Table 5 for study data on CBCs.

Statement 4.—Strong Recommendation.—The treating clinician or pathologist should obtain a fresh BM aspirate for all patients suspected of AL, a portion of which should be used to make BM aspirate specimens for morphologic evaluation. If performed, the pathologist should evaluate an adequate BM trephine core biopsy, BM trephine touch preparations, and/or marrow clots, in conjunction with the BM aspirates.

Note.—If BM aspirate material is inadequate or if there is compelling clinical reason to avoid BM examination, PB may

^{††} References 12-14, 16, 23, 24, 26, 29-31, 100-105, 109, 110, 113-123, 125-127, 129, 130.

^{‡‡} References 17-21, 91, 106, 108, 111, 112, 124, 128.

^{§§} References 11, 13, 15, 71, 101, 107, 111, 131.

Table 4. Continued, Extended		
Influence of Age (>10–20 y) on Outcome	Influence of Age (>20–65 y) on Outcome	Influence of Age (>65 y) on Outcome
...
...	OR for OS, 1.40 (95% CI, 1.27–1.54) $P < .001$; N = 1382	...
...	In MVA for OS, age above versus below median HR, 1.91 (95% CI, 1.3–2.81) $P = .001$; N = 249	...
...	OR for CR, 0.587, $P = .01$; DFS, 2.14, $P < .001$; OS, 1.85, $P < .001$; N = 1455	...
MVA showed age >15 y was an independent prognostic factor in predicting DFS ($P = .001$) and OS ($P = .001$) in the global series; N = 353 patients were 0.3–82 y. Of those, 179 were children (median age, 5 y; range, 0.3–14 y), and 174 adults (median age, 29 y; range, 15–82 y)
...	In MVAs, age HR, 1.02 (95% CI, 1.00–1.03) $P = .01$; for OS HR, 1.02 (95% CI, 1.01–1.04) $P = .001$; N = 454 patients were between 15–65 y	...
...	Cox regression model for OS showed: Age, +10 y; HR, 1.4 (95% CI, 1.2–1.5) $P < .001$; RFS HR, 1.2 (95% CI, 1.1–1.4) $P < .001$; N = 648 patients were 17–85 y	...

be used for diagnosis and ancillary studies if sufficient numbers of blasts are present. If a BM aspirate is unobtainable, touch-imprint preparations of a core biopsy should be prepared and evaluated, and an additional core biopsy may be submitted, unfixed, in tissue culture medium for disaggregation for flow and genetic studies. Optimally, the same physician should interpret the BM aspirate specimens and the core biopsy specimens, or the interpretations of these specimens should be correlated if performed by different physicians.

Numerous studies confirm the utility of BM aspirate specimens in the diagnosis of AL. The BM aspirate is the optimal specimen for both blast enumeration and dysplasia assessment for the myeloid and erythroid lineages.¹³² The confirmation of a blast percentage of at least 20% in blood or BM is a WHO requirement for AL diagnosis.¹³² A manual differential cell count performed on BM aspirate specimens is the standard procedure for blast enumeration.

An adequate BM trephine core biopsy provides essential diagnostic information in patients with AL, including overall cellularity, assessment of residual hematopoietic cells, extent of leukemia effacement of the BM as well as distinctive features that could affect treatment response, such as necrosis and fibrosis.^{132–138} In addition, numerous special

stains can be performed on a BM core biopsy section that can provide both diagnostic and prognostic information in patients with AL.^{132,135–138} The breadth of potential stains includes numerous immunohistochemical stains and some in situ hybridization stains (ones not adversely affected by decalcification), and DNA-based molecular studies can also be performed on the BM core or on clot biopsy sections. Core biopsy sections assume a greater role in leukemia diagnosis when an adequate BM aspirate is not obtained.

Adequacy of the BM trephine core biopsy specimen has been assessed in several studies. In general, an intact core biopsy specimen that is 1 cm or larger is considered optimal for diagnosis.^{133–135,137,139} This specimen cannot consist largely of cortical or subcortical regions but, instead, must contain intact hematopoietic regions of the BM.

Touch preparations of the BM core biopsy can potentially facilitate the AL diagnosis in a variety of ways.^{132–135,138} Because the touch preparations can be readily prepared before the BM core biopsy specimen is placed in fixative, they should be made every time a BM core biopsy is obtained. The cells in those touch preparations can be evaluated by Wright-Giemsa stain; differential cell counts can be performed as well as dysplasia assessment.^{132–134,138} In addition, these touch-preparation slides can be used for

Table 5. Summary of Study Data for Complete Blood Cell (CBC) Counts

Source, y	Study Design	Blood Blasts Influence on Outcome, %	WBC Count Influence on Outcome
Lange et al, ¹¹³ 2008	RCT	...	5-y EFS.—WBC × 1000/mm ³ <50, 45 ± 4; 50–100, 33 ± 9; >100, 32 ± 8; <i>P</i> < .001 5-y OS.—WBC × 1000/mm ³ <50, 56 ± 4; 50–100, 47 ± 10; >100, 41 ± 8; <i>P</i> < .001; <i>N</i> = 143/900
Schneider et al, ¹¹⁷ 2009	RCT	...	CR, OR, 0.53; <i>P</i> < .001
Oudot et al, ¹¹¹ 2008	NRCT	...	Patients with CR after induction: WBC × 1000/mm ³ <10, 47%; 10–50, 31%; 50–100, 9%; >100, 13%; <i>P</i> = .001; <i>N</i> = 1333/1386
Aricò et al, ¹¹² 2008	NRCT	...	5-y EFS.—WBC × 1000/mm ³ <20, 79.3 ± 1.2; 20–100, 74.5 ± 2.1; >100, 58.1 ± 3.7; HR, 0.70; <i>P</i> = .01; <i>N</i> = 177/1744
Damm et al, ¹¹ 2012	NRCT
Schwind et al, ¹³¹ 2011	NRCT	...	OR/HR, 1.22 (range, 1.09–1.35) for each 2-fold increase
Gaidzik et al, ¹⁰⁰ 2011	PCS	...	EFS.—Log ₁₀ WBC: HR, 1.25 (95% CI, 1.09–1.44) <i>P</i> = .002 RFS.—Log ₁₀ WBC: HR, 1.459 (95% CI, 1.20–1.770); <i>P</i> = .001
Metzeler et al, ¹⁰¹ 2011	PCS	...	OS.—Log ₁₀ WBC: HR, 1.45 (95% CI, 1.24–1.69); <i>P</i> = .001 EFS.—HR, 1.23; <i>P</i> < .001 CR.—OR, 0.71; <i>P</i> < .001 DFS.—HR, 1.37; <i>P</i> < .001; <i>N</i> = 427, 104 versus 323; 418 included in this analysis
Taskesen et al, ¹² 2011	PCS	...	OS.—HR, 1.35 (95% CI, 1.12–1.62); <i>P</i> < .001; <i>N</i> = 1182, 1031 versus 60 versus 91; (1143 included in this analysis)
Montesinos et al, ¹⁰² 2011	PCS	...	<i>P</i> = .03, <i>N</i> = 651; 72 versus 579
Stölzel et al, ¹⁰³ 2011	PCS	OS.— <i>P</i> = .04; HR, 1.47 (95% CI 1.01–2.13); <i>N</i> = 233 versus 72	OS.— <i>P</i> = .07 for all patients, but <i>P</i> < .01 for those ≤60 y; <i>N</i> = 305; 233 versus 72
Kayser et al, ⁷¹ 2011	PCS
Tallman et al, ¹⁰⁴ 2010	PCS	...	OS.—high versus low WBC: HR, 2.38 (95% CI, 1.71–3.32) <i>P</i> < .001 DFS.—HR, 2.70 (95% CI, 1.88–3.88) <i>P</i> < .001
Lugthart et al, ¹³ 2010	PCS	...	Median, Group A.—inv (3)/t(3;3) = 14.8 Group B.—t(3q26) = 7.2 Group C.—t(3q21) = 14.6 Group D.—Other 3q = 4.9; <i>N</i> = 288 OS.—HR = 1.25 (95% CI, 1.16–1.34) <i>P</i> < .001
Röllig et al, ¹⁴ 2010	PCS	Median, <i>N</i> = 906 BM blasts d 15.—CR, ≤10%, 58.6% versus >10%, 46.9%, <i>P</i> < .005; median DFS, ≤10%, 0.69 versus >10%, 0.98, <i>P</i> = .51; median OS, ≤10%, 0.81 versus >10%, 0.71, <i>P</i> = .37	Median, <i>N</i> = 906; WBC × 1000/mm ³ outcomes: CR, ≤20 × 1000/mm ³ , 53.6% versus >20 × 10 ⁹ /L, 44%, <i>P</i> = .005; median DFS, ≤20 × 1000/mm ³ , 0.95 versus >20 × 1000/mm ³ , 0.55, <i>P</i> = .01; median OS, ≤20, 0.90 versus >20, 0.56, <i>P</i> < .001
Ho et al, ¹⁰⁵ 2010	PCS	...	Median, <i>WT1</i> mutant = 35 × 1000/mm ³ <i>WT1</i> WT, 20.5 × 1000/mm ³ ; <i>N</i> = 388 EFS for WBC count >50 versus less, HR, 1.32 (95% CI, 1.13–1.56) <i>P</i> < .001
Wagner et al, ¹⁵ 2010	PCS
Groschel et al, ¹⁶ 2010	PCS	...	Median, Log ₁₀ (WBC); <i>N</i> = 1382 OS.—OR, 1.52 (95% CI, 1.35–1.70) <i>P</i> < .001
Kühnl et al, ¹⁷ 2010	PCS	...	OS.—OR, 1.52 (95% CI, 1.35–1.70) <i>P</i> < .001; <i>N</i> = 368 OS.—HR for WBC >30, 3.9 (95% CI, 2.2–6.9) <i>P</i> < .001

Table 5. Extended

Hgb Influence on Diagnosis	Hgb Influence on Outcome	Platelet Count Influence on Diagnosis	Platelet Count Influence on Outcome
...
...	<i>P</i> < .05; median, 92; CR, <i>P</i> = NS, NR; blast clearance, <i>P</i> = NS, NR	...	<i>P</i> < .05; median, 58; CR, <i>P</i> = NS, NR; blast clearance, <i>P</i> = NS, NR
...	Patients with CR after induction: <10 000 mg/dL, 62.5% versus >10 000 mg/dL, 18.5% versus undetermined, 19%; <i>P</i> = NS, NR; N = 1333/1386
...
...	Median in ID1, low, 45; ID1, high, 60; in MVA for OS, platelet count below versus above median HR, 1.56 (95% CI, 1.07–2.27), <i>P</i> = .02; for RFS HR, 1.56 (95% CI, 1.07–2.30), <i>P</i> = .022; N = 269
...	OR/HR, 1.15 (range, 1.05–1.25) each 50-unit increase
...
...	50-unit increase associated with EFS.—HR, 1.09; <i>P</i> = .01; OR, 0.83 CR.— <i>P</i> = .02 DFS.— <i>P</i> = .56; N = 427, 104 versus 323; 418 included in this analysis of patients with <i>TET2</i> ^{wt} versus <i>TET2</i> ^{mut}
...
...
Median, t-AML: 9.4 mg/dL versus de novo AML: 9.1; <i>P</i> = .04	...	Median, t-AML: 50.5 × 1000/mm ³ versus de novo AML: 55; <i>P</i> = .02	...
...
...	Median, Group A.—144; Group B.—55; Group C.—117; Group D.—65; N = 288 OS.—HR, 0.84 (95% CI, 0.74–0.94) <i>P</i> = .01
...
...
...	Median, <i>IDH1</i> SNP ⁻ , 53; <i>IDH1</i> SNP ⁺ , 47.5; N = 275; OS: HR, 0.70 (platelets above versus below median); 95% CI, 0.50–0.98; <i>P</i> = .04
...
...

Table 5. Continued

Source, y	Study Design	Blood Blasts Influence on Outcome, %	WBC Count Influence on Outcome
Escherich et al, ¹⁸ 2010	PCS	...	WBC \times 1000/mm ³ outcomes: EFS.—WBC < 50, 64.9% versus \geq 50, 57.2%, $P = .001$ OS.—WBC < 50, 80% versus \geq 50, 68.1%, $P = .001$; N = 1429/1818
Salzer et al, ¹⁹ 2010	PCS	...	Infant ALL.—WBC 50–100 versus <50 HR, 2.13 $P = .01$; Noninfant B-precursor ALL.—WBC 10–50 versus <10 HR, 1.43, $P < .001$; for T-ALL: $P = .004$ Infant ALL.—n = 148; B-precursor ALL.—n = 4959; T-cell ALL.—n = 705
Schmiegelow et al, ⁹¹ 2010	PCS	...	Patients in ALL-92 study and ALL-2000 study with higher WBC had poorer survival rates; EFS and OS, both $P < .001$ for both studies; N = 1645 for ALL-92 study; N = 358 for ALL-2000 study
Pui et al, ²⁰ 2010	PCS	...	WBC was a significant factor in studies 11, 12, and 13B for EFS and for studies 11 and 13B for OS
Marks et al, ²¹ 2009	PCS	...	For patients with T-cell, there was not a significant trend for diagnostic WBC to affect OS, although the 96 patients (27%) with a WBC >100 did have poorer OS at 5 y than patients with a WBC <100 had, $P = .03$; N = 1476
Metzeler et al, ¹⁰⁷ 2009	PCS
Karrman et al, ¹⁰⁸ 2009	PCS	...	Median, 66.5; N = 248; probability of EFS in WBC count <200 versus \geq 200, 0.67 versus 0.41, $P < .001$; probability of OS, 0.73 versus 0.41; $P < .001$
Gaidzik et al, ¹⁰⁹ 2009	PCS	...	WBC/MLL-PTD: RFS.—HR, 1.55; OS.—HR, 1.58; $P < .05$ for both
Virappane et al, ¹¹⁰ 2008	PCS	...	CR.—HR, 1.01 (1.01–1.02) $P < .001$; OS.—HR, 1.00 (1.00–1.00) $P < .001$
Lo-Coco et al, ²³ 2008	PCS	...	MVA for DFS.—WBC >50 HR, 1.82 (95% CI, 1.23–2.70), $P = .01$; the analysis of prognostic factors for DFS was carried out in 269 patients who achieved CR
Langer et al, ²⁴ 2008	PCS	...	Median, 26.1 (range, 0.8–295); log ₂ continuous, 2-fold increase in HR, 2.14, $P = .01$; N = 172
Gale et al, ²⁶ 2008	PCS	...	Median, 21.9; MVA for CR, WBC.—OR, 1.007 (95% CI, 1.004–1.009) $P < .001$; for OS.—OR, 1.002 (95% CI, 1.001–1.003) $P < .001$; N = 1425
Yanada et al, ¹¹⁴ 2007	PCS	...	OR, 3.61 (range, 1.14–11.4), $P = .03$; compared 20 \times 1000/mm ³ or higher versus <20 \times 1000/mm ³ (higher WBC associated with severe hemorrhage)
Damm et al, ²⁹ 2010	PCS	...	WT1 (AG/GG) group, 24.9; WT1 (AA) group, 26.6; in MVA for RFS, WBC count above versus below median HR, 1.56 (95% CI, 1.04–2.35); $P = .03$; N = 249
Pabst et al, ¹¹⁵ 2009	PCS	...	HR, 1.38 for OS; and 1.35 for DFS for WBC > 20 \times 1000/mm ³ compared with <20 \times 1000/mm ³
Marcucci et al, ¹¹⁶ 2008	PCS	...	CEBPA mutation associated with longer EFS after adjusting for WBC ($P = .03$)
Paschka et al, ¹¹⁸ 2008	PCS	...	50.9 \times 1000/mm ³ for WT1 mutated versus 23.8 for unmutated; on MVA log ₂ 2-fold increase had HR, 1.9; $P = .04$; N = 186
Damm et al, ¹¹⁹ 2011	PCS	...	\geq 25 \times 1000/mm ³ versus <25 \times 1000/mm ³ : HR, 1.6 (range, 1.03–2.38), $P = .04$
Becker et al, ¹²⁰ 2010	PCS	...	Median NPM1 mutated.—26.2; NPM1 WT, 7.0; WBC, each 50 unit increase.—OR for CR, 0.43, $P = .001$; HR for DFS, 1.78, $P = .01$; HR for OS, 1.19, $P = .01$
Rubnitz et al, ¹²¹ 2007	PCS	...	EFS HR for WBC \geq 50, 1.57 (95% CI, 1.02–2.43), $P = .04$; N = 191
Schwind et al, ³⁰ 2010	PCS	...	Median, 27.9; in MVA, in all patients for WBC, OS HR, 1.37 (95% CI, 1.13–1.67), $P = .01$; N = 187
Santamaria et al, ³¹ 2010	PCS	...	Median, 14.0 (range, 0.2–337); WBC >50 HR for OS, 1.7 (95% CI, 1.1–2.8), $P = .03$; N = 127
Jiao et al, ¹²² 2009	PCS	...	Median, 10.7; in UVA for OS, WBC \geq 1000/mm ³ versus <1000/mm ³ HR, 1.61 (95% CI, 1.00–2.60) $P = .05$; N = 118

Table 5. Continued

Source, y	Study Design	Blood Blasts Influence on Outcome, %	WBC Count Influence on Outcome
Johnston et al, ¹²³ 2010	PCS	...	Median, <i>CNS1</i> , 14.9; <i>CNS2</i> , 39; <i>CNS3</i> , 68.6; in MVA for OS WBC (< versus ≥100 000) HR, 1.52 (95% CI, 1.18–1.96) <i>P</i> = .001; for EFS HR, 1.59 (95% CI, 1.25–2.01), <i>P</i> < .001; <i>N</i> = 1459
Moorman et al, ¹²⁴ 2007	PCS	...	WBC (< 10 × 1000/mm ³ versus >10 × 1000/mm ³): EFS.—HR, 1.26 (range, 1.18–1.35), <i>P</i> < .001; OS.—HR, 1.36 (1.26–1.48), <i>P</i> < .001
Prébet et al, ¹²⁵ 2009	PCS	...	Median, 2700 mg/L; WBC, CR.—high count associated with lower CR (77% versus 92%, <i>P</i> = .02); WBC, OS.—high WBC associated with poorer OS (<i>P</i> = .02); high WBC: LFS.— <i>P</i> = .06
Santamaria et al, ¹²⁶ 2009	PCS	...	Median: 9.0; WBC >50 was an independent prognostic variable for a shorter OS (<i>P</i> = .01) and RFS (<i>P</i> = .01); <i>N</i> = 110
Schwind et al, ¹²⁷ 2010	PCS	...	Low BAALC, 27.8; high BAALC, 33.5; low ERG, 20.6; high ERG, 38.0; CR for WBC each 50-unit increase.—OR, 0.68 (95% CI, 0.49–0.93) <i>P</i> = .02; <i>N</i> = 158
Zachariadis et al, ¹²⁸ 2011	PCS	...	In MVA, WBC was the most powerful predictor of EFS (<i>P</i> < .01) and best predictor of OS (<i>P</i> < .01); <i>N</i> = 533
Wheatley et al, ¹²⁹ 2009	PCS	...	WBC × 1000/mm ³ (0–9.9, 10–49.9, 50–99.9, 100+).—Higher WBC count associated with poorer survival at 1-y in 3 of 4 studies (<i>P</i> < .001)
Renneville et al, ¹³⁰ 2012	PCS	...	Median, 11; WBC, EFS.—HR, 1.09 (1.04–1.14), <i>P</i> < .001; WBC, OS.—HR, 1.09 (1.04–1.15), <i>P</i> < .001

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BAALC, brain and acute leukemia cytoplasmic gene; BM, bone marrow; *CEBPA*, CCAAT/enhancer-binding protein α ; CNS, central nervous system; CR, complete remission; DFS, disease-free survival; EFS, event-free survival; ERG, erythroblast transformation-specific related gene; *FLT3*-ITD, fms-like tyrosine kinase 3-internal tandem duplication; Hgb, hemoglobin; HR, hazard ratio; ID1, inhibitor of deoxyribonucleic acid binding 1; *IDH1*, isocitrate dehydrogenase 1; LFS, leukemia-free survival; mut, mutant; MVA, multivariate analysis; . . ., not applicable; *NPM1*, nucleophosmin (nucleolar phosphoprotein B23, numatrin); NR, not reported; NRCT, nonrandomized clinical trial; NS, not significant; OR, odds ratio; OS, overall survival; PCS, prospective cohort study; PTD, partial tandem duplication; RCT, randomized, controlled trial; RFS, relapse-free survival; SNP, single-nucleotide polymorphisms; t-AML, therapy-related acute myeloid leukemia; TET2, tet methylcytosine dioxygenase-2; UVA, univariate analysis; WBC, white blood cell; WT, wild type; *WT1*, Wilms tumor 1.

cytochemical stains, fluorescence in situ hybridization (FISH), and molecular studies. Touch-preparation slides are even more essential in cases in which the BM aspiration is unsuccessful. This is not an uncommon event in AL cases, and, in that situation, the touch preparation slides become the key BM specimen for the blast enumeration, which is essential for AL diagnosis. The utility of the touch preparation in BM diagnosis has been confirmed by multiple comparative studies.^{132,135,138}

Once adequate numbers of BM aspirate specimen slides have been prepared, the remaining BM aspirate specimen clots into a semisolid tissue specimen, which can be wrapped in filter paper and submitted for routine tissue processing. Because there are no bony trabeculae in this coagulated aspirate specimen, decalcification is not necessary. Thus, the value of the clot sections is enhanced because it can be used for the full breadth of molecular studies as well as for all special stains, immunohistochemical stains, and for in situ hybridization.^{132–134,138}

The BM clot section can be used for any diagnostic technique, including many molecular diagnostic techniques that have been validated for formalin-fixed, paraffin-embedded (FFPE) tissue.^{132,133,138} The BM clot section assumes a greater role in AL diagnosis when an adequate BM core trephine biopsy specimen has not been obtained.

