

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, nondecalcified 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; <i>P</i> < .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; <i>P</i> = .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; <i>P</i> = .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; <i>P</i> = .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; <i>P</i> < .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; <i>P</i> < .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; <i>P</i> = .08; OS at 10 y: <1 y, 70 ± 13.6, 1–9 y, 89.3 ± 2.6, >10 y (73.5 ± 5.5; <i>P</i> = .004) |
| Scrideli et al, ²² 2009 | PCS | ... | 5-y EFS for 1–9 y, mean (SD) 80.2% (3.6) versus 70.5% (6.1) in >9 y; <i>P</i> = .02; N = 168 |
| 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, 3.09, 95% CI, 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); $P = NS$, 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); CBFβ-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

+++++ References 13, 17, 19–21, 31, 36, 71, 100, 122, 124, 127, 128, 158, 159, 171, 295, 296, 324, 330, 333, 339, 341, 350, 415, 417, 419, 421–423.

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

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

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

+++++ References 19, 20, 124, 128, 295, 330, 422.

***** 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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References

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias: French-American-British (FAB) co-operative group. *Br J Haematol.* 1976;33(4):451–458.
2. Bennett JM, Catovsky D, Daniel MT, et al. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-MO). *Br J Haematol.* 1991;78(3):325–329.
3. Bennett JM, Catovsky D, Daniel MT, et al. Criteria for the diagnosis of acute leukemia of megakaryocyte lineage (M7): a report of the French-American-British Cooperative Group. *Ann Intern Med.* 1985;103(3):460–462.
4. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001. *World Health Organization Classification of Tumours*.
5. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood.* 2009;114(5):937–951.
6. Abdel-Wahab O, Levine RL. Mutations in epigenetic modifiers in the pathogenesis and therapy of acute myeloid leukemia. *Blood.* 2013;121(18):3563–3572.
7. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia [published correction appears in *N Engl J Med.* 2013;369(1):98]. *N Engl J Med.* 2013;368(22):2059–2074.
8. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391–2405.
9. Graham R, Mancher M, Wolman DM, Greenfield S, Steinberg E, eds; Institute of Medicine; Board on Health Care Services; Committee on Standards for Developing Trustworthy Clinical Practice Guidelines. *Clinical Practice Guidelines We Can Trust*. Washington, DC: National Academies Press; 2011.
10. Mendler JH, Maharry K, Radmacher MD, et al. *RUNX1* mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and MicroRNA expression signatures. *J Clin Oncol.* 2012;30(25):3109–3118.
11. Damm F, Wagner K, Gorlich K, et al. ID1 expression associates with other molecular markers and is not an independent prognostic factor in cytogenetically normal acute myeloid leukaemia. *Br J Haematol.* 2012;158(2):208–215.
12. Taskesen E, Bullinger L, Corbacioglu A, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with *CEBPA* mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for *CEBPA* double mutant AML as a distinctive disease entity. *Blood.* 2011;117(8):2469–2475.
13. Lughart S, Groschel S, Beverloo HB, et al. Clinical, molecular, and prognostic significance of WHO type inv(3)(q21q26.2)/t(3;3)(q21;q26.2) and various other 3q abnormalities in acute myeloid leukemia. *J Clin Oncol.* 2010;28(24):3890–3898.
14. Röllig C, Thiede C, Gramatzki M, et al; Study Alliance Leukemia. A novel prognostic model in elderly patients with acute myeloid leukemia: results of 909 patients entered into the prospective AML96 trial. *Blood.* 2010;116(6):971–978.
15. Wagner K, Damm F, Gohring G, et al. Impact of *IDH1* R132 mutations and an *IDH1* single nucleotide polymorphism in cytogenetically normal acute myeloid leukemia: SNP rs11554137 is an adverse prognostic factor. *J Clin Oncol.* 2010;28(14):2356–2364.
16. Groschel S, Lughart S, Schlenk RF, et al. High *EV11* expression predicts outcome in younger adult patients with acute myeloid leukemia and is associated with distinct cytogenetic abnormalities. *J Clin Oncol.* 2010;28(12):2101–2107.
17. Kühnl A, Gokbuget N, Stroux A, et al. High BAALC expression predicts chemoresistance in adult B-precursor acute lymphoblastic leukemia. *Blood.* 2010;115(18):3737–3744.
18. Escherich G, Horstmann MA, Zimmermann M, Janka-Schaub GE; COALL Study Group. Cooperative study group for childhood acute lymphoblastic leukaemia (COALL): long-term results of trials 82,85,89,92, and 97. *Leukemia.* 2010;24(2):298–308.
19. Salzer WL, Devidas M, Carroll WL, et al. Long-term results of the pediatric oncology group studies for childhood acute lymphoblastic leukemia 1984–2001: a report from the children's oncology group. *Leukemia.* 2010;24(2):355–370.
20. Pui CH, Pei D, Sandlund JT, et al. Long-term results of St Jude total therapy studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. *Leukemia.* 2010;24(2):371–382.