Public Comment Response to Statement 4.—There were 186 respondents, 94.09% (*n* = 175) of whom agreed with this recommendation, and 5.91% (*n* = 11) who disagreed. The reasons for the disagreement were stated by only 7 respondents and were either (1) that, in some cases, the number of blasts in the PB were sufficient for diagnosis and for all required ancillary studies, and thus, BM studies were

not required (5 respondents); or (2) disagreement as to whether the specimens should be reviewed by a hematologist or a pathologist (2 respondents). These comments were considered in the final draft of statement 4 presented in this article.

Statement 5.—Strong Recommendation.—In addition to morphologic assessment (blood and BM), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (ie, karyotype), appropriate molecular-genetic and/or FISH testing, and FCI. The flow cytometry panel should be sufficient to distinguish acute myeloid leukemia (including acute promyelocytic leukemia), T-ALL (including early T-cell precursor leukemias), B-cell precursor ALL (B-ALL), and AL of ambiguous lineage for all patients diagnosed with AL. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis.

Note.—If sufficient BM aspirate or PB material is not available for FCI, immunohistochemical studies may be used as an alternative method for performing limited immunophenotyping. In addition, a second BM core biopsy can be obtained and submitted, unfixed, in tissue culture media for disaggregation for genetic studies and flow cytometry.

No studies from our SR directly informed this statement.

Specialized testing is essential in the diagnosis of AL and provides necessary prognostic information and a “fingerprint” of the neoplastic clone that can be used for optimal minimal residual disease (MRD) monitoring.

Flow cytometry immunophenotyping has an essential role in the diagnosis and classification of AL. Together with cytomorphology and cytochemistry, FCI is crucial for the

Table 5. Continued, Extended

Hgb Influence on Diagnosis	Hgb Influence on Outcome	Platelet Count Influence on Diagnosis	Platelet Count Influence on Outcome
...
...	Difference in baseline characteristics
...
...
...
...
...
...

detection of blasts and lineage assignment of blast cells and to define AL of ambiguous lineage. In addition, specific immunophenotypic profiles have been associated with prognosis and/or unique cytogenetic and molecular abnormalities, such as AML with t(15;17)(q24.1;q21.2), t(8;21)(q22;q22.1).

An adequate FCI panel should be able to determine not only AML, B-ALL, and T-ALL but also AL of ambiguous lineages, including acute, undifferentiated leukemia and MPAL. Acute leukemia of ambiguous lineage has been confirmed to be a poor-risk disease.¹⁴⁰ Furthermore, within each subset of AL, FCI panel/markers should be able to effectively subcategorize the blasts. In AML, FCI may help to determine blasts with monocytic differentiation and myeloperoxidase (MPO) expression¹⁴¹ and to recognize acute promyelocytic leukemia (APL) or APL mimics^{142–144} and blasts with erythroid¹⁴⁵ or megakaryoblastic differentiation.¹⁴⁶ In T-ALL, the FCI panel should include sufficient markers to identify early T-cell precursor lymphoblastic leukemia.¹⁴⁷ In addition, FCI may provide therapeutic marker measurement, such as CD20 in B-ALL, for a frontline rituximab-containing regimen.¹⁴⁸

Although no standard FCI panels are mandated for all laboratories, there are recommendations for instrumentation, preanalytic variables, panel design, data analysis, and validation by the EuroFlow Consortium (Leiden, the Netherlands),¹⁴⁹ the British Committee for Standards in Haematology (London, United Kingdom),¹⁵⁰ and the International Clinical Cytometry Society (Glenview, Illinois).^{151,152}

The role of cytogenetics in diagnosis, classification, and prognosis in AL is well established and predates the period used for our SR.^{153,154} During the past 30 years, cytogenetic studies have become an integral part of the diagnosis, prognosis, and treatment of AML and ALL in children and adults.^{155–157} Studies that preceded the dates of our SR and expert opinion informed this recommendation.

The value of conventional cytogenetic studies as a critical prognostic indicator in AL has been proven in numerous clinical trials in AML^{***} and ALL⁺⁺⁺ and now provides a basis for classification¹⁷⁴ and choice of initial and post-remission therapy.^{175–178} With testing widely available in academic and reference laboratories, conventional cytogenetics reveals a clonal abnormality in 40% to 50% of patients with AML and in 60% to 85% of patients with ALL; the success rate at diagnosis is typically in excess of 84% for ALL and 90% for AML in experienced laboratories.^{156,157,179} Importantly, new karyotypic abnormalities continue to be described that may not be apparent by other routine techniques.^{179,180} Including cytogenetic analysis as part of the diagnostic workup of AML, ALL, and AL of ambiguous lineage is endorsed by the National Comprehensive Cancer Network (Fort Washington, Pennsylvania) clinical practice guidelines¹⁸¹ and in the (now archived) British Committee for Standards in Haematology *Guidelines on the Management of Acute Myeloid Leukaemia in Adults*.¹⁸²

Molecular genetic and/or FISH testing should be considered complementary to an adequate conventional cytogenetic analysis. Unless the cytogenetic analysis is suboptimal because of poor chromosome morphology or insufficient cells for analysis or is completely unsuccessful because of no growth, FISH analysis may be an expensive, redundant technology, particularly in AML.^{183–185} On the other hand, certain abnormalities encountered in ALL, such as t(12;21)(p13.2;q22.1) *ETV6-RUNX1* fusion or intrachromosomal amplification of chromosome 21 (iAMP21), can be cytogenetically cryptic and are optimally detected by interphase or metaphase FISH.^{183,186} In general, the utility of FISH should be considered in the context of each case, and to that end, algorithmic approaches for using conven-

*** References 45, 113, 156, 158–168.

+++ References 106, 132, 156, 157, 169–173.

tional cytogenetics and FISH have been proposed by several groups.^{185,187–189}

Because of the critical importance of FCI and various genetic studies in AL diagnosis, prognosis, and disease monitoring, it is essential that a battery of specialized tests be performed on all cases. Consequently, a concerted effort to obtain adequate specimens for specialized studies is mandatory.

Public Comment Response to Statement 5.—There were 186 respondents, of whom, 94.09% (n = 175) agreed, and 5.91% (n = 11) disagreed with the statement. No clearly defined reasons for disagreeing were stated. There were 34 written comments, including one comment that only qualified cytogeneticists or pathologists subspecialized in cytogenetics, rather than “pathologists” should “perform” the cytogenetic studies, and another comment said that targeted FISH and molecular methodologies should replace conventional karyotyping for patients with ALL. The comments were considered in the final draft of statement 5 presented in this article.

Statement 6.—Expert Consensus Opinion.—For patients with suspected or confirmed AL, the pathologist may request and evaluate cytochemical studies to assist in the diagnosis and classification of AML.

The strength of evidence was *insufficient* for this statement.

No evidence from our SR informed this statement.

Cytochemical stains were historically the primary laboratory adjunct to routine morphology for classification of AL. Other techniques, particularly immunophenotyping, have largely supplanted them. Nonetheless, cytochemical studies still have some utility in some circumstances.

Evidence identified external to our SR indicated that 2 cytochemical stains have continued utility in AML diagnosis: MPO and nonspecific esterase stains. Both can be performed on air-dried, unfixed, unstained slides of blood, imprint specimens, and BM aspirate specimens.^{133,136,190} Myeloperoxidase is uniquely valuable because it can be performed within 5 to 10 minutes and is available 24 hours a day, 7 days a week in many laboratories.¹⁹⁰ The rapid diagnosis of APL is considered to be a medical emergency because of the risk of major hemorrhage,¹⁹¹ and MPO staining can be particularly helpful in cases of the microgranular variant of APL. The detection of MPO positivity in 3% or more of the blasts is a criteria for myeloid lineage delineation in AML.¹⁹² Similarly, nonspecific esterase positivity is one of the defining criteria for monoblast/promonocyte identification in AML.¹⁹² Other historically used stains, including Sudan black B, periodic acid–Schiff, or acid phosphatase, were not considered to have sufficient specificity for routine use in the classification of AL.

Public Comment Response to Statement 6.—There were 185 respondents, of whom, 92.97% (n = 172) agreed, and 7.03% (n = 13) disagreed. Although the reason for the disagreements was not clearly stated, there were 29 written comments. Three individuals explicitly commented that the recommendation should have been stronger, whereas another 3 commented that cytochemical stains were no longer necessary. These comments were considered in the final draft of statement 6 in this article.

In part because there are no studies showing the independent value of these stains, but recognizing that the speed and low cost of these techniques can have utility in some circumstances, expert consensus opinion supports the optional use of these stains.

Statement 7.—Recommendation.—The treating clinician or pathologist may use cryopreserved cells or nucleic acid, nondecalcified FFPE tissue, or unstained marrow aspirate or PB specimens obtained and prepared from PB, BM aspirate or other involved tissues for molecular or genetic studies in which the use of such material has been validated. Such specimens must be properly identified and stored under appropriate conditions in a laboratory that is in compliance with regulatory and/or accreditation requirements.

The strength of evidence was *adequate* to support this guideline statement.

This statement was supported by 4 PCSs^{193–196} that met the inclusion criteria for our SR. Two of the studies^{193,194} were deemed to have a low risk of bias, and the other 2^{195,196} were deemed to have a low to moderate risk of bias. None of these studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 10 for the quality-assessment results of studies included studies in statement 7.

The purpose of this guideline statement is to encourage preservation of cells from blood, BM, or other tissues that can be used to identify molecular, genetic, and/or antigenic abnormalities of leukemia cells that may be of further diagnostic or prognostic importance or that may be a target for a specific therapeutic agent but that were not assessed during the initial evaluation. The availability of preserved leukemic cells could spare the patient an additional BM procedure if there are no blasts in the PB, or, if, after therapy, there are few or no neoplastic cells in the marrow. This recommendation applies to initial diagnostic specimens as well as subsequent specimens with evidence of residual or recurrent disease.

Our SR provided no data that specifically informs this recommendation. Preserved cells are, however, invaluable for clinical research and are often used in cooperative group studies for clinical trials in which genetic abnormalities are performed in a central laboratory. Nevertheless, only a few studies in the SR specifically mention or allude to the use of preserved cells as the specimen source for the studies performed.^{193–196}

Historically, cryopreservation was the most commonly employed method of preserving cells.¹⁹⁷ Cryopreserved leukemia cells can be used for the extraction of DNA and RNA for molecular genetic studies.^{198–200} Although karyotypic analyses of cryopreserved cells have been reported, the number of analyzable metaphases is fewer and their quality is inferior to those from fresh samples.^{201,202} Thus, fresh cells are clearly preferable for karyotyping, and cryopreserved cells should be used only when no other cells are obtainable, and the results should be considered with caution. In contrast, cryopreserved cells can be successfully studied for specific chromosomal abnormalities by FISH,^{203,204} which can also be applied to properly stored specimens and cytospin or touch preparations on glass slides. Immunophenotyping by flow cytometry may be performed on cryopreserved cells, although some antigens or cells expressing those antigens may deteriorate during the freeze-thaw cycle. These latter, detrimental effects appear to depend, in part, on the protocols used for cryopreservation and cell storage and, perhaps, on the cell lineage.^{205–216}

Cryopreservation procedures require mononuclear cells be suspended in a solution containing a cryoprotective agent (usually dimethyl sulfoxide), cooled in a cooling device to the storage temperature, and then stored in liquid nitrogen (for use in studies in which viable cells are necessary) or a

–80° freezer, which is satisfactory for most molecular studies. Cryopreservation does require specialized equipment and storage facilities, which may not be available in all laboratories.

Nucleic acid extraction from FFPE tissues can be used for molecular studies as well, particularly for NGS and microarray technologies. In general, the use of FFPE cells has been most successful for DNA-based analyses,^{217–221} whereas RNA extracted from such specimens is fragmented by formalin fixation and is often of poor quality.^{222–225} Nevertheless, the expression pattern of small RNAs, eg, miRNAs, extracted from FFPE is reportedly similar to that derived from cryopreserved cells.^{226,227} DNA and RNA for molecular studies may also be obtained from archived cytology and specimen preparations on glass slides.^{228–230}

Public Comment Response to Statement 7.—There were 184 respondents to this statement, of whom, 97.28% (n = 179) agreed, and 2.72% (n = 5) disagreed. There was no specific issue identified by those who disagreed. However, there were 19 written comments, among which, were 4 that emphasized that the preserved specimens and cells should be held in Clinical Laboratory Improvement Amendments of 1988 (CLIA '88)–approved facilities under controlled conditions and should only be used for studies for which such specimens had been validated. These comments were considered in the final draft of statement 7 in this article.

Statement 8.—Strong Recommendation.—For patients with ALL receiving intrathecal therapy, the treating clinician should obtain a cerebrospinal fluid (CSF) sample. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist.

Acute lymphoblastic leukemia may involve the CNS, both at diagnosis and at relapse, and patients with CNS involvement require specific therapy. For that reason, knowledge of CSF status at the time of diagnosis has long been known to be important for proper management of patients. Because alteration of CNS therapy based on CSF findings has been standard practice for so long, there are no large-scale clinical trials using modern therapy that demonstrate adverse prognosis of CNS leukemia in the absence of therapy, although several have investigated the outcome of patients based on CSF involvement in the context of CNS-directed therapy.

Although there was no evidence from our SR to inform this statement, evidence obtained external to our SR and relevant to this statement includes 2 practice guidelines^{231,232} that speak to the need to perform CSF cell count and morphology on patients with ALL, resulting in the now standard classification of CNS involvement as CNS-1 (negative), CNS-2 (blasts with WBC counts <5/mm³), and CNS-3 (blasts with WBC counts ≥5/mm³).

Several retrospective reviews of RCTs of pediatric ALL^{233–235} specifically investigated the effect of CNS involvement and concluded that long-term outcome was not affected, provided appropriate therapy was given, although CNS relapse rates among patients with CNS-2 or CNS-3 disease have sometimes been found to be different.²³³ One older study²³⁶ showed that patients with blasts had an adverse prognosis, independent of blast count, but therapy was different in that study. Limited data were identified for adult patients with ALL, although one small study²³⁷ suggested that CSF blasts were associated with adverse outcome.

Public Comment Response to Statement 8.—There were 180 respondents, of whom, 92.22% (n = 166) agreed, and 7.78% (n = 14) disagreed. There were 20 written comments. Although a few respondents did not believe this to be required in all cases, others pointed out that there may be contraindications to obtaining a CSF in some patients, particularly when the peripheral blast count is high and that the initial CSF is typically obtained not at the time of diagnosis, but rather, after the diagnosis was made and intrathecal therapy planned, even if circumstances delayed the timing of the lumbar puncture. In the initial wording of this statement, there was also some confusion about who should obtain the CSF. The issue of obtaining flow cytometry on the CSF fluid was also raised. These important comments were considered in the final draft of statement 8 in this article. The wording was changed to reflect the clinical practice of obtaining CSF at the time of intrathecal therapy, and wording about the clinical contraindication was not included because that was presumed to be covered by the association with administration of intrathecal therapy. Also raised in commentary was the need for flow cytometry, but that is covered in statement 10.

Irrespective of conclusions regarding the independent prognostic significance of finding blasts, it is clear that classification of CNS status requires knowledge of both the cell count and the morphologic assessment for blasts and affects most ALL protocols; thus, we consider this a strong recommendation.

Statement 9.—Expert Consensus Opinion.—For patients with AL, other than those with ALL, receiving intrathecal therapy, the treating clinician may, under certain circumstances, obtain a CSF sample when there is no clinical contraindication. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist.

The strength of evidence was *insufficient* to support this guideline statement. No data from the SR informed this statement.

This statement is regarding CSF evaluation at the time of diagnosis for patients with AL, other than those who have ALL, undergoing intrathecal therapy/prophylaxis and covered in statement 8. This recommendation is based on expert consensus opinion arising from controversial issues regarding clinical significance, indications, and timing of CSF examination in AL.

The CSF evaluation may be indicated for patients with any CNS signs and symptoms, for those who are suspected of having ocular involvement, for patients with increased risk of CNS involvement or later CNS relapse, or per protocol requirement. Central nervous system involvement at the time AML is diagnosed is uncommon in adults, and the risk factors include younger age, high leukocyte count, high lactate dehydrogenase (LDH) level, African American ethnicity, and 11q23.3/*KMT2A* abnormalities.^{238–241} Because of a low incidence of CNS involvement, routine evaluation is often not recommended for adult patients with asymptomatic AML. The reported incidence of CNS involvement in childhood AML ranges from 6% to 29%^{123,242} and is higher in patients younger than 2 years old, in patients with AML and high WBC and peripheral blast counts, in patients with AML and monocytic differentiation, in patients with AML and inv(16)(p13.1;q22) or t(16;16)(p13.1;q22), and in patients with AML and hyperdiploidy.^{123,242,243} Rarely, CNS disease may present as extramedullary involvement that precedes

clinically evident AML.^{244–246} Clinical risks for lumbar puncture (LP) in patients diagnosed with AL include increased intracranial pressure and severe coagulopathy. The presence of high numbers of circulating blasts may introduce blasts in the CNS if a traumatic LP occurs.^{247,248} An LP should be performed only when there is no clinical contraindication.

If an LP is performed, CSF should be sent for WBC count with differential, total RBC count, and a cytocentrifuge preparation, similar to any other routine CSF examination for suspected CNS tumor involvement. The pathologist should review the slides to determine the presence or absence of blasts via light microscopy. The characteristic features of leukemia cells, either lymphoblasts or myeloblasts, are best seen on Wright-Giemsa-stained preparations, although use of other stains, such as Papanicolaou and Diff-Quik, varies in clinical laboratories. In addition, the detection of blasts via cytomorphology in a low-cell-count, CSF specimen may depend on the sensitivity of the cytocentrifuge used for preparation of the specimen.²⁴⁹

Although CNS involvement in ALL is classified into 3 categories as CNS1, CNS2, and CNS3, according to total WBC and RBC counts and the presence or absence of blasts,^{231,233} the threshold for a definitive, positive CNS involvement in nonlymphoblastic leukemia varies among different groups. The Italian Cooperative Study Group on Chronic Myeloid Leukemia (Bologna, Italy),²⁵⁰ the Pediatric Oncology Group (Monrovia, California),²⁵¹ and St Jude Children's Research Hospital²³⁸ define CNS involvement as the presence of any blasts, regardless of total cell count, whereas the Children's Cancer Group (Monrovia, California)²⁵² and the International Berlin-Frankfurt-Münster Study Group (Kiel, Germany)²⁵³ use a WBC threshold of 5 and 10/mm³ with the presence of blasts, respectively. In children, CNS involvement at the time of AML diagnosis is often thought to confer a worse prognosis^{252,254,255}; however, recent studies showed that CNS involvement might not have significant effect on survival.^{123,242,256,257} A large cohort study by the Children's Oncology Group (Monrovia, California)¹²³ showed that, although CNS involvement at AML diagnosis had no effect on OS, affected patients did have an increased risk of isolated CNS relapse and had an inferior leukemia EFS. On the other hand, the prognostic significance of CNS involvement at the time of AML diagnosis in adults is controversial.^{240,241,258} The use of chemotherapeutic agents that offer greater penetration of the CNS, such as high-dose cytarabine and cladribine, may have increased the eradication of low-level CNS involvement without requiring additional CNS-directed therapy.^{259,260} Of note, recent induction chemotherapy regimens designed primarily for elderly patients with AML do not include high-dose cytarabine, and whether those induction protocols would result in an increased risk for CNS relapse is unknown.²⁴¹

Public Comment Response to Statement 9.—There were 184 respondents, 96.2% (n = 177) of whom agreed with the statement, and 3.8% (n = 7) disagreed. There were 31 written comments. The comments received during the open comment period were related to the indications, timing, and risks of LP. Specifically, a number of commenters suggested deferring LP until after the first cycle of chemotherapy to avoid a traumatic LP in patients with high circulating blasts. Some also advocated performing CSF evaluation in all patients with AL. These comments and concerns have been

incorporated in the final draft of statement 9 and are addressed in the preceding text.

Statement 10.—Recommendation.—For patients with suspected or confirmed AL, the pathologist may use flow cytometry in the evaluation of CSF.

As discussed in statements 8 and 9, examination of the CSF is indicated in cases of ALL and may be indicated in some cases of AML. Definitive determination of CSF involvement by AL is based on identification of blasts by visual inspection of a CSF cytocentrifuge preparation; however, flow cytometry may provide immunophenotypic information to confirm the morphologic impression of the presence of blasts.

No evidence from our SR informed this statement; however, evidence identified outside our SR indicated that flow cytometry can effectively detect disease in CSF samples from patients with ALL^{261,262} and can detect subtle leukemic involvement in some cytologically negative CSF samples from both pediatric and adult patients with B-ALL and T-ALL.^{263,264} Moreover, patients with ALL and CSF disease not detected by visual inspection of cytocentrifuge preparations but detectable by flow cytometry involvement have shorter OS times than do those with no involvement detected by flow cytometry ($P = .01$ on multivariate analysis).²⁶⁵ Based on this evidence, a recommendation is made that flow cytometry be performed on CSF samples taken to evaluate for leukemic involvement in patients diagnosed with ALL; although the expert opinion is that AML blasts can also be detected in the CSF by flow cytometry, we identified one study to support that practice for AML.²⁶⁶

Public Comment Response to Statement 10.—There were 181 respondents, of whom, 92.82% (n = 168) agreed, and 7.18% (n = 13) who disagreed. There were 27 written comments. Of the 27 who wrote specific comments, 15 thought that the words “pathologists may use flow cytometry” should be changed to “should use flow cytometry.” The question in the survey was, however, slightly different and indicated that “the pathologists may use flow cytometry in the evaluation of CSF when sufficient cells are available.” These comments were taken into consideration in the final draft of statement 10 in this article.

Statement 11.—Strong Recommendation.—For patients who present with extramedullary disease without BM or blood involvement, the pathologist should evaluate a tissue biopsy and process it for morphologic, immunophenotypic, cytogenetic, and molecular genetic studies, as recommended for the BM.

Note.—Additional biopsies may be indicated to obtain fresh material for ancillary testing.

No evidence from our SR informed this statement.

Patients with AL may present initially with extramedullary disease. Extramedullary AL (myeloid sarcoma) has been shown to present months, or even years, before AML becomes evident in the BM or PB in some patients.^{267–271} Myeloid sarcoma also can occur in patients with MDS, myeloproliferative neoplasms, or myelodysplastic/myeloproliferative neoplasms whose BM or PB may never meet the diagnostic criteria for AML. The incidence of extramedullary leukemic involvement varies widely among reports in the literature.^{268,269,272–275} Sites of isolated myeloid sarcoma can occur in the bone, periosteum, soft tissues, lymph nodes, CNS, orbit, intestine, mediastinum, epidural region, testis, uterus, or ovary. Similarly, patients with acute lymphoblastic leukemia/lymphoma (ALL/LBL) may present

with extramedullary disease. Patients with T-cell ALL/LBL often present with a mediastinal mass, lymphadenopathy, or other extranodal tissue mass.^{276,277} Most patients with B-ALL present with PB and BM leukemia, but about 10% of patients may have an isolated extramedullary presentation.^{278,279} Skin, bone, and soft tissue are the most frequently reported sites of extramedullary involvement in B-cell ALL/LBL.²⁸⁰

Fresh specimens, either fine-needle aspirate, excisional biopsy, or body-effusion fluids, are adequate for leukemia workup.^{267,281–286} Similar to the analysis for BM and PB samples, the workup includes morphologic examination, FCI, and cytogenetic and molecular studies. In patients with suspected CNS leukemia,²⁸⁷ a CSF sample may be obtained. Cyto centrifuge preparation with Wright-Giemsa staining should be performed for morphologic examination, and, if sufficient numbers of blasts are present, CSF can be used for ancillary studies. Fine-needle aspiration samples or body fluids with cell-block preparation and excisional or core needle biopsy with a portion of the tissue fixed and paraffin embedded can be used for morphologic examination, immunohistochemistry and targeted FISH, and some mutational studies. The targeted FISH panel and molecular studies for these tissue samples should be performed according to the recommendations for the BM and PB workup for different types of leukemia. For myeloid sarcoma, the frequent cytogenetic abnormalities found in adults are +8, *KMT2A* translocations, inv(16), +4, -16, del(16q)/loss of 16q, del(5q), del(20q), and +11.^{255,286,288} Nucleophosmin (nucleolar phosphoprotein B23, numatrin) (*NPM1*) mutations have been reported in 15% of patients with extramedullary myeloid tumors, as determined by immunohistochemical studies,²⁸⁹ and *fms*-related tyrosine kinase 3 internal tandem duplication (*FLT3*-ITD) mutations have been reported in 15% of cases of myeloid sarcoma.²⁹⁰

Public Comment Response to Statement 11.—There were 185 respondents, of whom, 97.84% (n = 181) who agreed, and 2.16% (n = 4) who disagreed. There were 17 written comments. Concerns raised in these comments revolved around several practical issues. First, what are the indications to prompt an AL workup on tissue biopsies or fluid specimens when BM and PB have no sign to suggest AL? Air-dried touch imprints of tissue biopsy specimens or smears/cyto centrifuge preparations of fluids may have great utility in that scenario. Wright-Giemsa–stained slides allow for the assessment of the cytologic features. Cytochemical studies for MPO or nonspecific esterase can also be performed to raise the suspicion, thereby facilitating triage of the specimen for further workup. Touch imprints can also be used for FISH studies when a diagnosis of AL is confirmed.²⁷¹ Of note, in reviews of myeloid sarcoma published in the literature,^{272,275,291} nearly 50% of cases were initially misdiagnosed, most often as malignant lymphoma. With the advent of immunohistochemistry, most cases can be diagnosed correctly by applying proper immunohistochemical stains. A number of immunohistochemical markers have been shown to have diagnostic utility in extramedullary leukemia,^{267,281,292,293} including immature hematopoietic markers, such as CD34, CD117, and TdT; lineage markers, such as CD3, PAX5, CD19, CD79a, MPO, and CD61; monocytic markers; and markers for blastic plasmacytoid dendritic cell neoplasms. The second concern raised was that the biopsy tissue sample may be small, especially in patients with cutaneous involvement, and there may not be sufficient tissue for a

complete AL workup. Paraffin-embedded tissue samples should be the priority in such circumstances. Paraffin-embedded tissue is adequate for immunohistochemical analysis, FISH, and some molecular testing.^{267,281–284,286} When a diagnosis of extramedullary leukemia is confirmed or highly suspected based on the initial biopsy, additional biopsies may be indicated to obtain fresh material for ancillary testing.

Some comments also suggested that BM or PB that contains a substantial proportion of blasts, even less than 20%, is a more desirable material than tissue/fluids for an AL workup. Although this is largely true for lymphoblastic lymphoma/leukemia, it is less clear in myeloid sarcoma. Pileri and colleagues²⁸⁶ compared the results of FISH performed on myeloid sarcoma tissues and conventional cytogenetic analysis performed on BM or PB. A full concordance between the FISH and conventional cytogenetic results was found in 71% of patients with available results. This finding suggests that conventional cytogenetic studies on BM or PB and targeted FISH analysis on myeloid sarcoma are complementary and may be pursued in the appropriate clinical setting. Some responders also suggested that extramedullary tumors need a complete workup, even in patients with 20% or more blasts in BM and PB. Although there is no clear evidence to support or reject that approach, the consensus is that, in a case with a full leukemia workup completed using BM and PB, the workup using tissue samples may primarily focus on confirming the diagnosis.

Based on those findings, the EP concluded that, for patients who present with extramedullary disease without BM or PB involvement, the pathologist should evaluate a tissue biopsy specimen and process it for morphologic, immunophenotypic, cytogenetic, and molecular genetic studies, as recommended for the PB and/or BM.

Statement 12.—Strong Recommendation.—For patients with suspected or confirmed AL, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of MRD.

The strength of evidence was *convincing* to support this guideline statement.

This recommendation was supported by 15 studies that met the inclusion criteria for our SR,^{18,20,22,91,294–304} comprising one RCT³⁰⁴ and 14 PCSs.^{18,20,22,91,294–303} The single RCT, reported by Yin et al³⁰⁴ in 2012, was deemed to have a moderate risk of bias. For the 14 PCSs, 2 studies^{91,299} were deemed to have a low risk of bias, 10 were deemed to have a low to moderate risk of bias,^{†††} and 2 were deemed to have a moderate risk of bias.^{22,294} Overall, none of these 15 studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 11 for the quality-assessment results of studies for statement 12.

Minimal residual disease is a powerful predictor of adverse outcome in patients with AL. Although measurement of MRD per se is outside the scope of this recommendation, because it is, by definition, not performed at the time of diagnosis, it is important that the initial diagnostic material be handled in such a way that MRD testing on subsequent samples is possible. Minimal residual disease may be measured by flow cytometry or by molecular testing; the latter encompasses a variety of techniques,

††† References 18, 20, 295–298, 300–303.

including quantitative PCR detection of antigen-receptor rearrangements, of fusion transcripts of leukemic translocations, or of mutated genes. Next-generation sequencing has increasingly been used as a tool for MRD detection.

Nine studies demonstrated the importance of detecting MRD in ALL.^{§§§} The studies differed in methodology (PCR versus flow cytometry), the timing of the sample, cutoff values, and the outcome variable measured (EFS, relapse rate, OS), but all showed a statistically significant effect on the outcome variable measured.

There were 4 studies that demonstrated the importance of detecting MRD in AML.^{294,298,301,304} Two of those studies used reverse transcription-PCR (RT-PCR) and were limited to core-binding factor (CBF) AML.^{298,304} The other 2 used flow cytometry.^{294,301} All showed that MRD was an important factor in relapse-free survival (RFS) and, in some studies, OS.

One study demonstrated the prognostic significance of MRD detection in MPAL.²⁹⁶

Three studies addressed the question of comparing the value of MRD detection by flow cytometry compared with molecular studies in ALL.^{22,297,300} In all 3, neither method was found to be superior. There were no studies identified that addressed that question in AML.

Numerous publications were identified external to our SR, which offered evidence that information obtained from diagnostic material was important for the subsequent detection of MRD, and only a few are referenced here. Flow cytometric MRD detection in both ALL and AML often depends on the persistence of cells with a particular leukemia-associated immunophenotype identified at the time of diagnosis.^{305–308} Even when flow cytometry methods that depend on recognizing differences between normal and abnormal cells are used,^{309,310} it is advantageous to be able to compare initial and posttreatment phenotypes because those often change in predictable ways. Molecular methods of detecting MRD in both ALL and AML require that leukemic cells be characterized and sequenced at diagnosis, whether MRD detection is performed by conventional PCR-based techniques^{311–313} or by NGS.^{314,315}

Public Comment Response to Statement 12.—There were 179 respondents, of whom, 94.41% (n = 169) agreed with the statement, and 5.59% (n = 10) who disagreed. There were 19 written comments. However, for the open comment period, the original draft statement was written to address only patients with suspected or confirmed ALL. Comments offered were largely directed at the conduct and/or timing of subsequent MRD studies and were considered in the final draft of statement 12 for this article.

This statement is designated as having a strong recommendation. Clinicians and pathologists should be mindful, at the time of initial workup of AL, of the requirements for subsequent MRD studies. Much of the molecular testing required can be performed on preserved material obtained as specified in statement 7. However, material is frequently not preserved in such a way that flow cytometry can be readily performed after the fact. Thus, it is imperative that, in settings in which flow cytometric MRD detection is contemplated, initial immunophenotyping be performed in such a manner so as to optimize that testing. Refer to Table 6 for study data on MRD.

Statement 13.—*Strong Recommendation.*—For pediatric patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(12;21)(p13.2;q22.1); *ETV6-RUNX1*, t(9;22)(q34.1;q11.2); *BCR-ABL1*, *KMT2A* (previously *MLL*) translocation; *iAMP21*; and trisomy 4 and 10 is performed.

The strength of evidence was considered *adequate* to support this guideline statement.

This statement is supported by 6 PCSs^{18–20,91,124,316} that met the inclusion criteria for our SR. Risk of bias assessments ranged from low^{91,316} to low to moderate.^{18–20,124} None of those studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 12 for the quality-assessment results of studies included for statement 13.

Prognosis in pediatric patients with B-ALL varies with low-risk patient 5-year EFS approaching 90%, whereas high-risk patient 5-year EFS was less than 45%.¹⁷⁰ Risk stratification supports risk-directed therapy to optimize patient care while minimizing unnecessary risks associated with treatment. Factors used for risk assessment include age, WBC count, genetic abnormalities (as in this recommendation), early response to therapy, CNS involvement, and MRD level.

Systematic literature review shows several important markers for risk stratification in pediatric B-ALL, including t(12;21)(p13.2;q22.1); *ETV6-RUNX1*, t(9;22)(q34.1;q11.2); *BCR-ABL1*, *KMT2A* (previously *MLL*) translocation; *iAMP21*; and trisomy 4 and 10. The PCSs showed specific prognostic information allowing risk stratification associated with each of those markers. In the most recent clinical trial from each article, the presence of t(12;21)(p13.2;q22.1); *ETV6-RUNX1*^{18,19,91,316} or trisomy of the 4 and 10 chromosomes¹⁹ conferred improved 5-year EFS ($P < .001$ for both) and OS ($P < .001$ for both) in cooperative group studies. Interestingly, the more-recent studies (St Jude studies 13A and 13B) in another series failed to show significant prognostic impact for t(12;21) on EFS or OS,²⁰ perhaps because of improved OS for patients with B-ALL resulting from therapeutic improvements and the introduction of MRD evaluation for risk stratification. The t(12;21) is the most common recurrent cytogenetic abnormality in pediatric B-ALL (approximately 25% of patients) and is cryptic by classic cytogenetic evaluation. An alternate method, such as FISH or multiplex RT-PCR, is required for detection of that abnormality and should be performed in all pediatric patients with B-ALL.

Other abnormalities conferring a poor prognosis include t(9;22); *BCR-ABL1*; hypodiploidy (or decreased DNA index); *KMT2A* (previously *MLL*) translocation with slow early treatment response; and *iAMP21*. However, recent evidence indicates that tyrosine kinase inhibitor (TKI) therapy, combined with intensive chemotherapy, leads to a good outcome in children and adolescents with B-ALL who have the *BCR-ABL1* mutation as their sole abnormality.³¹⁷ Fortunately, *BCR-ABL1*⁺ B-ALL is significantly less common in children than it is in adults, accounting for only 2% to 4% in that patient population. Our SR included PCSs demonstrating decreased 5-year EFS ($P < .01$)^{18–20,91} and OS ($P < .01$)^{18–20} in patients with t(9;22); *BCR-ABL1*; and t(4;11) but not other 11q23 (*KMT2A/MLL*) abnormalities.^{18–20,91} In addition, *iAMP21* was associated with decreased EFS ($P < .001$)^{124,318} and OS ($P = .01$)¹²⁴, ($P = .02$)³¹⁸ in pediatric patients with B-ALL, as well as separately in the subset of

§§§ References 18, 20, 22, 91, 295, 297, 299, 302, 303.

standard-risk but not high-risk patients.³¹⁸ In addition, intensifying treatment for patients with iAMP21 reduced the likelihood of relapse and improved survival.³¹⁹

Pediatric treatment algorithms rely on accurate risk stratification so that patients with higher-risk disease receive appropriately intensified therapy. Genetic abnormalities, as defined in this statement, are an important aspect of therapeutic decision making with t(12;21) and trisomies 4 and 10 conferring improved prognosis, whereas t(9;22)(q34.1;q11.2); *BCR-ABL1*; *KMT2A* (previously *MLL*) translocation; and iAMP21 conferring poor prognosis requiring intensification of therapy. Other genetic markers associated with adult and pediatric B-ALL are discussed in statement 15.

Refer to Table 7 for study data for t(12;21)(p13.2;q22.1); *ETV6-RUNX1*, t(9;22)(q34.1;q11.2); *BCR-ABL1*, *KMT2A* (previously *MLL*) translocation; iAMP21; and trisomy 4 and 10.

Public Comment Response to Statement 13.—There were 172 respondents, 94.19% (n = 162) of whom agreed with the statement, and 5.81% (n = 10) who disagreed. There were 39 written comments, most of which were supportive but which covered a range of issues including queries regarding specific methodology for detection of the genetic abnormalities, detection of Philadelphia chromosome (Ph)-like B-ALL, the necessity for obtaining *BCR-ABL1* transcripts at diagnosis, and the costs of these tests. These comments were taken into consideration in the final draft statement for this article.

Statement 14.—*Strong Recommendation for Testing for t(9;22)(q34.1;q11.2); BCR-ABL1; Recommendation for Testing for KMT2A (previously MLL) Translocations.*—For adult patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2); *BCR-ABL1* is performed. In addition, testing for *KMT2A* (previously *MLL*) translocations may be performed.

The strength of evidence was *adequate* to support this guideline statement.

This statement was supported by 2 PCSs^{17,171} that met the inclusion criteria for our SR. These studies were deemed to have a low to moderate risk of bias. None of these studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 13 for the quality-assessment results of studies included for statement 14.

The presence of the Ph chromosome or *BCR-ABL1* fusion was the most-common, recurrent abnormality in adult B-ALL affecting approximately 25% of patients. This abnormality is an independent risk factor conferring poor prognosis, as demonstrated by several studies and was supported by the Moorman et al¹⁷¹ study that was part of our SR. The t(9;22)(q34.1;q11.2), associated with the *BCR-ABL1* fusion, is detected by conventional cytogenetic studies in approximately 95% of patients; however, a molecular genetic method, such as RT-PCR or FISH analysis, is required for detection in the remaining, approximately 5%, of cases. In addition, with the incorporation of *BCR-ABL1* TKIs as front-line therapy for Ph⁺ B-ALL, rapid detection of the abnormality is often required and may be best obtained using RT-PCR or FISH analysis, thus, allowing a more-rapid treatment decision in those patients. Demonstrating the *BCR-ABL1* fusion by quantitative RT-PCR at the time of diagnosis is necessary if subsequent MRD monitoring by the same method will be used.

In the PCS by Moorman et al,¹⁷¹ 1522 patients with ALL were studied, and *BCR-ABL1* fusion detected was detected in 19% by conventional cytogenetics, RT-PCR, and/or FISH. Patients with Ph⁺ ALL had significantly inferior 5-year EFS (16% versus 36%) and OS (22% versus 41%) (both $P < .001$ adjusting for age, sex, and WBC count)¹⁷¹ in comparison to patients lacking the *BCR-ABL1* fusion. Those patients were treated on protocols before the incorporation of imatinib for patients with Ph⁺ disease. Studies incorporating TKIs into therapeutic regimens show improved outcome in adults with Ph⁺ B-ALL in comparison to Ph⁺ patients not receiving TKIs^{320,321} and suggest that improvement can be enhanced by the addition of hematopoietic cell transplantation.^{322,323} Thus, the detection of *BCR-ABL1* fusion in adults with B-ALL is essential to determine prognosis and to identify those patients who will benefit from a *BCR-ABL1* TKI.

KMT2A (previously *MLL*) translocations, an abnormality present in approximately 10% of adult patients with ALL, are also considered a poor-risk abnormality in adult patients with B-ALL. In the study by Moorman et al,¹⁷¹ patients with a cytogenetic presence of t(4;11) had significantly inferior EFS ($P < .001$) and OS ($P < .001$) when compared with patients with Ph⁻ disease. Other *KMT2A* translocations did not show statistically significant differences.

The SR also included studies showing the possible prognostic effect of other markers, although those markers did not reach the level of evidence required for recommendation. Of note, one study¹⁷ investigating brain and acute leukemia cytoplasmic (BAALC) expression by RT-PCR showed elevated levels were associated with an immature phenotype and primary therapy resistance in adult patients with B-ALL ($P = .01$). In addition, patients with *BCR-ABL1*⁻ or *KMT2A*⁻ disease with higher BAALC expression had shorter OS rates ($P = .03$).¹⁷

Predicting the prognosis and determining the optimal therapy is important for all patients with AL. In adults with B-ALL, the most significant prognostic factor is the presence of the *BCR-ABL1* fusion, a finding associated with a poor prognosis. Optimal therapy for that patient subset requires identification of *BCR-ABL1* and initiation of an appropriate TKI therapy. Other genetic markers associated with adult and pediatric B-ALL are discussed in statement 15.