21. Marks DI, Paietta EM, Moorman AV, et al. T-cell acute lymphoblastic leukemia in adults: clinical features, immunophenotype, cytogenetics, and outcome from the large randomized prospective trial (UKALL XII/ECOG 2993). *Blood.* 2009;114(25):5136–5145.
22. Scrideli CA, Assumpção JG, Ganazza MA, et al. A simplified minimal residual disease polymerase chain reaction method at early treatment points can stratify children with acute lymphoblastic leukemia into good and poor outcome groups. *Haematologica.* 2009;94(6):781–789.
23. Lo-Coco F, Cuneo A, Pane F, et al; Acute Leukemia Working Party of the GIMEMA group. Prognostic impact of genetic characterization in the GIMEMA LAM99P multicenter study for newly diagnosed acute myeloid leukemia. *Haematologica.* 2008;93(7):1017–1024.
24. Langer C, Radmacher MD, Ruppert AS, et al; Cancer and Leukemia Group B (CALGB). High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: a Cancer and Leukemia Group B (CALGB) study. *Blood.* 2008;111(11):5371–5379.
25. Wandt H, Schäkel U, Kroschinsky F, et al. MLD according to the WHO classification in AML has no correlation with age and no independent prognostic relevance as analyzed in 1766 patients. *Blood.* 2008;111(4):1855–1861.
26. Gale RE, Green C, Allen C, et al; Medical Research Council Adult Leukaemia Working Party. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with *NPM1* mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood.* 2008;111(5):2776–2784.
27. Roman-Gomez J, Cordeu L, Agirre X, et al. Epigenetic regulation of Wnt-signaling pathway in acute lymphoblastic leukemia [retracted in: *Blood.* 2012;120(17):3625]. *Blood.* 2007;109(8):3462–3469.
28. Dufour A, Schneider F, Metzeler KH, et al. Acute myeloid leukemia with biallelic *CEBPA* gene mutations and normal karyotype represents a distinct genetic entity associated with a favorable clinical outcome. *J Clin Oncol.* 2010;28(4):570–577.
29. Damm F, Heuser M, Morgan M, et al. Single nucleotide polymorphism in the mutational hotspot of *WT1* predicts a favorable outcome in patients with cytogenetically normal acute myeloid leukemia. *J Clin Oncol.* 2010;28(4):578–585.
30. Schwind S, Maharry K, Radmacher MD, et al. Prognostic significance of expression of a single microRNA, miR-181a, in cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol.* 2010;28(36):5257–5264.
31. Santamaria C, Chillón MC, Garcia-Sanz R, et al. BAALC is an important predictor of refractoriness to chemotherapy and poor survival in intermediate-risk acute myeloid leukemia (AML). *Ann Hematol.* 2010;89(5):453–458.
32. Seifert H, Mohr B, Thiede C, et al; Study Alliance Leukemia (SAL). The prognostic impact of 17p (p53) deletion in 2272 adults with acute myeloid leukemia. *Leukemia.* 2009;23(4):656–663.
33. Medeiros BC, Othman M, Fang M, Roulston D, Appelbaum FR. Prognostic impact of monosomal karyotype in young adult and elderly acute myeloid leukemia: the Southwest Oncology Group (SWOG) experience. *Blood.* 2010;116(13):2224–2228.
34. Roman-Gomez J, Agirre X, Jiménez-Velasco A, et al. Epigenetic regulation of microRNAs in acute lymphoblastic leukemia. *J Clin Oncol.* 2009;27(8):1316–1322.
35. Tauchi H, Tomizawa D, Eguchi M, et al. Clinical features and outcome of *MLL* gene rearranged acute lymphoblastic leukemia in infants with additional chromosomal abnormalities other than 11q23 translocation. *Leuk Res.* 2008;32(10):1523–1529.
36. Moorman AV, Schwab C, Ensor HM, et al. IGH@ translocations, *CRLF2* deregulation, and microdeletions in adolescents and adults with acute lymphoblastic leukemia. *J Clin Oncol.* 2012;30(25):3100–3108.
37. Schneider F, Hoster E, Unterhalt M, et al. The FLT3ITD mRNA level has a high prognostic impact in *NPM1* mutated, but not in *NPM1* unmutated, AML with a normal karyotype. *Blood.* 2012;119(19):4383–4386.
38. Alford KA, Reinhardt K, Garnett C, et al; International Myeloid Leukemia–Down Syndrome Study Group. Analysis of *GATA1* mutations in Down syndrome transient myeloproliferative disorder and myeloid leukemia. *Blood.* 2011;118(8):2222–2238.
39. Bruwier A, Chantrain CF. Hematological disorders and leukemia in children with Down syndrome. *Eur J Pediatr.* 2012;171(9):1301–1307.
40. Gamis AS, Alonzo TA, Gerbing RB, et al. Natural history of transient myeloproliferative disorder clinically diagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. *Blood.* 2011;118(26):6752–6759.
41. Godley LA. Inherited predisposition to acute myeloid leukemia. *Semin Hematol.* 2014;51(4):306–321.
42. Chessells JM, Richards SM, Bailey CC, Lilleyman JS, Eden OB. Gender and treatment outcome in childhood lymphoblastic leukaemia: report from the MRC UKALL trials. *Br J Haematol.* 1995;89(2):364–372.
43. Rowe JM. Prognostic factors in adult acute lymphoblastic leukaemia. *Br J Haematol.* 2010;150(4):389–405.
44. Jiang A, Jiang H, Brandwein J, Kamel-Reid S, Chang H. Prognostic factors in normal karyotype acute myeloid leukemia in the absence of the *FLT3*-ITD mutation. *Leuk Res.* 2011;35(4):492–498.
45. Farag SS, Archer KJ, Mrózek K, et al; Cancer and Leukemia Group B 8461. Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. *Blood.* 2006;108(1):63–73.
46. Pabst T, Eysel M, Haefliger S, Schardt J, Mueller BU. Somatic *CEBPA* mutations are a frequent second event in families with germline *CEBPA* mutations and familial acute myeloid leukemia. *J Clin Oncol.* 2008;26(31):5088–5093.