Public Comment Response to Statement 14.—There were 180 respondents, of whom, 95% (n = 171) agreed, and 5% (n = 9) disagreed. There were 26 written comments that were, generally, supportive but were similar to those for statement 13 regarding clarification of specific methodology and the necessity of *BCR-ABL1* transcripts at diagnosis for *BCR-ABL1*⁺ ALL. These comments were considered in the final draft of statement 14 for this article.

Statement 15.—*Recommendation.*—For patients with suspected or confirmed ALL, the pathologist or treating clinician may order appropriate mutational analyses for selected genes that influence diagnosis, prognosis, and/or therapeutic management, which includes, but is not limited to, *PAX5*, *JAK1*, *JAK2*, and/or *IKZF1* for B-ALL and *NOTCH1* and/or *FBXW7* for T-ALL. Testing for overexpression of CRLF2 may also be performed for B-ALL.

The strength of evidence was *adequate* to support this guideline statement.

This recommendation was supported by 14 PCSs.^{****} One of the studies was deemed to have a low risk of bias,³³⁴

**** References 21, 36, 302, 324–334.

Table 6. Summary of Study Data on Minimal Residual Disease (MRD)

Source, y	Study Design	MRD Methodology	Specimen
ALL			
Maloney et al, ²⁹⁵ 2010	PCS	Flow cytometry	BM d 29
Escherich et al, ¹⁸ 2010	PCS	Antigen receptor PCR	BM d 29, d 43, wk 12
Schmiegelow et al, ⁹¹ 2010	PCS	Flow cytometry (B-ALL); antigen receptor PCR (T-ALL)	BM
Pui et al, ²⁰ 2010	PCS	Flow cytometry	BM d 19
Basso et al, ²⁹⁷ 2009	PCS	PCR (NR), flow cytometry MRD on d 15, PCR-MRD on d 33 (TP1) and 78 (TP2); standard risk, PCR ⁻ at TP1 and TP2; high risk (PCR >1 × 10 ⁻³ at TP2); others, intermediate risk; flow (flow versus molecular, <i>P</i> = NS)	BM d 15, 33 and 78
Scrideli et al, ²² 2009	PCS	Antigen receptor PCR and flow cytometry; flow (for MRD; flow versus molecular, <i>P</i> = NS)	BM d 14 and 28
Zhou et al, ²⁹⁹ 2007	PCS	Antigen receptor PCR	BM end of induction
Mullighan et al, ³⁰² 2009	PCS	Flow cytometry	BM and PB, original cohort.—d 8 PB and d 29 BM; validation cohort.—d 19 BM and d 46 BM
Waanders et al, ³⁰³ 2011	PCS	Antigen receptor PCR d 42 and 84; MRD low.—MRD ⁻ at both times; MRD medium.—MRD ⁺ at 1 or both times, but MRD <5 × 10 ⁻⁴ at d 84; MRD high.—MRD >5 × 10 ⁻⁴ at d 84	BM d 42 and 84
Patel et al, ²⁹⁶ 2010	PCS	PCR (NR): Flow	BM and PB; antigen receptor PCR: 5 wk; 10 wk; 17 wk; 6–9 mo
AML			
Yin et al, ³⁰⁴ 2012	RCT	PCR (NR)	BM and PB qRT-PCR for patients with CBF-AML; multiple time points
Markova et al, ²⁹⁸ 2009	PCS	PCR (NR)	BM and PB RT-PCR for patients with CBF-AML; multiple time points
Buccisano et al, ²⁹⁴ 2010	PCS	Flow cytometry	BM postinduction and postconsolidation
Maurillo et al, ³⁰¹ 2008	PCS	Flow cytometry	BM

Abbreviations: AML, acute myeloid leukemia; B-ALL, B-cell precursor acute lymphoblastic leukemia; BM, bone marrow; CBF, core binding factor; CI, confidence interval; DFS, disease-free survival; EFS, event-free survival; HR, hazard ratio; MRD, minimal residual disease; NR, not reported; OS, overall survival; PB, peripheral blood; PCR, polymerase chain reaction; PCS, prospective cohort study; RFS, relapse-free survival; RT-PCR, reverse transcription-polymerase chain reaction; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RCT, randomized, controlled trial; T-ALL, T-cell precursor acute lymphoblastic leukemia; TP, time period; WBC, white blood cell count.

10 were deemed to have a low to moderate risk of bias,⁺⁺⁺ and 3 were deemed to have a moderate risk of bias.^{327,331,332} None of those studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 14 for the quality-assessment results of studies included for statement 15.

In addition to gene fusions from chromosomal translocations, such as *BCR-ABL1*, and numerical abnormalities, such as trisomy 4 and 10 in pediatric B-ALL, a number of gene

alterations have also been shown to have independent prognostic and therapeutic effect in ALL. Literature review revealed several genes that may contribute to risk stratification, including *PAX5*, Janus kinase 1 (*JAK1*), Janus kinase 2 (*JAK2*), IKAROS family zinc finger 1 (*IKZF1*), cytokine receptor-like factor 2 (*CRLF2*), notch homolog 1 (*NOTCH1*), and F-box and WD repeat domain containing 7 (*FBXW7*). We also recognize that information regarding genetic information in ALL is rapidly expanding, and evidence supporting disease-related, relevant markers continues to evolve. Additional markers may also be useful.

⁺⁺⁺ References 21, 36, 302, 324–326, 328–330, 333.

Table 6. Extended

Patient Population	MRD Outcome Data
Pediatric	EFS (5-y EFS for patients with Down syndrome with d 29 MRD <0.01% was 81.9% ± 10.1% versus 49.5% ± 24.9% for those with MRD ≥0.01%; <i>P</i> = .03)
Pediatric	EFS (B-precursor: 10-y EFS, 0.92 ± 4.0 for MRD ⁻ d 29 versus 0.71 ± 5.0 for MRD ⁺ results); T-ALL: 10-y EFS, 0.81 ± 7.0 for MRD <10 ⁻³ versus 0.48 ± 8.0 for patients with MRD ≥10 ⁻³ at d 29
Pediatric	EFS (≥5%, 0.45 ± .07; ≥0.1 to <5%, 0.74 ± .04; <0.1%, 0.86 ± .02, <i>P</i> < .001); OS (≥5%, 0.60 ± 0.08; ≥0.1 to <5%, 0.90 ± .03; <0.1%, 0.93 ± 0.01; <i>P</i> < .001)
Pediatric	EFS (5-y for MRD, <0.01% at d 19, 87.1 ± 4.3; 10-y, 85.5 ± 4.8; <i>P</i> = .003 for both) versus MRD >0.01+ OS (5-y for MRD, <0.01% at d 19 was 95.2 ± 2.7; 10-y, 93.5 ± 3.3; <i>P</i> = .001 for both versus MRD >0.01+)
Pediatric	HR: model 1, flow cytometry, d 15.—for 0.1 to <10%, 1.87 (95% CI, 1.05–3.34), <i>P</i> = .03; for >0%, 4.91 (95% CI, 2.35–10.27), <i>P</i> < .001; model 2, PCR d 33 and 78.—for IR, 3.59 (95% CI, 1.77–7.3), <i>P</i> < .001; for HR, 3.99 (95% CI, 1.56–10.2), <i>P</i> = .004; mean (SE) EFS (flow cytometry d 15 only: in <0.1%, 89.9% (1.7); 0.1%–10%, 79.3% (2.3); ≥10%, 46.1% (5.9), <i>P</i> < .001)
Pediatric	EFS (MRD d 28: 5-y EFS in negative, 82.9% ± 3.0; in positive, 27.8% ± 12.0, <i>P</i> < .001; in patients negative on d 14, 85.0% ± 3.2; in patients positive d 14 and negative d 28, 76.0% ± 8.0; in patients positive d 28, 27.8% ± 12.0; <i>P</i> < .001)
Pediatric	MRD ⁻ , 62% (n = 179); MRD ⁺ , 38% (n = 108); 5-y freedom from relapse rates, MRD ⁻ , 95% ± 2%; MRD ⁺ , 56% ± 5%; <i>P</i> < .001
Pediatric	Risk of relapse: original cohort.—MRD ⁻ versus MRD ⁺ ; validation cohort.—MRD ⁻ versus MRD ⁺ ; <i>P</i> < .001 for both comparisons; HR (original cohort only), d 29, MRD >1.0% versus d 29 MRD ≤ 0.01% HR, 2.55 (95% CI, 1.34–4.85) <i>P</i> < .005; 0.01% < d 29 MRD < 1.0% versus d 29 MRD ≤ 0.01% HR, 2.33 (95% CI 1.31–4.15); <i>P</i> < .005
Pediatric	Nonrelapse versus relapse by MRD status: MRD-low.—29.9% versus 8.3%; MRD-medium.—65.4% versus 45.8%; MRD-high.—4.7% versus 45.8%; <i>P</i> < .001; 9 y RFS: MRD-low.—94%; MRD-medium.—86%; MRD-high.—31%; <i>P</i> < .001
Adult	RFS (MRD ⁺ > 10 ⁻⁴) ⁺ versus MRD ⁻ (<10 ⁻⁴): 5 wk.—42% (95% CI, 23–61) versus 69% (95% CI, 55–83), <i>P</i> = .03; HR, 2.36 (95% CI, 1.11–5.04); 10 wk.—HR, 4.99 (95% CI, 1.96–12.65); 17 wk.—HR, 5.18 (95% CI, 2.15–12.48); for patients with standard risk (age < 35 y and WBC < 30 × 1000/mm ³): 10 wk.—14% (95% CI, 0–38) versus 80% (95% CI, 62–98); <i>P</i> < .001; 17 wk.—25% (95% CI, 1–50) versus 73% (95% CI, 56–90); <i>P</i> < .001
Adult	Relapse rates t(8;21): BM.—MRD ⁻ , 5.3%; MRD ⁺ , 93.3%; <i>P</i> < .001; PB.—MRD ⁻ , 6%; MRD ⁺ , 93.3%; <i>P</i> < .001; Relapse rates inv(16): BM.—MRD ⁻ , 6.4%; MRD ⁺ , 82.4%; <i>P</i> < .001; PB.—MRD ⁻ , 4.8%; MRD ⁺ , 81.8%; <i>P</i> < .001
Mixed adult and pediatric	6-y DFS: MRD ⁻ , 95.2% versus MRD ⁺ , 68.8%; OS (MRD ⁻ , <i>P</i> = .044); RFS (MRD ⁻ , <i>P</i> = .008)
Adult	MRD ⁺ defined as >3.5 × 10 ⁻⁴ residual leukemic cells: OS.—MRD postconsolidation HR, 2.38 (95% CI, 1.03–5.45) <i>P</i> = .04; RFS.—MRD postconsolidation HR, 2.68 (95% CI, 1.27–5.67) <i>P</i> = .01; Cumulative incidence of relapse: cytogenetic intermediate risk MRD ⁻ and MRD ⁺ .—OS, 67% versus 23%, <i>P</i> = .01; cytogenetic good risk MRD ⁻ and MRD ⁺ .—OS, 84% versus 38%, <i>P</i> = .01; good risk MRD ⁺ .—n = 8; good risk MRD ⁻ .—n = 14; intermediate risk MRD ⁺ .—n = 86; intermediate risk MRD ⁻ .—n = 29; intermediate risk MRD ⁻ and MRD ⁺ .—4-y RFS, 63% versus 17%, <i>P</i> < .001; good risk MRD ⁻ and MRD ⁺ .—4-y OS, 70% versus 15%, <i>P</i> = .001
Adult	MRD ⁺ defined as >3.5 × 10 ⁻⁴ residual leukemic cells postconsolidation; RFS (5-y, <i>P</i> < .001); OS (62% MRD ⁻ versus 23% MRD ⁺ ; <i>P</i> = .001); in multivariable analysis, MRD ⁺ significant for worse outcome, HR, 3.56 (95% CI 1.50–8.43) <i>P</i> = .004

PAX5, a transcription factor required for B-lymphoid development and located on chromosome arm 9p, may be important for leukemogenesis, although evidence for independent contribution of *PAX5* alterations to patient prognosis has varied. *PAX5* alterations occur in approximately 30% to 35% of B-ALL by deletion, fusion translocations, or point mutations. Deletions of *PAX5* occur in the greatest proportion of patients when concurrent *BCR-ABL1* or *TCF3-PBX1* is present.

Our SR included 4 PCs that addressed *PAX5* mutation.^{36,302,325,327} Only one study³⁶ on adolescent/adult *BCR-ABL1*⁻ B-ALL showed significantly improved prognosis in a multivariate analysis for patients with *PAX5* alterations, including improved EFS (*P* = .02), RFS (*P* = .05), and OS (*P* = .03). Another study in adult B-ALL demonstrated improved

complete response (CR) rate (*P* = .03), although *PAX5* alterations had no significant effect on cumulative incidence of relapse and disease-free survival (DFS).³²⁵ The 2 pediatric studies showed no significant difference in prognosis in high-risk B-ALL³⁰² and no difference in *PAX5* alterations when comparing a small relapse-prone cohort with an unselected B-ALL cohort.³²⁷ Although *PAX5* abnormalities are common in B-ALL, more data may be needed to determine whether these abnormalities assist with risk stratification. Of note, a small subset of patients with B-ALL (2%–3%) show structural rearrangements resulting in fusion proteins, with *PAX5-JAK2* identified as a recurrent abnormality. The presence of *PAX5-JAK2* fusion protein can result in the constitutively activated *JAK-STAT* pathway raising the possibility that this represents a TKI target for therapeutic intervention.

Table 7. Summary of Study Data for t(12;21)(p13.2;q22.1); ETV6-RUNX1, t(9;22)(q34.1;q11.2); BCR-ABL1, KMT2A (MLL) Translocation, iAMP21, and Trisomy 4 and 10

Source, y	Study Design	t(12;21) (p13.2;q22.1); ETV6-RUNX1	t(9;22) (q34.1;q11.2); BCR-ABL1
Escherich et al, ¹⁸ 2010	PCS	Negative versus positive OS, $P < .001$; EFS, $P = .001$; in favor of positive	Negative versus positive OS, $P = .001$; EFS, $P = .001$; in favor of negative
Salzer et al, ¹⁹ 2010	PCS	Present versus absent EFS, $P < .001$; OS, $P < .001$; in favor of present	Present versus absent OS, $P < .001$; EFS, $P < .001$; in favor of absent
Schmiegelow et al, ⁹¹ 2010	PCS	Present versus absent EFS, $P \leq .001$; OS, $P < .001$; in favor of present	Present versus absent EFS, $P = .01$; OS, NS (few patients with positive results in study); in favor of absent
Pui et al, ²⁰ 2010	PCS	ETV6-RUNX1; present versus absent; EFS, $P = .05$; OS, $P = .04$	Present versus absent EFS, $P < .001$; OS, $P < .001$; in favor of absent
Rubnitz et al, ³¹⁶ 2008	PCS	TEL (ETV6) rearrangement; present versus absent EFS, $P < .0001$; in favor of present	...
Moorman et al, ¹²⁴ 2007	PCS

Abbreviations: *ABL1*, Abelson murine leukemia viral oncogene homolog 1; *BCR*, breakpoint cluster region protein; EFS, event-free survival; *ETV6*, ETS variant 6; *iAMP21*, intrachromosomal amplification of chromosome 21; *KMT2A/MLL*, mixed-lineage leukemia; . . ., not available; OS, overall survival; PCS, prospective cohort study; *RUNX1*, runt-related transcription factor 1; TEL, translocation-ETS-leukemia.

IKZF1 encodes for the IKAROS zinc finger binding protein and is associated with a poor prognosis in B-ALL. Alterations in *IKZF1* occur in more than 80% of patients with *BCR-ABL1*⁺ ALL who may be associated with resistance to TKIs.³³⁵ Our SR included 3 PCSs (1 adolescent/adult³⁶ and 2 pediatric^{302,327}) and overall supported the poor prognosis of *IKZF1* alterations in *BCR-ABL1*⁻ B-ALL. In adolescent/adult patients with Ph⁻ B-ALL, *IKZF1* alterations were associated with inferior EFS ($P = .01$), DFS ($P = .06$), and OS ($P = .10$) in univariate analysis; although significance was not demonstrated in multivariate analysis.³⁶ In pediatric patients with high-risk B-ALL (very high risk and *BCR-ABL1* excluded), *IKZF1* alteration was associated with elevated MRD ($P = .04$, day 8; $P = .001$, day 29) and increased incidence of relapse ($P < .001$).³⁰² That study noted similarity between the gene expression signature of high-risk *BCR-ABL1*⁻ ALL from the original cohort and the gene signature of *BCR-ABL1*⁺ ALL in the validation cohort. The poor prognosis of *IKZF1* was independent of *BCR-ABL1* status. Another pediatric study³²⁷ supported the poor prognosis of *IKZF1* alterations with an increased proportion of patients with *IKZF1* deletion in their small cohort of relapse-prone ALL.

Overexpression of the cytokine receptor *CRLF2* is associated with a poor prognosis in B-ALL and often results from translocations of *CRLF2* with partner genes, such as the immunoglobulin heavy-chain gene (*IGH*) or *P2RY8*. *CRLF2* overexpression is seen in 5% to 16% of pediatric and adult B-ALL, more than 50% of Down syndrome ALL, and approximately 50% of *BCR-ABL1*-like B-ALL (see discussion of *BCR-ABL1*-like B-ALL below). *CRLF2* alterations are also associated with concurrent *IKZF1* deletion and/or mutation, *JAK1/JAK2* mutations, and a poor prognosis in adult and pediatric B-ALL.^{36,330,336} Our SR included 3 PCSs (1 adolescent/adult³⁶ and 2 pediatric^{330,332}). *CRLF2* deregulation in adolescents/adults was associated with decreased 5-year RFS ($P = .03$) and OS ($P = .04$).³⁶ In pediatric patients, *CRLF2* high-level expression was associated with worse EFS rates ($P = .01$) and greater cumulative risk of relapse ($P = .01$), mainly because of the high incidence of relapse in non-high-risk patients with *P2RY8-CRLF2*.³³⁰ Gene expression

profiling in pediatric patients with high-risk B-ALL showed an expression cluster with *CRLF2* rearrangements, *JAK* mutations, *IKZF1* deletions, *BCR-ABL1*-like signature, and a very poor prognosis.³³² Increasing interest in *CRLF2* status has occurred, particularly in light of its high incidence in *BCR-ABL1*-like ALL. *CRLF2* overexpression may be detected by flow cytometry or FISH assays.

Regarding the prognostic significance of *JAK1* and *JAK2* mutations alone in ALL (without *CRLF2*), no data were available from our SR.

BCR-ABL1-like (or Ph-like) ALL has been recently recognized and is of particular prognostic importance. These leukemias lack the *BCR-ABL1* fusion but have a gene expression profile similar to *BCR-ABL1*⁺ leukemia and are associated with a poor prognosis. One expression array study in pediatric B-ALL patients identified the *BCR-ABL1*-like phenotype and showed an increased relapse rate ($P < .05$) and decreased 5-year DFS ($P < .03$) in that subset of patients when compared with other forms of B-ALL (*BCR-ABL1*⁺ ALL excluded).³²⁸ A separate, large study³³⁷ of B-ALL reported a frequency of *BCR-ABL1*-like ALL ranging from 10% for standard-risk, pediatric patients with ALL, up to 27% among young adults with ALL. In most of those patients (91%) a kinase-activating alteration, such as *ABL1*, *ABL2*, *CRLF2*, *JAK2*, or platelet-derived growth factor receptor β (*PDGFRB*), was identified suggesting that at least some patients may benefit from TKI therapy. The most common gene expression alteration identified in *BCR-ABL1*-like ALL, as well as Down syndrome-associated ALL, was elevated *CRLF2* expression, occurring in approximately 50% of patients. Concurrent *JAK2* or *JAK1*, *IL7R*, *FLT3*, *SH2B3*, and *NRAS* mutations were also present in 30% to 55% of patients with *CRLF2* overexpression. Deletions involving *IKZF1*, *PAX5*, and *EBF1* were also detected in patients with Ph-like ALL.⁸

For T-ALL, our SR revealed data about alterations of several genes.

NOTCH1 and *FBXW7* mutations frequently occur in T-ALL, and both result in decreased *NOTCH1* activity. Some studies suggest an improved early response to therapy and prognosis in these patients, although others lack prognostic

Table 7. Extended

KMT2A/MLL Translocations	iAMP21	Trisomy 4 and 10
11q23.3; t(4;11), <i>MLL</i> ⁻ versus <i>MLL</i> ⁺ OS, <i>P</i> < .001; EFS, <i>P</i> = .001; in favor of <i>MLL</i> ⁻
11q23.3; t(4;11), present versus absent; OS, <i>P</i> < .001; EFS, <i>P</i> < .001; in favor of absent	...	Present versus absent OS, <i>P</i> < .001; EFS, <i>P</i> < .001; in favor of present
11q23.3; t(4;11); present versus absent EFS, <i>P</i> < .001; OS, <i>P</i> < .001; in favor of absent
11q23.3; t(4;11); Present versus absent EFS, <i>P</i> = .002; OS, <i>P</i> < .001; No difference detected
...
...	Present versus absent EFS observed-expected ratio <i>P</i> < .001; OS observed-expected ratio <i>P</i> = .01; in favor of absent	...

significance. Those discrepancies may be the result of different treatment regimens. Our SR included 5 PCSs (3 adult; 2 pediatric). Of those studies, results of studies of adults with T-ALL and *NOTCH1* and/or *FBXW7* mutations include one showing an improved median EFS (*P* = .02) and OS (*P* = .01) in multivariate analysis,³²⁶ whereas 2 showed no prognostic significance,^{21,329} although a trend toward improved EFS was seen in one (*P* = .10).²¹ In pediatric T-ALL, one study identified an improved early response to therapy (*P* < .01), decreased early (*FBXW7* and/or *NOTCH1*) and late MRD (*NOTCH1*, *P* < .01), and improved EFS (*NOTCH1*; *P* = .01). Patients with both *NOTCH1* and *FBXW7* mutations had similar outcomes to those with *NOTCH1* mutations alone.³³⁴ The second pediatric study showed improved early response to therapy with *NOTCH1* and/or *FBXW7* mutation but no difference in EFS or OS.³²⁴

JAK1 mutations were associated with reduced DFS (*P* = .01) and OS (*P* < .01) in a small cohort of patients with T-ALL,³³¹ but no recommendation regarding *JAK1* mutation testing in T-ALL was made because of a lack of multiple or larger studies confirming that report.

Refer to Table 8 for study data on *PAX5* and other mutations in patients with B-ALL. Refer to Table 9 for study data on *NOTCH1* and *FBXW7* mutations in patients with T-ALL.

Public Comment Response for Statement 15.—There were 174 respondents, 90.8% (n = 158) of whom agreed, and 9.2% (n = 16) who disagreed with the statement. There were 32 written comments, most of which were very supportive, but some of which expressed that the panel was too limited, others questioning its clinical utility, and others suggesting the inclusion of copy number aberrations and genetic abnormalities characterizing Ph-like ALL be added.

Statement 16.—*Strong Recommendation for Testing for FLT3-ITD; Recommendation for Testing for Other Mutational Analysis.*—For pediatric and adult patients with suspected or confirmed AML of any type, the pathologist or treating clinician should ensure that testing for *FLT3-ITD* is performed. The pathologist or treating clinician may order mutational analysis that includes, but is not limited to, *IDH1*, *IDH2*, *TET2*, *WT1*, *DNMT3A*, and/or *TP53* for prognostic and/or therapeutic purposes.

The strength of evidence was *adequate* to support this guideline statement.

The recommendation for *FLT3-ITD* testing was supported by 13 PCSs^{****} that met the inclusion criteria for our SR and 8 other studies^{26,343–349} that were found external to our systematic search (or did not meet the inclusion criteria) but were retained for discussion. Of the 13 studies, one was deemed to have a low risk of bias,³⁴² 10 were deemed to have a low to moderate risk of bias,^{§§§§} and 2 were deemed to have a moderate risk of bias.^{14,294} None of these studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 15 for the quality-assessment results of studies included on *FLT3-ITD* testing.

The discovery of gene mutations that affect prognosis in AML was a major advance of the past decade, and the more-recent use of NGS techniques has increased access to mutation panels in the diagnostic setting. Although the prognostic significance of gene mutations were first recognized in patients with normal karyotype AML (NK-AML), it is now recognized that some mutations may define specific disease-classification groups, such as AML with mutated *NPM1*, AML with biallelic mutations of *CEBPA*, and the provisional entity of AML with mutated *RUNX1*, whereas others, such as *FLT3*, may provide prognostic information across different classification groups.⁸ Mutations in *FLT3* most commonly result in ITDs but may also be point mutations in the tyrosine kinase domain. Many gene mutations are now, however, reported in AML,^{7,349} creating challenges in understanding which individual genes and/or gene combinations are significant in the disease and warrant testing. Although NGS panels may allow for routine study of multiple genes, the literature review tended to focus on the significance of individual genes. It is understood that, with more study, stronger recommendations for genetic testing in AML may be appropriate in the near future.

Mutations in *FLT3-ITD* are now recognized as predictors of a poor prognosis in AML, especially in NK-AML. Most patient cohort studies have found a worse DFS or OS in

**** References 14, 16, 71, 100, 105, 122, 294, 298, 338–342.

§§§§ References 16, 71, 100, 105, 122, 298, 338–341.

Table 8. Summary of Study Data for PAX5 and Other Mutations in Patients With Acute Lymphoblastic Leukemia (ALL)

Source, y	Study Design	PAX5 Mutations	Other Mutations
Moorman et al, ³⁶ 2012	PCS	In MVA, PAX5 deletions in patients with BCR-ABL1 ⁻ B-ALL had improved survival outcomes: EFS (<i>P</i> = .02), RFS (<i>P</i> = .05), and OS (<i>P</i> = .03)	CRLF2 deregulations showed worsened survival outcomes.—5-y RFS HR, 2.04, 95% CI, 1.07–3.89 (<i>P</i> = .03); 5-y OS HR, 1.78, 95% CI, 1.04–3.07 (<i>P</i> = .04) IKZF1 deletions showed worsened survival outcomes.—5-y EFS HR, 1.54, 95% CI, 1.12–2.12 (<i>P</i> = .01); 5-y OS HR, 1.55, 95% CI, 1.11–2.16 (<i>P</i> = .01)
Familiades et al, ³²⁵ 2009	PCS	Associated with improved CR rates (<i>P</i> = .03), but no significant effect on cumulative rate of relapse or disease-free survival	...
Mullighan et al, ³⁰² 2009	PCS	No independent association between PAX5 alterations and outcome observed in high-risk pediatric B-ALL (very high risk subtypes excluded)	Patients with IKZF1 alteration showed increase in hematologic relapse; original cohort incidence at 4 or 5 y, 55.2 ± 8.6 (<i>P</i> < .001); validation cohort incidence at 10 y, 46.3 ± 8.4 (<i>P</i> = .01); <i>N</i> = 27 of 67
Kuiper et al, ³²⁷ 2010	PCS	<i>P</i> = .59	Higher proportion IKZF1 deletions in small, relapsed cohort (compared with unselected cohort); <i>P</i> = .002
Cario et al, ³³⁰ 2010	PCS	...	High CRLF2 expression had a worse 6-y EFS probability compared with patients with low CRLF2 expression (61% ± 8% versus 83% ± 2%; <i>P</i> = .003)
Harvey et al, ³³² 2010	PCS	...	Gene expression profiling in pediatric patients with high-risk B-ALL showed an expression cluster with CRLF2 rearrangements, JAK mutations, IKZF1 deletions, BCR-ABL1-like signature, and very poor prognosis

Abbreviations: B-ALL, B-cell precursor acute lymphoblastic leukemia; BCR-ABL1, breakpoint cluster region protein–Abelson murine leukemia viral oncogene homolog 1; CR, complete remission; CRLF2, cytokine receptor-like factor 2; EFS, event-free survival; HR, hazard ratio; IKZF1, IKAROS family zinc finger 1; JAK, Janus kinase; MVA, multivariate analysis; . . . , not available; OS, overall survival; PAX5, paired box 5; PCS, prospective cohort study; RFS, relapse-free survival.

patients with this mutation, although differences in CR are not always present.***** Similar findings are found in young adult patients with AML and cytogenetic abnormalities, including t(15;17)(q24.1;q21.2), t(8;21)(q22;q22.1), and t(6;9)(p23;q34.1), as well as mutations of NPM1 and CEBPA.^{100,349} Fewer studies have failed to find mutations of FLT3-ITD to be associated with prognosis, and the significance may be less in pediatric AML.^{122,298,338–340} The mutation level was also directly associated with worse survival,^{26,342,343} including 2 patient cohort studies, and the level of mutation should be investigated in cases with a mutation detected.

Refer to Table 10 for study data on NPM1, FLT3-ITD, CEBPA, KIT, and RUNX1 testing.

The recommendation for testing of other mutations in AML is supported by 21 studies,^{††††} comprising one SR based meta-analysis,³⁵³ 3 NRCTs,^{10,11,354} and 17 PCSs.^{††††} The meta-analysis, reported by Zhou et al³⁵³ was deemed to have a low risk of bias. The 3 NRCTs^{10,11,354} were all deemed to have a low to moderate risk of bias. For the 17 PCSs, 3 were deemed to have a low risk of bias,^{30,101,119} 13 were deemed to have a low to moderate risk of bias,^{§§§§§} and one was deemed to have a moderate risk of bias.²⁹ None of those studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 16 for the quality-assessment results for all other molecular tests, excluding FLT3-ITD testing.

***** References 14, 16, 71, 100, 105, 294, 341, 344–349.

†††† References 10, 11, 15, 29, 30, 101, 109, 110, 116, 118–120, 130, 195, 338, 340, 350–354.

†††† References 15, 29, 30, 101, 109, 110, 116, 118–120, 130, 195, 338, 340, 350–352.

§§§§§ References 15, 109, 110, 116, 118, 120, 130, 195, 338, 340, 350–352.

Among studies of the effect of isocitrate dehydrogenase 1 (IDH1) mutation R132 in adult AML, one meta-analysis³⁵³ found the mutation associated with a worse EFS, but not associated with OS. One NRCT³⁵⁴ found the mutation to be associated with a worse OS and DFS, but that study combined the results of IDH1 and IDH2 mutations. Three PCSs^{340,351,352} found IDH1 mutation to be associated with a worse prognosis in patients with AML, who have intermediate-risk cytogenetics or NK-AML, but one of those studies³⁵¹ also combined the results of both IDH1 and IDH2 mutations for analysis. Three PCSs found no prognostic significance to detecting this mutation in adult AML.^{15,119,338} A single PCS of pediatric AML did not detect the R132 mutation in any cases.³³⁸ One PCS of the IDH1 single-nucleotide polymorphism (SNP) rs11554137 found the presence of that SNP to be associated with a worse OS.¹⁵

For mutations in IDH2 in adult AML, most studies combined the results of the R140 and R172 mutations, and some combined IDH2 results with those of IDH1. One meta-analysis³⁵³ found improved OS, but no effect on EFS in AML with mutated IDH2. Two NRCTs^{10,354} and 2 PCSs^{351,352} found a worse prognosis with that mutation, although the worse prognosis was only associated with the R172 mutation in one study.³⁵² Two PCSs^{119,340} found no prognostic significance to the presence of an IDH2 mutation in AML, and one³⁵² found no prognostic significance when the R140 mutation was present in AML.

One NRCT and one PCS studied the prognostic significance of tet methylcytosine dioxygenase 2 (TET2) mutations in AML. The NRCT³⁵⁴ found no prognostic significance to the detection of that mutation, whereas the PCS¹⁰¹ found TET2 mutations to be associated with a worse outcome.

Mutations of Wilms tumor 1 (WT1), usually involving exons 7 and 9, were evaluated in 12 studies and found to be associated with a significantly worse prognosis in AML,

Table 9. Summary of Study Data for NOTCH1 and FBXW7 Mutations in Patients With T-Cell Acute Lymphoblastic Leukemia (T-ALL)

Source, y	Study Design	Age, y, Range (Median)	NOTCH1 Mutations	FBXW7 Mutations
Marks et al, ²¹ 2009	PCS	15–59 (29)	Mutation in NOTCH1 pathway (NOTCH1 and/or FBXW7) had higher EFS but not statistically significant ($P = .1$)	
Asnafi et al, ³²⁶ 2009	PCS	15–58 (28)	By MVA, NOTCH1 and/or FBXW7 mutations were associated with improved survival outcomes compared with patients lacking these mutations: EFS.—HR, 0.58, 95% CI, 0.37–0.92 ($P = .02$); OS.—HR, 0.54, 95% CI, 0.33–0.87 ($P = .01$)	
Baldus et al, ³²⁹ 2009	PCS	16–66 (30)	NOTCH1 and/or FBXW7 mutations CR ($P = .5$), relapse ($P = .76$), and EFS ($P = .39$)	
Clappier et al, ³²⁴ 2010	PCS	1–17 (8)	NOTCH pathway mutations associated with early response to therapy ($P = .02$), but similar EFS and OS rates	
Kox et al, ³³⁴ 2010	PCS	<18 (NR)	Mutation correlated with better outcomes: EFS 87% versus 74% in nonmutated group ($P = .01$); relapse 7% versus 17% in nonmutated group ($P = .01$)	Mutation correlated with better early response to therapy: 88% versus 55% in nonmutated group ($P < .001$); similar EFS and relapse rate with and without FBXW7 mutations

Abbreviations: CR, complete remission; EFS, event-free survival; FBXW7, F-box and WD repeat domain containing 7; HR, hazard ratio; MVA, multivariate analysis; NOTCH1, notch homolog 1; NR, not reported; OS, overall survival; PCS, prospective cohort study.

usually in NK-AML, in 8 of the 12 studies—one NRCT¹⁰ and 7 PCSs,^{*****}—and was not prognostically significant in the other 4 PCSs.^{29,109,119,120} One positive PCS was in pediatric patients.¹⁹⁵

The prognostic significance of detecting the WT1 SNP rs16754 was evaluated in one NRCT¹¹ and 3 PCSs^{15,29,119} and was found to be significantly associated with an improved prognosis in all studies.

One PCS in adult AML evaluated the prognostic significance of DNA methyltransferase 3 alpha (DNMT3A) mutations in AML and found mutations to be significantly associated with a worse OS and EFS.¹³⁰

No studies were identified with the search parameters of the SR evaluating additional sex combs like 1, transcriptional regulator (ASXL1), mutations in AML, although a recent study found a worse OS in patients with AML with myelodysplasia-related changes (AML-MRC), and ASXL1 mutations compared with patients with AML-MRC and no such mutation ($P = .01$).³⁵⁵ Future studies may clarify the significance of that gene mutation in AML.

Two PCSs evaluated the prognostic significance of KMT2A (MLL)-PTD (partial tandem duplication) mutations in AML. One found that mutation to be associated with a worse progression-free survival,¹⁰⁹ and one found no significance to that mutation on OS in NK-AML.¹¹⁹

One PCS evaluated the prognostic significance of neuroblastoma RAS viral oncogene homolog (NRAS) mutations in NK-AML and found no effect on OS.¹¹⁹

Additional studies on mutations in DNMT3A, usually R882, show variable results, with some also showing such mutations associated with shorter remission and survival,^{356,357} whereas others showed no effect on remission or survival duration.^{357,358} Mutations may be associated with a worse outcome in younger adults (younger than 60 years),^{130,358} but one study suggests worse DFS and OS in older adults with the R882 mutation.³⁵⁶

Mutations in tumor protein p53 (TP53) were highly associated with complex karyotypes and other myelodysplasia-related cytogenetic abnormalities that involve chromosomes 5, 7, and 17.^{359–361} Such cases were either therapy-related AML or AML-MRC, but the mutations may be germline. TP53 mutations are associated with worse RFS, EFS, and OS. However, the effect of that mutation may not

be significant, with complex karyotypes defined as 5 or more abnormalities.³⁶⁰

Refer to Table 11 for study data for IDH1, IDH2, TET2, WT1, DNMT3A testing.

Public Comment Response for Statement 16.—There were 172 respondents, 65.7% (n = 113) of whom agreed, 1.74% (n = 3) who disagreed, and 32.56% (n = 56) who did not commit but responded with written comments to the initial draft statement. The comments included suggestions to include more mutation analysis as well as suggestions that fewer mutation studies should be listed. Some confusion arose from the omission of mutation studies for NPM1 and CEBPA in this statement, but those studies were recommended in statement 19. Some respondents suggested restricting that statement only to NK-AML, and others wanted to expand testing to a large NGS panel for all AML cases. Several objected to the listing of specific protein expression assays that were included in the original statement because such tests were not widely available, and others suggested adding testing for other proteins. Those comments were considered in the final draft of statement 16 in this article.

Statement 17.—**Strong Recommendation for Testing for KIT Mutation in Adult Patients With CBF-AML; Expert Consensus Opinion for Testing for KIT Mutation in Pediatric Patients With CBF AML.**—For adult patients with confirmed CBF-AML—AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22)/t(16;16)(p13.1;q22); CBFβ-MYH11—the pathologist or treating clinician should ensure that an appropriate mutational analysis for KIT is performed. For pediatric patients with confirmed CBF-AML—AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22)/t(16;16)(p13.1;q22); CBFβ-MYH11—the pathologist or treating clinician may ensure that appropriate mutational analysis for KIT is performed.

The strength of evidence was adequate to support testing for KIT in adult patients with CBF-AML, but insufficient to support testing for KIT in pediatric patients with CBF-AML.

This recommendation was supported by 2 PCSs^{298,339} obtained from our SR. The risk of bias assessment for both was low to moderate. Overall, none of the studies providing the evidence base for statement 17 were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 17 for the quality-assessment results for the studies included for statement 17.

***** References 30, 101, 110, 116, 118, 195, 350.

Table 10. Summary of Study Data for *NPM1*, *FLT3* Internal Tandem Duplication (ITD), *CEBPA*, *KIT*, and *RUNX1*

Source, y	Study Design	<i>NPM1</i>	<i>FLT3</i> ITD
Gaidzik et al, ¹⁰⁰ 2011	PCS	<i>RUNX1</i> mutations (N = 53) and <i>NPM1</i> mutated (5 of 53) versus <i>RUNX1</i> wild-type (N = 831) and <i>NPM1</i> mutated (307 of 831); <i>P</i> < .001	...
Kayser et al, ⁷¹ 2011	PCS	MVA for t-AML relapse: HR, 0.69 (<i>P</i> < .001); Death in CR.—HR, 0.67 (<i>P</i> = .04); OS.—HR, 0.78 (<i>P</i> < .001)	MVA for t-AML relapse.—HR, 1.4 (<i>P</i> < .001); Death in CR.—HR, 1.61 (<i>P</i> = .01); OS.—HR, 1.51 (<i>P</i> < .001)
Buccisano et al, ²⁹⁴ 2010	PCS	...	Patients with <i>FLT3</i> -ITD who were also MRD ⁻ had better 4-y RFS and OS rates than did patients with <i>FLT3</i> -ITD and MRD ⁺ disease; RFS.—54% versus 17% (<i>P</i> < .001); OS.—60% versus 23% (<i>P</i> = .01)
Röllig et al, ¹⁴ 2010	PCS	CR.— <i>NPM1</i> ⁻ / <i>FLT3</i> -ITD ⁻ , OR, 1.00; <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁻ , OR, 2.49, 95% CI, 1.48–4.18 (<i>P</i> = .001); <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁺ , OR, 1.71–5.59 (<i>P</i> < .001); <i>NPM1</i> ⁻ / <i>FLT3</i> -ITD ⁺ , OR, 1.1, 95% CI, 0.60–2.00 (<i>P</i> = .76) DFS.— <i>NPM1</i> ⁻ / <i>FLT3</i> -ITD ⁻ , HR, 1; <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁻ , HR, 0.48, 95% CI, 0.33–0.71 (<i>P</i> < .001); <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁺ , HR, 0.59, 95% CI, 0.37–0.92 (<i>P</i> = .02); <i>NPM1</i> ⁻ / <i>FLT3</i> -ITD ⁺ , HR, 2.28, 95% CI, 1.27–4.09 (<i>P</i> = .006) OS.— <i>NPM1</i> ⁻ / <i>FLT3</i> -ITD ⁻ , HR, 1; <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁻ , HR, 0.69, 95% CI, 0.53–0.91 (<i>P</i> = .007); <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁺ , HR, 0.74, 95% CI, 0.55–0.99 (<i>P</i> = .04); <i>NPM1</i> ⁻ / <i>FLT3</i> -ITD ⁺ , HR, 0.94, 95% CI, 0.70–1.26 (<i>P</i> = .67) Note: All MVA	...
Groschel et al, ¹⁶ 2010	PCS	...	<i>MECOM/EV11</i> ⁻ (N = 1234) and <i>FLT3</i> -ITD mutation (N = 325; 26%) versus <i>MECOM/EV11</i> ⁺ (N = 148) and <i>FLT3</i> -ITD mutation (N = 22; 15%) (<i>P</i> = .002)
Kayser et al, ³⁴¹ 2009	PCS	...	<i>FLT3</i> -ITD insertions in β1-sheet associated with inferior RFS (<i>P</i> = .001) and OS (<i>P</i> = .01)
Ho et al, ¹⁰⁵ 2010	PCS	...	Patients with <i>WT1</i> mutations and <i>FLT3</i> -ITD ⁻ had superior CR rates to patients with <i>WT1</i> 82.2% versus 52.2% (<i>P</i> = .02); patients with <i>WT1</i> mutations/ <i>FLT3</i> -ITD ⁺ had poorer mean OS (15 versus 56; <i>P</i> = .001) and mean EFS (15 versus 35; <i>P</i> = .02) compared with patients with <i>WT1</i> mutations/ <i>FLT3</i> -ITD ⁻
Abbas et al, ³⁴⁰ 2010	PCS	<i>NPM1</i> mutations associated with IDH wild-type.— <i>NPM1</i> ⁺ / <i>IDH1</i> ⁺ (35 of 893); <i>NPM1</i> ⁺ / <i>IDH2</i> ⁺ (40 of 893); <i>NPM1</i> ⁺ / <i>IDH1/2</i> ⁺ (191 of 893); <i>P</i> = .001	<i>P</i> = .09
Ho et al, ³³⁸ 2010	PCS	...	<i>P</i> = .35 (no difference detected between <i>FLT3</i> -ITD R132 versus wild-type
Pollard et al, ³³⁹ 2010	PCS
Markova et al, ²⁹⁸ 2009	PCS	...	<i>P</i> = .08 RFS in patients with CBF-AML with various <i>FLT3</i> Asp835 mutations
Jiao et al, ¹²² 2009	PCS

Abbreviations: AML, acute myeloid leukemia; Asp, aspartic acid; CBF, core-binding factor; *CEBPA*, CCAAT/enhancer binding protein; CN, cytogenetically normal; CR, complete response; DFS, disease-free survival; EFS, event-free survival; HR, hazard ratio; *IDH*, isocitrate dehydrogenase; *FLT3*, fms-related tyrosine kinase 3; *KIT*, proto-oncogene receptor tyrosine kinase; *MECOM/EV11*, MDS1 and EV11 complex locus; MRD, minimal residual disease; mut, mutant; MVA, multivariate analysis; . . . , not available; *NPM1*, nucleophosmin (nucleolar phosphoprotein B23, numatrin); OR, odds ratio; OS, overall survival; PCS, prospective cohort study; RFS, relapse-free survival; *RUNX1*, runt-related transcription factor 1; t-AML, therapy-related acute myeloid leukemia; *WT1*, Wilms tumor 1.

Table 10. Extended

<i>CEBPA</i>	<i>KIT</i>	<i>RUNX1</i>
...	...	<i>RUNX1</i> associated with inferior survival (4-y survival rates): EFS ($P < .001$), RFS ($P = .02$); <i>RUNX1</i> mutations are inversely associated with <i>NPM1</i> mutations ($P < .001$)
...
...
...
...
...
<i>CEBPA</i> ^{mut} associated with superior OS in patients with CN-AML (HR, 0.26; 95% CI, 0.10–0.72) and in all patients with AML (HR, 0.31; 95% CI, 0.12–0.83) ($P < .05$), NR
...
...
...
...	In 1 of 4 clinical trials of patients with CBF-AML, <i>KIT</i> mutation was associated with poorer 5-y survival ($P = .01$)	...
...	$P = .33$ for relapse and OS in patients with CBF-AML and <i>KIT</i> mutations; in patients with <i>RUNX1-RUNX1T1</i> ⁺ , OS in patients with <i>KIT</i> mutation (6 of 31) was inferior to patients without mutation ($P = .14$)	...
...	<i>KIT</i> was associated with overexpression of <i>RUNX1-RUNX1T19a</i> ($P = .02$) and overexpression of the gene resulted in shorter EFS ($P = .01$) and OS ($P = .01$)	...

This recommendation for adult patients is evidence based and is supported by multiple cohort studies outside of our SR. Core-binding factor AML is a distinct cytogenetic subtype of AML that is characterized by the presence of t(8;21)(q22;q22.1) *RUNX1-RUNX1T1* or inv(16)(p13.1q22)/

t(16;16)(p13.1;q22) *CBFB-MYH11* and, in general, is considered a favorable risk.^{156,162} However, *KIT* mutations were identified in approximately 20% to 30% of CBF-AML^{298,362,363} and are associated with increased recurrence risk and decreased survival. In adults, available data

Table 11. Summary of Study Data for *IDH1*, *IDH2*, *TET2*, *RUNX1*, *WT1*, *DNMT3A*

Source, y	Study Design	<i>IDH1</i>	<i>IDH2</i>
Zhou et al, ³⁵³ 2012	M/A	...	OS benefit, <i>P</i> = .01
Nomdedéu et al, ³⁵⁴ 2012	NRCT
Mendler et al, ¹⁰ 2012	NRCT	...	<i>P</i> = .84 (no correlation with <i>RUNX1</i>)
Damm et al, ¹¹ 2012	NRCT
Paschka et al, ³⁵¹ 2010	PCS	<i>P</i> = NS overall in CN AML (RFS, <i>P</i> = .72; OS, <i>P</i> = .44), but the presence of <i>IDH1</i> or <i>IDH2</i> mutations in the CN <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁻ group associated with poorer EFS (<i>P</i> = .02) and OS (<i>P</i> = .03)	<i>P</i> = NS overall in CN AML (RFS, <i>P</i> = .72; OS, <i>P</i> = .44), but the presence of <i>IDH1</i> or <i>IDH2</i> mutations in the CN <i>NPM1</i> ⁺ / <i>FLT3</i> -ITD ⁻ group associated with poorer EFS <i>P</i> = .02 and OS <i>P</i> = .03
Abbas et al, ³⁴⁰ 2010	PCS	Mutations associated with poorer EFS (<i>P</i> = .005) and OS (<i>P</i> = .03) in patients with <i>FLT3</i> ^{wt} / <i>NPM1</i> ^{wt}	NR
Marcucci et al, ³⁵² 2010	PCS	<i>IDH1</i> mutations associated with poorer DFS (<i>P</i> = .046)	<i>IDH2</i> mutations associated with poorer CR rates (<i>P</i> = .01)
Wagner et al, ¹⁵ 2010	PCS	<i>P</i> = .49 (R132) <i>P</i> = .04 (SNP)	...
Damm et al, ¹¹⁹ 2011	PCS	<i>P</i> = NS, NR	<i>P</i> = NS, NR
Metzeler et al, ¹⁰¹ 2011	PCS
Becker et al, ³⁵⁰ 2010	PCS
Virappane et al, ¹¹⁰ 2008	PCS
Marcucci et al, ¹¹⁶ 2008	PCS
Paschka et al, ¹¹⁸ 2008	PCS
Schwind et al, ³⁰ 2010	PCS	<i>P</i> = .01	<i>P</i> = .88 (no difference between healthy and mutation expression in patients with CN-AML and miR-181a)
Hollink et al, ¹⁹⁵ 2009	PCS
Gaidzik et al, ¹⁰⁹ 2009	PCS
Damm et al, ²⁹ 2010	PCS
Becker et al, ¹²⁰ 2010	PCS
Renneville et al, ¹³⁰ 2012	PCS

Abbreviations: AML, acute myeloid leukemia; *CEBPA*, CCAAT/enhancer-binding protein α ; CN, cytogenetically normal; CR, complete response; DFS, disease-free survival; *DNMT3*, D... (cytosine-5-)-methyltransferase 3; EFS, event-free survival; *FLT3*, fms-related tyrosine kinase 3; *IDH1*, isocitrate dehydrogenase 1; *IDH2*, isocitrate dehydrogenase 2; M/A, meta-analysis; mut, mutant; ..., not available; *NPM1*, nucleophosmin (nucleolar phosphoprotein B23, numatrin); NRCT, nonrandomized clinical trial; NR, not reported; NS, not significant; OR, odds ratio; OS, overall survival; PCS, prospective cohort study; PR, partial response; RFS, relapse-free survival; *RUNX1*, runt-related transcription factor 1; SNP, single nucleotide polymorphism; *TET2*, tet methylcytosine dioxygenase 2; wt, wild-type; *WT1*, Wilms tumor 1.

supported an adverse prognostic effect from *KIT* mutations in CBF-AML.³⁶²⁻³⁶⁴ In one study of 33 patients,³⁶⁴ the few patients (n = 8; 24.2%) with t(8;21) and a *KIT* mutation had a significantly lower EFS (244 days versus 744 days); that difference was not seen in patients with NK-AML. A similar result was seen in a larger study of 110 adult patients with CBF-AML enrolled in multiple RCTs.³⁶³ In AML with inversion 16, the *KIT* mutation was associated with a greater 5-year cumulative incidence of relapse (56% versus

29%; *P* = .05) and a worse OS when adjusted for sex (*P* = .01), compared with cases without the *KIT* mutation. In AML with t(8;21), the *KIT* mutation was associated with increased 5-year cumulative incidence of relapse (70% versus 36%; *P* = .02), but there was no statistically significant difference in OS. A third, larger study³⁶² of 354 patients with CBF-AML also showed a higher incidence of relapse among the 99 patients with *KIT* mutations, but further showed that

Table 11. Extended

<i>TET2</i>	<i>RUNX1</i>	<i>WT1</i>	<i>DNMT3A</i>
OS ($P = .68$); DFS ($P = .43$); PR ($P = .27$) for <i>TET2</i> ^{wt} versus <i>TET2</i> ^{mut} in CN patients	...	OS ($P = .99$); DFS ($P = .69$); PR ($P = .8$) for <i>WT1</i> ^{wt} versus <i>WT1</i> ^{mut} in patients with normal karyotype and AML	...
...	Mutation associated with lower CR rates ($P = .01$), poorer EFS ($P = .001$), and poorer OS ($P = .01$)	$P = .61$	$P = .15$ (did not correlate with <i>RUNX1</i>)
...	...	<i>WT1</i> SNP rs16754: ≥ 1 minor allele versus homozygous for major allele associated with an increase in CR ($P = .03$), OS ($P = .01$), and RFS ($P = .01$)	...
...
...
...	...	$P = .31$...
$P < .001$...	$P = .003$ (SNP); $P = \text{NS}$, NR (mutation) $P = .01$; OR, 0.03	...
...	...	$P < .001$...
...	...	Mutations associated with inferior response ($P = .02$), RFS ($P = .01$), and OS ($P = .01$)	...
...	...	EFS ($P = .03$) (<i>CEBPA</i> ^{mut} + <i>WT1</i> ^{mut}); OS ($P = .002$) (<i>CEBPA</i> ^{mut} + <i>WT1</i> ^{mut})	...
...	...	$P < .001$...
...	...	$P = .16$ (no difference between normal and mutation expression in patients with CN-AML and miR-181a)	...
...	...	Mutations associated with poorer EFS ($P < .001$) and OS ($P = .01$)	...
...	...	RFS ($P = .4$); OS ($P = .62$)	...
...	...	CR ($P = .15$); RFS ($P = .57$); OS ($P = .24$) (<i>WT1</i> versus <i>WT1</i> ^{mut})	...
...	...	$P = .73$ (<i>WT1</i> versus <i>WT1</i> ^{mut} in patients with <i>NPM1</i> ^{mut} and <i>NPM1</i>)	...
...	Mutations associated with poorer EFS ($P = .02$) and OS ($P = .02$)

effect was only significant in multivariate analysis if it was limited to those patients with higher levels of the mutation.

The prognostic effect of *KIT* mutations in pediatric CBF-AML is more controversial. One study met inclusion criteria in our SR.³³⁹ In that retrospective study of 203 pediatric patients with CBF amassed from several different trials, 38 showed *KIT* mutations, but there was no difference in the 5-year EFS between patients with and those without *KIT* mutations, either when they were looked at as a whole or when they were divided separately into those with t(8;21) or inv(16) leukemia. In contrast, a separate pediatric study from the external review of t(8;21) AML showed 94.7% DFS for the 38 patients without *KIT* mutations compared with 37.5% for the 8 patients with the mutations ($P \leq .001$).³⁶⁵ Significant differences in 4-year OS were also observed

between patients with the *KIT* mutation (50.0%) and those without *KIT* mutation (97.4%; $P = .001$). The outcome of the *KIT*⁻ t(8;21) patients in this study was much better than the 59% 5-year EFS reported in the larger CBF study, suggesting that the prognostic significance may be different in the context of different therapies. *KIT* was not prognostic in a separate pediatric AML study reviewed in the external review, which did not look separately at patients with CBF leukemia.³⁶⁶

One study from our SR included both pediatric and adult CBF AML.²⁹⁸ For combined adult/pediatric patients with AML and t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*, this study showed no significant association of *KIT* mutation and outcome ($P = \text{not significant [NS]}$ for relapse rate [$P = .39$] or OS [$P = .58$]), although with a trend for inferior OS in

patients with the *KIT* mutation ($P = .14$). For combined adult/pediatric patients with AML and *inv(16)(p13.1q22)/t(16;16)(p13.1;q22)*; *CBFB-MYH11*, this study showed no significant association of the *KIT* mutation with relapse rate ($P = .41$) or OS ($P = .70$).²⁹⁸

Based on those findings, the EP concluded that *KIT* mutation testing should be performed in cases of confirmed CBF leukemia for further prognostication of this AML category. The evidence is strongest in adult patients, but there are data to suggest a negative prognostic effect in the pediatric population.

Public Comment Response to Statement 17.—There were 173 respondents, with 84.39% ($n = 146$) who agreed, 3.47% ($n = 6$) who disagreed, and 12.14% ($n = 21$) who wrote comments. There was strong support for the recommendation, especially for the adult population. The prognostic significance of the *KIT* mutation in the pediatric AML was felt to be more controversial, and after review of the comments, the recommendation to test in the pediatric population was changed from *should* to *may*. Some felt that this testing should be performed only in patients in whom it affects clinical management or those who are transplant candidates. The public comments were taken into consideration in the final draft of statement 17 in this article.

Statement 18.—Strong Recommendation.—For patients with suspected APL, the pathologist or treating physician should also ensure that rapid detection of *PML-RARA* is performed. The treating physician should also order appropriate coagulation studies to evaluate for disseminated intravascular coagulation (DIC).

APL is defined by the presence of *PML-RARA* rearrangement. Because APL is treated differently from other AML subtypes, rapid diagnosis of this type of AML is critical. Although no evidence from our SR informs this statement, evidence external to our SR indicates that conventional karyotyping should be performed in all patients with suspected APL, but the testing may miss rare, cryptic *PML-RARA* rearrangements.³⁶⁷ Reverse transcription PCR for *PML-RARA* can rapidly confirm a diagnosis of APL, even in patients who are leukopenic and in those with cytogenetically cryptic *PML-RARA* rearrangements.^{367–369} Interphase FISH studies using dual-fusion probes for *PML* and *RARA* can also be used to confirm the rearrangement.³⁷⁰ Immunofluorescence staining methods for PML protein can rapidly confirm the presence of *PML-RARA* rearrangement because of the differential nuclear distribution of the PML protein in APL, but that staining is not widely available and may miss variant *RARA* translocations.^{367,371,372} The body of evidence supports a strong recommendation for the use of a rapid-detection method to confirm *PML-RARA* rearrangement in APL; the determination of which of the several alternate methods to use should be made by each individual laboratory.

Patients with APL are at high risk for DIC, which can be evaluated by coagulation studies. No evidence from our SR informed this statement. Evidence outside our SR indicates that compared with other AML subtypes, APL is more often associated with DIC, has more fibrin degradation products, higher D-dimer levels, and lower fibrinogen levels.³⁷³ In patients with APL, a prolonged prothrombin time has been associated with greater risk of clinical bleeding³⁷⁴ and a high International Society of Thrombosis and Hemostasis (Carrboro, North Carolina) DIC score (based on platelet count, D-dimer level, prothrombin time, and fibrinogen level)³⁷⁵ has been associated with a greater risk of fatal bleeding

events.³⁷⁶ Based on that evidence, a strong recommendation was made to perform coagulation testing in patients with suspected APL.

Public Comment Response to Statement 18.—There were 172 respondents, of whom, 89% ($n = 154$) agreed, 1.74% ($n = 3$) disagreed, and 8.72% ($n = 14$) wrote comments, among which were a more-precise definition of *rapid* and the preferred methodology for detection of *PML-RARA* rearrangement. The comments were considered in the final draft of statement 18 in this article.

Statement 19.—Strong Recommendation.—For patients other than those with confirmed CBF-AML, APL, or AML-MRC cytogenetic abnormalities, the pathologist or treating clinician should ensure that mutational analysis for *NPM1*, *CEBPA*, and *RUNX1* is performed.

The strength of evidence was *adequate* to support this guideline statement.

This statement was supported by 2 PCSs^{100,339} that met the inclusion criteria for our SR. Both of those studies were deemed to have a low to moderate risk of bias. Neither of the studies was found to have methodological flaws that would raise concerns about their findings. Refer to Supplemental Table 18 for the quality-assessment results for the studies included for statement 19.

Acute myeloid leukemia with mutated *NPM1* defines a specific and unique category of AML under the WHO classification.¹³² Mutations in *NPM1*, a nucleocytoplasmic shuttling protein, are the most-common mutations in adult AML, occurring in 27% to 35% of cases.^{377,378} Frameshift mutations in exon 12 (chromosome band 5q35) result in an elongated protein that is retained in the cytoplasm.³⁷⁷ Those mutations are most frequent in NK-AML (45%–60%),^{377–379} and occur only rarely in association with the recurrent cytogenetic abnormalities that define CBF-AML—AML with *t(8;21)* or *inv(16)/t(16;16)*—or APL.^{377,378,380,381} This was supported by 2 studies from our SR, for both adult (Gaidzik et al¹⁰⁰ reported $P < .05$ incidence of *NPM1* mutations compared with other AML subtypes) and pediatric patients (Pollard et al³³⁹ reported 0% incidence of *NPM1* and *CEBPA* mutations). Cytogenetic abnormalities associated with *NPM1* mutations are most frequently single genetic abnormalities (ie, +8, +4, -Y, del(9q), +21)^{378,382} and are only rarely associated with a complex karyotype ($P < .001$).³⁷⁸ These mutations are less frequent in childhood AML (6.5%–8%) but occur primarily in pediatric cases with NK-AML (22%–27.1%).^{383–385}

The favorable prognostic effect of the *NPM1* mutation has been shown in multiple cohort studies and is strongest when combined with a lack of the *FLT3-ITD* mutation. In NK-AML, the *NPM1* mutation alone is associated, in some studies, with improved CR ($P < .03$) without significant effect on OS.^{378,380} However, when evaluated in context of the *FLT3* mutation, the *NPM1* mutation in the absence of the *FLT3* mutation is associated with significantly higher OS ($P < .03$),^{378–380} DFS ($P < .04$),³⁷⁸ EFS ($P = .01$),³⁸⁰ and RFS ($P < .001$)^{379,380} compared with all other *NPM1/FLT3* groups. In addition, the availability of a human leukocyte antigen-matched family donor in the *NPM1*⁺*FLT3*⁻ patient group does not affect RFS ($P = .57$) but was shown to significantly affect RFS in all other groups ($P = .001$),³⁷⁹ suggesting those patients should be excluded from transplant as first-line therapy. Studies in the pediatric population are limited by small patient numbers. In one pediatric AML study, the *NPM1* mutation in the absence of *FLT3-ITD* mutation ($n = 13$) was associated with a trend toward a favorable 5-year

EFS ($P = .51$).³⁸³ Within the *FLT3*⁻ subset, the *NPM1* mutation was shown to have similar outcome to t(8;21) and inversion 16 AML. In a second cohort study, *NPM1* mutation was associated with favorable EFS ($P = .02$ overall; $P = .01$ in NK-AML).³⁸⁵ *FLT3* did not appear to affect outcome, but analysis was limited by small numbers ($n = 10$ of 25 patients with the *NPM1* mutation). The favorable prognostic effect of the *NPM1* mutation was not altered by an aberrant, non-MDS karyotype³⁸² or by multilineage dysplasia³⁸⁶ in de novo AML. The AML with mutated *CEBPA* also defined a specific and unique category of AML under the WHO classification, but classification under that category is now restricted to cases with biallelic mutation.^{8,132} *CEBPA* mutations have also been associated with a favorable prognosis in AML. *CEBPA* belongs to the CCAAT/enhancer binding protein family of transcription factors, is expressed exclusively by myelomonocytic cells, and is upregulated in granulocyte differentiation.³⁸⁷

Mutations in *CEBPA* are reported in 10% to 15% of patients with AML, and the most frequent mutations include either N-terminal frameshift mutations or C-terminal in-frame insertions/deletions.^{388,389}

Three mutational patterns have been identified: single-mutated (involving one allele), double-mutated (typically biallelic), and homozygous *CEBPA* mutation because of a loss of heterozygosity.^{390,391} These mutations occur most frequently in NK-AML (70%) and less frequently in AML with intermediate risk (most frequently trisomy 8) or unfavorable cytogenetic abnormalities.³⁹² These mutations do not occur with favorable, recurrent cytogenetic abnormalities—t(15;17);*PML-RARA*, t(16;16) or inv(16); *CBFB-MYH11*, or t(8;21); *RUNX1-RUNX1T1*.³⁸⁹ *FLT3*-ITD and *NPM1* mutations rarely occur in combination with biallelic-mutated *CEBPA*.³⁹²

The favorable prognosis associated with *CEBPA* mutations has been shown in multiple cohort studies and is confined to biallelic-mutated *CEBPA*. In a prospective, multicenter clinical trial of 135 patients with AML, the presence of a *CEBPA* mutation (single or biallelic mutated) compared with wild-type *CEBPA* was associated with longer OS ($P = .04$), EFS ($P = .04$), and DFS ($P = .05$).³⁹³ A second prospective trial of 224 patients with AML demonstrated that the favorable prognostic significance of *CEBPA* mutation was confined to cases that were biallelic mutations.¹¹⁵ Biallelic *CEBPA* mutations were associated with improved OS ($P = .01$) and DFS ($P = .01$) compared with a single *CEBPA* mutation. There was no difference in OS or DFS in AML with a single *CEBPA* mutation when compared with wild-type *CEBPA*. These findings were further supported by a large, multicenter study, which included 2296 patients with AML enrolled in 2 large, prospective clinical trials.³⁹² Biallelic *CEBPA* mutations, but not single *CEBPA* mutations, were verified as an independent favorable prognostic factor. When compared with single-mutated and wild-type *CEBPA*, biallelic-mutated *CEBPA* was associated with improved OS ($P < .001$ and $P < .002$) and longer EFS ($P = .008$ and $P = .012$). There was no difference in outcome between NK-AML and AML with intermediate-risk cytogenetic abnormality for both biallelic *CEBPA* mutations and single *CEBPA* mutations.

One NRCT¹⁰ and one RCS¹⁰⁰ evaluated the prognostic significance of *RUNX1* mutations in AML and both found worse OS and EFS in patients with that mutation. Unlike *NPM1* and *CEBPA* mutations, mutations of *RUNX1* were relatively commonly associated with MDS-related cyto-

netic abnormalities or prior therapy, features that continue to take precedence over the *RUNX1* mutation for disease classification. Based on those studies, the EP recommends testing for *NPM1*, *CEBPA*, and *RUNX1* mutations in AML other than APL, CBF-AML, or AML with MRC cytogenetic abnormalities. Cases meeting criteria for AML-MRC based on multilineage dysplasia alone should also be tested. In addition to defining specific categories of AML under the WHO classification, mutations of *NPM1* and biallelic mutations of *CEBPA* were associated with a favorable risk. The favorable prognosis for *NPM1* mutation was confined to cases that lacked *FLT3*-ITD and, for *CEBPA*, to the group with biallelic mutations.

Public Comment Response to Statement 19.—There were 172 respondents, of whom 75.58% ($n = 130$) agreed, and 4.07% ($n = 7$) disagreed. However, 20.35% ($n = 35$) of the respondents provided a range of comments. Most of the comments referred to the necessity of evaluating *NPM1* and *CEBPA* mutations in the context of the *FLT3* mutation, which was addressed in the discussion and in statement 16. Several respondents suggested restriction of those markers for NK-AML or *FLT3*-ITD⁻ AML. Those comments were addressed in the discussion and were considered in the final draft of statement 19 for this article.

Statement 20.—No Recommendation.—For patients with confirmed AL, no recommendation is made for or against the use of global/gene-specific methylation, miRNA expression, or gene expression analysis for diagnosis or prognosis.

The strength of evidence was *insufficient* to support this guideline statement.

This statement was supported by 10 studies,⁺⁺⁺⁺⁺ comprising 2 NRCTs^{11,131} and 8 PCSs.⁺⁺⁺⁺⁺ Both of the nonrandomized studies were deemed to have a low to moderate risk of bias assessment. For the PCSs, the risk of bias assessments ranged from low^{24,30,101,193,395} to low to moderate.^{34,127,394} None of those studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 19 for the quality-assessment results of studies included for statement 20.

The interplay between mutational analysis and gene expression profiling in predicting prognosis is an area of ongoing research, especially in patients with cytogenetically normal AML. Although protein and gene-expression profiling studies for ERG, BAALC, or MECOM/EVI1 have reported prognostic significance for outcome in some studies, overall, independent, prognostic significance may not be present when mutational analysis is integrated into multivariate analyses.¹¹ Often, the prognostically significant effect of deregulated expression of a specific gene was most apparent in highly selected patient cohorts, such as patients older than 70 years with cytogenetically normal AML.¹³¹ In AML with *KMT2A (MLL)*-rearrangement, overexpression of MECOM/EVI1 is associated with an inferior prognosis ($P = .01$ for OS).³⁹⁶ In one study, a 24-gene prognostic signature independently predicted OS and EFS in AML ($P < .001$).³⁹⁷

In studies outside our SR, low global DNA methylation (as assayed by a luminometric methylation assay) was associated with favorable outcome in non-APL de novo AML, independent of karyotype risk and *NPM1*, *FLT3*, and *CEBPA* mutation status.³⁹⁸ High methylation of polycomb

+++++ References 11, 24, 30, 34, 101, 127, 131, 193, 394, 395.

+++++ References 24, 30, 34, 101, 127, 193, 394, 395.

target genes was associated with better progression-free survival and OS in cytogenetically normal AML.³⁹⁹ Within our SR, 2 studies were identified for gene-specific methylation, one finding no significant prognostic effect of BMP/retinoic acid inducible neural specific 1 (*BRINP1/DBC1*) methylation in AML³⁹⁴ and one of which found that methylation status of any of 9 specific genes adversely affected OS in ALL ($P < .05$).³⁴

In our SR, 5 studies found a significant effect of miRNA expression levels on outcome in adult AML ($P < .05$),^{24,30,127,193,395} including one in which miRNA expression patterns were correlated with expression of other prognostic markers,¹²⁷ whereas one study found no significant effect on outcome.¹⁰¹ One study found significant association of miRNA with OS in adult and pediatric ALL ($P < .05$).³⁴

Overall, many studies identified both within, and outside of, our SR indicated that deregulated gene expression, miRNA expression, and global as well as gene-specific methylation may affect outcome in AL. However, much of the data are relatively recent; moreover, those studies are not currently standard clinical laboratory tests, even in reference laboratories. Thus, no recommendation was made for, or against, those specialized tests at the time of AL diagnosis. With technological advances, it is possible that these specialized studies will become more widespread in clinical practice, similar to the current standardized assessment for mutations in key leukemia-associated genes recommended in statements 15, 16, and 19.

Refer to Supplemental Table 4 for study data on global/gene-specific methylation, miRNA expression, and gene expression analysis.

Public Comment Response to Statement 20.—There were 162 respondents, of whom, 58.64% ($n = 95$) agreed, and 11.11% ($n = 18$) who disagreed. However, 30.25% ($n = 49$) provided written comments, which indicated they thought the clinical utility of gene expression, miRNA expression, and global/gene-specific methylation studies currently have limited clinical utility and that the studies were not widely available for clinical use. Those comments were considered in the final draft of statement 20 in this article.

Statement 21.—*Strong Recommendation.*—For patients with confirmed MPAL, the pathologist or treating clinician should ensure that testing for $t(9;22)(q34.1;q11.2)$; *BCR-ABL1*, and *KMT2A (MLL)* translocations is performed.

Although one study from our SR informed this recommendation,¹⁴⁰ the guideline statement is evidence based and supported by 2 additional studies outside our SR.^{400,401}

Mixed-phenotype acute leukemia is associated with a variety of cytogenetic abnormalities, among which, are included $t(9;22)(q34.1;q11.2)$; *BCR-ABL1* and *KMT2A (MLL)* translocations.^{140,02} Those cytogenetic abnormalities are associated with distinctive features and define specific entities under the WHO classification: MPAL with $t(9;22)$; *BCR-ABL1* (Ph^+) and MPAL with $t(v;11q23.3)$; *KMT2A (MLL)* rearranged.¹³²

In studies using 2008 WHO criteria for MPAL, $t(9;22)(q34.1;q11.2)$ was identified in 15% to 20% of cases, and *KMT2A* rearrangement in 4% to 8% of cases.^{140,403}

With the advent of TKIs, it has become important to recognize the *BCR-ABL1* fusion in cases of AL. Three studies were identified that specifically addressed the question of the outcome of MPAL with $t(9;22)(q34.1;q11.2)$ (Ph^+). Overall, those patients have a poorer outcome and, in the preimatinib era, had the worst prognosis of any cytogenetic group in MPAL.¹⁴⁰ In one small (nonrandomized) series of

21 patients with adult MPAL, the poor general outcome was confirmed, and patients receiving imatinib fared better with a 1-year OS of 43% in the imatinib group compared with no survivors in the chemotherapy-alone group.⁴⁰⁰ In a different series, which compared the outcome of 42 patients with Ph^+ AL treated with imatinib-containing regimens, the 5-year OS and DFS of the 13 patients with MPAL was no different from that of the patients with Ph^+ ALL, suggesting that imatinib therapy improved the outcome of the patients with Ph^+ MPAL and should be considered the standard of care.⁴⁰¹ Similarly, *KMT2A (MLL)* rearrangement was associated with reduced survival.⁴⁰⁴

Based on those data, the EP recommends testing for $t(9;22)(q34.1;q11.2)$; *BCR-ABL1* and *KMT2A (MLL)* translocations in MPAL.

Public Comment Response to Statement 21.—There were 174 respondents, 89.66% ($n = 156$) of whom agreed, 1.15% ($n = 2$) who disagreed, and 9.2% ($n = 16$) who wrote comments. In response to the comments, language regarding the specific methodology to test for these rearrangements was removed from the final draft of statement 21 in this article.

Statement 22.—*Strong Recommendation.*—All laboratory testing performed for the initial workup and diagnosis of a patient with AL must be performed in a laboratory that is in compliance with regulatory and/or accreditation requirements.

The strength of evidence was *insufficient* to support this guideline statement, but its justification seems intuitive because laboratory testing occurs in a highly regulated environment. No evidence-based data were available from our SR.

This guideline statement was based on expert consensus opinion and codifies the importance of good laboratory practices in patient care. In the United States, clinical laboratory testing is regulated under CLIA '88, as administered by the Centers for Medicare and Medicaid Services (Baltimore, Maryland); the amendments were updated in 2003.^{405,406} Certain medical devices and laboratory tests used in an evaluation for AL have been approved and are regulated by the US Food and Drug Administration, whereas others have been developed by, and are performed within, accredited laboratories (laboratory-developed tests). It is important to ensure that laboratory-developed tests have been appropriately validated and performance characteristics established before being used in patient care. The penalties for regulatory noncompliance are significant and can include loss of laboratory director responsibilities for 2 years, monetary fines, closure of the laboratory, and inability to receive Medicare reimbursement. The EP included this guideline statement to ensure that tests performed in research laboratories (eg, in the United States, non-CLIA-approved laboratories) would not be used for patient care or be included in the medical record.

Public Comment Response to Statement 22.—There were 172 respondents, 97.67% ($n = 168$) agreed, and 2.33% ($n = 4$) who disagreed. There were 5 comments that were generally supportive, although they raised the question of an emergency situation that might arise outside of the availability of an accredited laboratory. The final draft statement in this document was modified to apply to national and international situations by not specifying individual regulatory and/or accrediting agencies. It is the laboratory director's responsibility to be aware of applicable regulations.⁴⁰⁷

Statement 23.—Strong Recommendation.—If, after examination of a PB specimen, it is determined that the patient will require immediate referral to another institution with expertise in the management of AL for treatment, the initial institution should, whenever possible, defer invasive procedures, including BM aspiration and biopsies, to the treatment center to avoid duplicate procedures, associated patient discomfort, and additional costs.

The strength of evidence was *adequate* to support this guideline statement.

This statement was supported by 28 studies,^{§§§§§§} comprising 2 meta-analyses,^{408,410} one RCT,⁴¹⁴ 2 NRCTs,^{106,112} and 23 PCSs.^{*****} For the 2 meta-analyses, both were deemed to have a low to moderate risk of bias. One trial, an RCT reported by Vance et al⁴¹⁴ was deemed to have a moderate to high risk of bias. For the 2 NRCTs,^{106,112} the trial by Moorman et al¹⁰⁶ was deemed to have a moderate risk of bias. The trial reported by Aricò et al¹¹² was deemed to have a low to moderate risk of bias. For the PCSs, 3 were deemed to have a low risk of bias,^{101,102,194} 14 were deemed to have a low to moderate risk of bias,^{††††††} and 6 were deemed to have a moderate risk of bias.^{‡‡‡‡‡‡} None of those studies were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 20 for the quality-assessment results of studies included for statement 23.

The level of expertise for the diagnosis and treatment of AL varies, with some centers having experience in virtually all case types, and some never treating such patients. In centers that do not routinely treat patients with AL and do not offer most of the testing needed to make a comprehensive diagnosis of AL, it is recommended that the patient be transferred to a treating center before the complete diagnostic evaluation is performed. This helps to reduce duplication of testing and the associated expense and discomfort that occur with such testing. It is recognized that some centers may determine that transfer of care is appropriate only after a complete diagnostic workup is completed or will need to make a rapid diagnosis to initiate therapy before transfer, such as in the diagnosis of APL with *PML-RARA*, and this guideline statement should not interfere with testing that is considered emergent before transfer.

Review of diagnostic material for the diagnosis of AL varies in the literature. At diagnosis, morphologic evaluation is often performed and interpreted at the local institution,^{§§§§§§} often, with review of slides in a central laboratory or tertiary care center if part of a clinical trial,^{25,411,415} or samples may be prepared and interpreted at a central laboratory or tertiary care center,^{112,297,340,412,413} including any immunophenotypic or other studies required for diagnosis.⁴¹⁵ A discrepancy rate of 12% was reported between local and central review of AML diagnoses.⁴¹¹ At diagnosis, flow cytometry testing may be performed at the primary institution, with results reviewed by a central laboratory or tertiary care center if part of a clinical

trial,^{14,324,415} or flow cytometry may be performed and interpreted at a central laboratory.^{297,413} At diagnosis, cytogenetic testing may be performed and karyotypes interpreted at the primary institution^{*****}; cytogenetics may be performed, but karyotype images be reviewed at a central laboratory or tertiary care center,^{††††††} or all cytogenetic testing and interpretation may be performed at a central laboratory or a tertiary care center.^{‡‡‡‡‡‡} In one study, 32% of AML and 38% of ALL karyotypes were revised or rejected as inadequate upon central review of the local karyotype images.¹⁷⁹ Fluorescence in situ hybridization studies as part of clinical trials were typically performed at a central laboratory or tertiary care center.^{106,112,413,414} Molecular studies confirming mutations and gene rearrangements as part of clinical trials were typically performed at a central laboratory or tertiary care center^{§§§§§§} as were DNA methylation studies.¹⁹⁶

If testing involves making specimens for routine or cytochemical staining or performing flow cytometry on BM at a central laboratory, the sample should be shipped overnight and processed within 24 hours of being obtained.^{112,297} For testing involving cytogenetics performed centrally, overnight shipping of a heparinized BM sample is recommended.²³ Molecular genetic testing may be performed on samples that are cryopreserved,^{194,324,411,412} with some studies recommending the use of Trizol reagent (Thermo Fisher Scientific, Waltham, Massachusetts)¹⁹⁴ and storage of samples in liquid nitrogen,^{324,412} and others recommending overnight shipping of sodium-citrate, anticoagulated samples for preparation of DNA (dry pellets stored at -80°C) or RNA (pellets in 4M guanidium isothiocyanate stored at -20°C).²³ For DNA methylation testing, BM samples should be shipped to the central laboratory in heparinized tubes for processing within 24 to 36 hours of being obtained, should be frozen immediately upon receipt (2–10 million cells), and should be stored at -70°C .¹⁹⁶

Public Comment Response for Statement 23.—There were 171 respondents, of whom, 91.81% (n = 157) agreed, and 8.19% (n = 14) disagreed. Despite the support for the initial draft of this statement, there were 32 written comments that raised several patient concerns. Most of those concerns related to delays in transfer of patients or the inability to obtain acceptance for transfer without a complete diagnosis. In addition, the need for a rapid diagnosis of APL was raised by several commenters. Based on those comments, the final draft of statement 23 in this article was altered to clarify that it applies to patients needing immediate transfer.

Refer to Supplemental Table 5 for study data informing this statement.

Statement 24.—Strong Recommendation.—If a patient is referred to another institution for treatment, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogenetic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded when they become available.

The strength of evidence was *insufficient* to support this guideline statement.

§§§§§§ References 14, 22, 23, 25, 33, 71, 101, 102, 106, 112, 115, 116, 159, 171, 179, 194, 196, 297, 324, 340, 408–415.

***** References 14, 22, 23, 25, 33, 71, 101, 102, 115, 116, 159, 171, 179, 194, 196, 297, 324, 340, 409, 411–413, 415.

†††††† References 23, 25, 71, 115, 116, 159, 171, 196, 297, 324, 340, 409, 411, 415.

‡‡‡‡‡‡ References 14, 22, 33, 179, 412, 413.

§§§§§§ References 14, 33, 102, 112, 159, 411.

***** References 22, 33, 102, 106, 115, 159.

†††††† References 14, 101, 116, 171, 194, 324, 409, 414.

‡‡‡‡‡‡ References 71, 297, 340, 408, 410, 412, 413.

§§§§§§ References 23, 25, 71, 115, 116, 194, 297, 340, 411–413.

This statement was supported by 2 PCSs^{179,411} that met the inclusion criteria for our SR. The study by Barbaric et al⁴¹¹ was deemed to have a low to moderate risk of bias, and the study reported by Mrozek et al¹⁷⁹ was deemed to have moderate risk of bias. Neither of the studies was found to have methodological flaws that would raise concerns about their findings. Refer to Supplemental Table 21 for the quality-assessment results of studies included for statement 24.

This guideline statement was based on expert consensus opinion that knowledge of test results performed at the primary institution is optimal to rapidly confirm a diagnosis of AL and to allow more cost-effective management of the patient at the referral institution. Morphologic, flow cytometric, cytogenetic, and molecular genetic studies can pose significant cost to the health care system, especially if they are repeated without knowledge of the initial results. Certain tests may not need to be repeated at the referral institution if the information from the primary institution is available for review in a timely manner, and the findings are confirmed. We found no studies that compared interinstitutional results; however, comparisons between local institution and central review in the context of clinical trials were available for morphologic and cytogenetic reviews, and, as mentioned previously, indicated a discrepancy rate of 12% between local and central review of AML diagnoses,⁴¹¹ and in one study, 32% of AML and 38% of ALL karyotypes were revised or rejected as inadequate upon central review.¹⁷⁹ These findings support the need for confirming the results of diagnostic testing at the referral institution, with repeat testing employed judiciously.

Public Comment Response to Statement 24.—There were 169 respondents, 98.22% (n = 166) of whom agreed, and 1.78% (n = 3) who disagreed. There were 9 written comments. Those comments generally dealt with logistical issues regarding communication between the primary institution and treatment center. However, as one respondent indicated, “seems simple, but in the real world it is often difficult.” In the era of shared medical records, these procedures may become easier. The comments received were considered and are reflected in the final draft of statement 24 in this article.

Statement 25.—Strong Recommendation.—In the initial report, the pathologist should include laboratory, morphologic, immunophenotypic, and, if performed, cytochemical data on which the diagnosis was based, along with a list of any pending tests. The pathologist should issue addenda/amended reports when the results of additional tests become available.

Our SR provided no data to inform this statement. However, the panel believed that the benefits of implementing the recommendation vastly outweighed the harms and thus designated this guideline statement with a strong recommendation.

Both routine and more-specialized testing results must be incorporated into initial and subsequent, integrated reports. Because morphology, cytochemical stains, FCI, and immunohistochemical stains are typically available within a day or so after the BM has been obtained, the interpretation and integration of those results should be included in the initial reports.

Specialized testing is an integral component of AL diagnosis and is required in virtually all cases of AL to provide either diagnostic or prognostic information. In

addition, specialized tests may provide evidence to validate the use of a specific type of targeted therapy for an individual patient. Based on an assessment of the morphologic and immunophenotypic features of the AL, genetic testing must be performed in a cost-effective manner using evidence based criteria. Conventional karyotyping is considered the standard of care for all cases of AL. In addition, some molecular genetic tests are also considered to be warranted in cases of AL meeting specific criteria as listed in this clinical practice guideline.

Consolidating all routine and specialized test results into an integrated consultation report is optimal for effective communication with treating physicians and patients and for optimal therapy.⁴¹⁶ Because of the time delays inherent in some molecular genetic testing, pathologists need to have mechanisms to track pending test results. Those results need to be integrated with morphologic and immunophenotypic data to enhance the original diagnosis, using current WHO classification criteria, as well as to provide prognostic information. Either an addendum format or, more optimally, an integrated, interpretive format is optimal.⁴¹⁶

Public Comment Response to Statement 25.—There were 171 respondents, 94.15% (n = 161) of whom agreed, and 5.85% (n = 10) who disagreed. Despite the overwhelming support for this statement, several of the comments recognized the difficulty of getting all the data into the electronic medical record. Those comments were taken into account for the final statement in this article.

Statement 26.—Strong Recommendation.—The pathologist and treating clinician should coordinate and ensure that all tests performed for classification, management, predicting prognosis, and disease monitoring are entered into the patient’s medical records.

Note.—This information should include the sample source, adequacy, and collection information, as applicable.

Our SR provided no data related to this key question or statement. The EP, however, strongly recommended that all critical information related to the diagnosis and prognosis of a patient with AL be available in the medical record. Ideally, this would be summarized in a single consolidated report.⁴¹⁶ However, it is recognized that, in some settings, not all information needed for pathologists to generate such a report might be available because of some testing being sent directly to other laboratories by the treating physician. Therefore, all this information should be either interfaced with, or scanned into, the medical record, so that all the material needed for the determination of a comprehensive diagnosis is present in the patient’s record. It is recognized that improved and more-integrated pathology information systems that directly interface with the electronic medical record are needed to allow for optimal patient care.

Public Comment Response for Statement 26.—There were 169 respondents, 99.41% (n = 168) of whom agreed, and 0.59% (n = 1) of whom disagreed. There were 8 written comments, all of which endorsed this concept.

Statement 27.—Strong Recommendation.—Treating physicians and pathologists should use the current WHO terminology for the final diagnosis and classification of AL.

The strength of evidence was *convincing* to support this guideline statement.

This guideline statement was supported by 40 PCSs obtained in our SR.***** The risk of bias-assessment scores were low,^{12,101} low to moderate,⁺⁺⁺⁺⁺⁺ and moderate.⁺⁺⁺⁺⁺⁺ Overall, none of the studies providing the evidence base for statement 27 were found to have methodological flaws that would raise concerns about the studies' findings. Refer to Supplemental Table 22 for the quality-assessment results for the studies included for statement 27.

A review of the studies published in recent years shows that various classification systems have been used for the diagnosis and subclassification of AL. In AML, the classification systems used included the FAB system,^{ssssssss} the WHO classification (2001 version),^{417,425} and the WHO classification (2008 version),^{*****} or more than one classification system because of lengthy study periods.^{13,71,159,341,423} For studies that did not include a classification system,^{31,127,129,419,421} cytogenetic-defining groups were variably used. For acute B-ALL, most studies⁺⁺⁺⁺⁺⁺ used the 2008 WHO classification system or a cytogenetic classification system similar to the 2008 WHO system. The National Cancer Institute (Frederick, Maryland) risk classification¹⁶⁷ of ALL was used in conjunction with the cytogenetic risk classification in some studies.^{19,20,295,330} The FAB system alone was mostly used to describe morphology.¹²⁴ A few studies used immunophenotypic classification only^{17,296,426} or no classification system at all.^{171,327} The T-ALL classification was mainly based on immunophenotyping alone, including a system proposed by the European Group for the Immunological Characterization of Leukemias (Nancy, France).⁺⁺⁺⁺⁺⁺

A uniform categorization of AL is essential to facilitate understanding between health care workers and to provide a framework for clinical practice, data comparison, epidemiologic studies, and new genetic and molecular investigations. The WHO classification of neoplasms of the hematopoietic and lymphoid tissues, published in 2001,⁴²⁵ and updated in 2008,⁵ with a new revision recently summarized,⁸ represents a worldwide consensus on the diagnosis of hematopoietic tumors. That classification derives from numerous published clinical and scientific studies and is the result of collaboration and consensus among pathologists, cytogeneticists, and treating physicians. The WHO classification attempts to incorporate the disease characteristics that have been proven to have clinical and biologic relevance into a useful working nomenclature. A retrospective study of 5848 adult patients with AML showed that the FAB morphologic subclassification did not provide prognostic information if the specific genetic and morphologic WHO categories and WHO provisional entities, such as "AML with mutated *NPM1*" and "AML with mutated *CEBPA*," were included.⁴²⁸ The applicability of the WHO classification in pediatric AML, on the other hand, may

require additional molecular genetic data for further disease delineation.⁴²⁹ Nevertheless, since the first edition in 2001, the WHO classification system has been adopted for numerous studies, and its clinical practicality and reproducibility has been demonstrated in diverse international settings. Treating physicians and pathologists should use the most current WHO terminology for the diagnosis and classification of AL, including adoption of the current revision.

Public Comment Response to Statement 27.—There were 167 respondents, 98.8% (n = 165) of whom agreed, and 1.2% (n = 2) who disagreed. There were 11 written comments. Some commented that the WHO classification of AL requires cytogenetic and molecular data, which are often not available at the time of diagnosis or before the initiation of treatment. The other concern was that the WHO classification system was not always up to date. Since the last updates in 2008, numerous molecular genetic discoveries have been published, some of which have been shown to have a significant effect on the treatment and prognosis of AL, and more discoveries will certainly be described after the 2016 classification. Therefore, the final report should incorporate those new data for the purposes of therapy and prognosis. New technologies in molecular genetic discovery are evolving quickly and provide new insights in disease biology, thereby refining disease classifications and guiding clinical practice. The panel acknowledged those comments, which are reflected in the final statement in this article.

Refer to Table 12 for study data that informed this guideline statement.

CONCLUSIONS

The 27 statements that comprise the ASH/CAP guideline for the initial diagnostic workup of AL address the 6 key questions initially proposed:

1. What clinical and laboratory information should be available? (Statements 1 and 2.)
2. What samples and specimen types should be evaluated? (Statements 3, 4, 7, 8, and 11.)
3. What tests are required for all patients during the initial evaluation? (Statements 3, 5, 6, 9, and 12.)
4. What tests are required for only a subset of patients? (Statements 10, 14, 13, 16, 17, 18, 19, 20, and 21.)
5. Where should laboratory testing be performed? (Statements 22, 23, and 24.)
6. How should the results be reported? (Statements 25, 26, and 27.)

As noted at the beginning of this article, the initial workup and evaluation of AL has become increasingly complex during the past decade, due, in part, to the availability of new laboratory techniques—particularly genetic studies—that have resulted in better characterization of AL and in classification schemes with improved clinical and scientific relevance. However, not only is the diagnosis and classification of AL important but also of importance is the identification of prognostic factors, antigens, or genetic abnormalities that may be targets for specific therapy, and markers that can be used to follow the response to therapy and monitor residual disease. In addition, the workup must be performed quickly, efficiently, and at a reasonable cost. When all these factors are considered, along with the realization that the recent revision of the 4th edition of the WHO classification of AL has nearly 50 distinct subtypes of

***** References 12–14, 17, 19–21, 31, 36, 71, 100, 101, 122, 124, 127–129, 158, 159, 171, 294–296, 324, 327, 330, 333, 339, 341, 350, 386, 415, 417–424

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++++++ References 14, 129, 294, 327, 386, 418, 420, 424.

ssssssss References 3, 14, 100, 101, 294, 350.

***** References 5, 12, 122, 339, 386, 424.

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***** References 20, 21, 324, 333, 415, 427.

Table 12. Study Data on Classification Scheme

Source, y	Study Design	Classification Scheme Used for the Final Diagnosis
Metzeler et al, ¹⁰¹ 2011	PCS	FAB
Kayser et al, ³⁴¹ 2009	PCS	WHO 2001, WHO 2008, FAB
Meshinchi et al, ⁴²¹ 2008	PCS	Pediatric AML
Marks et al, ²¹ 2009	PCS	NR, but immunophenotype was used for diagnosis
Taskesen et al, ¹² 2011	PCS	WHO 2008
Gaidzik et al, ¹⁰⁰ 2011	PCS	FAB
Clappier et al, ³²⁴ 2010	PCS	EGIL
Kühnl et al, ¹⁷ 2010	PCS	EGIL
Pollard et al, ³³⁹ 2010	PCS	CBF + AML
Santamaria et al, ⁴¹⁷ 2008	PCS	WHO 2001
de Jonge et al, ⁴¹⁹ 2010	PCS	WHO 2008
Santamaria et al, ³¹ 2010	PCS	NR, but AML, excluding APL, was used for diagnosis
Csinady et al, ⁴²⁰ 2009	PCS	NR, but B-precursor ALL was used for diagnosis
Falini et al, ³⁸⁶ 2010	PCS	WHO 2008
Heesch et al, ³³³ 2010	PCS	T-cell subclassification by early, thymic, or mature
Jiao et al, ¹²² 2009	PCS	FAB and WHO 2008
Moorman et al, ¹²⁴ 2007	PCS	NR
Ongaro et al, ⁴²² 2009	PCS	WHO 2008
Zachariadis et al, ¹²⁸ 2011	PCS	NR, but risk stratification used cytogenetic categorization
Kuiper et al, ³²⁷ 2010	PCS	NR, partially incorporated cytogenetic categorization
Kayser et al, ⁷¹ 2011	PCS	WHO 2001, FAB
Röllig et al, ¹⁴ 2010	PCS	FAB and ECOG
Harrison et al, ¹⁵⁸ 2010	PCS	FAB
Buccisano et al, ²⁹⁴ 2010	PCS	FAB, cytogenetic risk stratification
Becker et al, ³⁵⁰ 2010	PCS	FAB
Patel et al, ²⁹⁶ 2010	PCS	NR, but immunophenotype was used for diagnosis
Schwind et al, ¹²⁷ 2010	PCS	NR, but adult AML was used for diagnosis
Lugthart et al, ¹³ 2010	PCS	FAB
Maloney et al, ²⁹⁵ 2010	PCS	NR, but for Down syndrome, precursor B-ALL was used for diagnosis
Salzer et al, ¹⁹ 2010	PCS	NR, but immunophenotype was used for diagnosis
Pui et al, ²⁰ 2010	PCS	NR, but immunophenotype was used for diagnosis
Cario et al, ³³⁰ 2010	PCS	NR, but pediatric B-ALL was used for diagnosis (ie, NCI risk and cytogenetic risk were used)
Wheatley et al, ¹²⁹ 2009	PCS	NR, but AML was used for diagnosis of elderly patients (ie, implying FAB)
Grimwade et al, ¹⁵⁹ 2010	PCS	WHO 2001, FAB
Busse et al, ⁴¹⁸ 2009	PCS	NR, but adult B-ALL was used for diagnosis
Suela et al, ⁴²³ 2007	PCS	WHO 2001
Moorman et al, ¹⁷¹ 2007	PCS	NR
Gönen et al, ⁴²⁴ 2012	PCS	WHO 2008
Moorman et al, ³⁶ 2012	PCS	NR, but precursor ALL and mature B-ALL were used for diagnosis
Patel et al, ⁴¹⁵ 2012	PCS	NR, but T-ALL was used for diagnosis

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; B-ALL, B-cell precursor acute lymphoblastic leukemia; CBF, core-binding factor; ECOG, Eastern Cooperative Oncology Group classification; EGIL, European Group for the Immunological Characterization of Leukemias; FAB, French–American–British classification; PCS, prospective cohort study; NCI, National Cancer Institute classification; NR, not reported; T-ALL, T cell precursor acute lymphoblastic leukemia; WHO, World Health Organization classification.

AL,⁸ the value of the guidelines described in this article and their applicability for the workup of AL can be readily appreciated.

Although many pathologists and clinicians will consider the most crucial guidelines to be those indicated that relate to specific tests necessary to make an accurate diagnosis and to identify prognostic factors, the guidelines also highlight recommendations for general and logistic considerations that may be neglected in the urgency of the initial workup of AL. For example, statement 1 emphasizes the value of the patient’s history, particularly of any predisposing conditions or syndromes, previous therapies, and any family history of leukemia or other neoplasms. This latter recommendation is important in view of the inclusion in the revised WHO classification of the provisional entity *myeloid neoplasms with germline predisposition*, which is likely more common than currently recognized. In addition, recommendations are made for preservation of cells and tissue from the initial diagnostic specimen for any future studies that may be relevant for prognosis or therapy (statement 7). For patients who are transferred from one institution to another, the

guidelines recommend the avoidance of duplicate testing and invasive procedures whenever possible (statement 23), and the transfer of all test results and tests in progress, along with the diagnostic specimens, to the receiving institution (statement 24). The guidelines also indicate the central role of the pathologist in issuing the diagnostic reports, and for updating those reports as additional data are accumulated (statements 25 and 26). Thus, the recommendations are comprehensive in providing guidelines from the time a patient suspected of having AL is first encountered until the final diagnostic reports are generated.

The ASH and the CAP have cooperated in developing this guideline. That joint effort underscores the cooperation that is necessary between the treating clinician and the pathologist in the workup, diagnosis, and care of patients presenting with a suspected diagnosis of AL.

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APPENDIX. Conflicts of Interest of the Expert Panel^a

Name	Interest/Activity Type	Institution	
Daniel A. Arber, MD	Consultancies	Celgene, Summit, New Jersey Gerson Lehrman Group, New York, New York Glenview Capital, New York, New York United States Diagnostic Standards, Rockville, Maryland	
	Board or advisory board	Clariant, Muttentz, Switzerland DAVA Oncology, Dallas, Texas Bristol-Myers Squibb, New York, New York Agiros Pharmaceuticals, Cambridge, Massachusetts Celgene, Summit, New Jersey Novartis, Deerfield, Illinois	
	Lecture fees paid by entity (honoraria)	AMP, Bethesda, Maryland California Society of Pathologists, Sacramento CAP, Northfield, Illinois Emory University, Atlanta, Georgia Minnesota Society of Pathologists, Minneapolis, Minnesota South Bay Pathology Society, San Jose, California USCAP, Palm Springs, California University of Calgary, Calgary, Alberta, Canada Montefiore Medical Center, Bronx, New York Brigham and Women's Hospital, Boston, Massachusetts Cleveland Clinic, Cleveland, Ohio Tutorial in Neoplastic Hematopathology, Miami, Florida (2017) University of Chicago, Chicago, Illinois ASCP, Chicago, Illinois The France Foundation, Old Lyme, Connecticut ISLH, Glenview, Illinois Memorial Sloan Kettering Cancer Center, New York, New York Medical University of South Carolina, Charleston University of Texas Southwestern Medical Center, Dallas Children's Hospital of Philadelphia, Philadelphia, Pennsylvania Georgia Regents University Medical Center, Augusta AAOMP, Wheaton, Illinois	
	Expert witness	Baumgartner Nelson & Wagner PLLC, Vancouver, Washington Dade County, Florida/Fowler White Burnett, Miami, Florida The Markham Group, Spokane, Washington The Berkowitz Law Firm LLC, Stamford, Connecticut Malkmus Law Firm LLC, Springfield, Missouri Gary D. McCallister & Associates LLC, Chicago, Illinois Christie Pabarue Mortensen & Young, Philadelphia, Pennsylvania	
	Royalties	ASCP Press, Chicago, Illinois Elsevier, Atlanta, Georgia Lippincott Williams & Wilkins (now Wolters Kluwer), Riverwoods, Illinois	
	Leadership in other associations	Society for Hematopathology, Chicago, Illinois USCAP, Palm Springs, California	
	Michael J. Borowitz, MD, PhD	Consultancies	HTG Molecular Diagnostics, Inc, Tucson, Arizona
		Board or advisory board	BeaconLBS/LabCorp, Montvale, New Jersey
		Leadership in other associations	Children's Oncology Group, Philadelphia, Pennsylvania International Clinical Cytometry Society, Glenview, Illinois US FDA, Silver Spring, Maryland
		Lecture fees paid by entity (honoraria)	Alexion Pharmaceuticals, New Haven, Connecticut
		Grants	Amgen, Thousand Oaks, California Beckman Coulter, Brea, California Becton Dickinson Biosciences, Franklin Lakes, New Jersey Bristol-Myers Squibb, New York, New York Genzyme, Cambridge, Massachusetts MedImmune, Gaithersburg, Maryland Micromet, Inc, Rockville, Maryland Children's Oncology Group, Philadelphia, Pennsylvania
	Elected or appointed positions in other national/international medical organizations		
	Melissa Cessna, MD	Grants	Intermountain Research and Medical Foundation, Salt Lake City, Utah

APPENDIX. Continued

Name	Interest/Activity Type	Institution
Joan Etzell, MD	Lecture Fees Paid By Entity (honoraria)	AACC, Washington, DC ASCP, Chicago, Illinois ASH, Washington, DC CAP, Northfield, Illinois The France Foundation, Old Lyme, Connecticut
Kathryn Foucar, MD	Grants Consultancies Lecture fees paid by entity (honoraria)	Abbott Laboratories, Abbott Park, Illinois Celgene, Summit, New Jersey Scientific Symposium (Institute for Healthcare Improvement), Cambridge, Massachusetts Tutorial on Neoplastic Hematopathology, Miami, Florida (2017) California Society of Pathologists Educational Symposia, San Francisco, California (2017)
	Royalties	ARP, Rockville, Maryland Amirsys (Elsevier), Salt Lake City, Utah ASCP Press, Chicago, Illinois
Robert P. Hasserjian, MD	Consultancies	Alliance Oncology, Newport Beach, California Amgen, Thousand Oaks, California Cancer and Leukemia Group B, Chicago, Illinois Genzyme, Cambridge, Massachusetts Incyte, Wilmington, Delaware Infinity Pharmaceuticals, Cambridge, Massachusetts Promedior, Lexington, Massachusetts Sanofi, Paris, France
	Lecture fees paid by entity (honoraria)	Colorado Society of Clinical Pathologists, Franktown University of San Francisco, San Francisco, California USCAP, Palm Springs, California
	Leadership in other associations	Society for Hematopathology, Chicago, Illinois USCAP, Palm Springs, California
J. Douglas Rizzo, MD	Grants	HRSA, Rockville, Maryland NIH/NCI, Bethesda, Maryland NIH/NHLBI, Bethesda, Maryland
	Elected or appointed positions in other national/international medical organizations	ASH, Washington, DC ASBMT, Arlington Heights, Illinois MEDCAC, Baltimore, Maryland
R. Bryan Rumble, MSc	Consultancies	AMP, Bethesda, Maryland ASH, Washington, DC ASCO, Alexandria, Virginia ASCP, Chicago, Illinois
Karl Theil, MD, PhD	Vendor Vendor Elected or appointed positions in other national/international medical organizations	CAP, Northfield, Illinois CAP, Northfield, Illinois CLSI, Wayne, Pennsylvania ASCP, Chicago, Illinois
Nicole E. Thomas, MPH, CT(ASCP) ^{cm}	Grants/cooperative agreements	CDC, Atlanta, Georgia
James W. Vardiman, MD	Consultancies Board or advisory board Royalties	Celgene, Summit, New Jersey <i>Leukemia Research</i> Editorial Board, Elsevier, Chennai, India Elsevier, Atlanta, Georgia ASCP, Chicago, Illinois
Sa A. Wang, MD	Consultancies Lecture fees paid by entity (honoraria)	Genzyme, Cambridge, Massachusetts ASCP, Chicago, Illinois
	Board or advisory board	Seattle Genetics, Bothell, Washington
	Grants	Seattle Genetics, Bothell, Washington Cancer Incite LLC, San Antonio, Texas GlaxoSmithKline Inc, Brentford, Middlesex, United Kingdom
	Royalties	Amirsys, Salt Lake City, Utah

Abbreviations: AACC, American Association for Clinical Chemistry; AAOMP, American Association of Oral & Maxillofacial Pathology; ASCP, American Society for Clinical Pathology; AMP, Association for Molecular Pathology; ARP, American Registry of Pathology; ASBMT, American Society for Blood and Marrow Transplantation; ASCO, American Society of Clinical Oncology; ASH, American Society of Hematology; CAP, College of American Pathologists; CDC, Centers for Disease Control and Prevention; CLSI, Clinical & Laboratory Standards Institute; HRSA, Health Resources and Services Administration; ISLH, International Society for Laboratory Hematology; MEDCAC, Medicare Evidence Development & Coverage Advisory Committee; NCI, National Cancer Institute; NIH, National Institutes of Health; NHLBI, National Heart, Lung, and Blood Institute; USCAP, United States and Canadian Academy of Pathology; US FDA, United States Food and Drug Administration.

^a Anthony T. Smith, MLS has no reported conflicts of interest to disclose.