47. Renneville A, Mialou V, Philippe N, et al. Another pedigree with familial acute myeloid leukemia and germline *CEBPA* mutation. *Leukemia*. 2009;23(4):804–806.
48. Buijs A, Poddighe P, van Wijk R, et al. A novel *CBFA2* single-nucleotide mutation in familial platelet disorder with propensity to develop myeloid malignancies. *Blood*. 2001;98(9):2856–2858.
49. Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited *RUNX1* mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood*. 2008;112(12):4639–4645.
50. Shinawi M, Erez A, Shardy DL, et al. Syndromic thrombocytopenia and predisposition to acute myelogenous leukemia caused by constitutional microdeletions on chromosome 21q. *Blood*. 2008;112(4):1042–1047.
51. Song WJ, Sullivan MG, Legare RD, et al. Haploinsufficiency of *CBFA2* causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet*. 1999;23(2):166–175.
52. Bluteau D, Balduini A, Balayn N, et al. Thrombocytopenia-associated mutations in the *ANKRD26* regulatory region induce MAPK hyperactivation. *J Clin Invest*. 2014;124(2):580–591.
53. Noris P, Perrotta S, Seri M, et al. Mutations in *ANKRD26* are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. *Blood*. 2011;117(24):6673–6680.
54. Hahn CN, Chong CE, Carmichael CL, et al. Heritable *GATA2* mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet*. 2011;43(10):1012–1017.
55. Ishida H, Imai K, Honma K, et al. *GATA-2* anomaly and clinical phenotype of a sporadic case of lymphedema, dendritic cell, monocyte, B- and NK-cell (DCML) deficiency, and myelodysplasia. *Eur J Pediatr*. 2012;171(8):1273–1276.
56. Rodrigues NP, Janzen V, Forkert R, et al. Haploinsufficiency of *GATA-2* perturbs adult hematopoietic stem-cell homeostasis. *Blood*. 2005;106(2):477–484.
57. Tsai FY, Keller G, Kuo FC, et al. An early haematopoietic defect in mice lacking the transcription factor *GATA-2*. *Nature*. 1994;371(6494):221–226.
58. West RR, Hsu AP, Holland SM, Cuellar-Rodriguez J, Hickstein DD. Acquired *ASXL1* mutations are common in patients with inherited *GATA2* mutations and correlate with myeloid transformation. *Haematologica*. 2014;99(2):276–281.
59. Alter BP, Giri N, Savage SA, et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. *Br J Haematol*. 2010;150(2):179–188.
60. Kirwan M, Vulliamy T, Marrone A, et al. Defining the pathogenic role of telomerase mutations in myelodysplastic syndrome and acute myeloid leukemia. *Hum Mutat*. 2009;30(11):1567–1573.
61. Churpek JE, Lorenz R, Nedumgottil S, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. *Leuk Lymphoma*. 2013;54(1):28–35.
62. Seif AE. Pediatric leukemia predisposition syndromes: clues to understanding leukemogenesis. *Cancer Genet*. 2011;204(5):227–244.
63. Cadman EC, Capiizzi RL, Bertino JR. Acute nonlymphocytic leukemia: a delayed complication of Hodgkin's disease therapy: analysis of 109 cases. *Cancer*. 1977;40(3):1280–1296.
64. Kaldor JM, Day NE, Band P, et al. Second malignancies following testicular cancer, ovarian cancer and Hodgkin's disease: an international collaborative study among cancer registries. *Int J Cancer*. 1987;39(5):571–585.
65. Le Beau MM, Albain KS, Larson RA, et al. Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: further evidence for characteristic abnormalities of chromosomes no. 5 and 7. *J Clin Oncol*. 1986;4(3):325–345.
66. Rosner F, Grünwald H. Hodgkin's disease and acute leukemia: report of eight cases and review of the literature. *Am J Med*. 1975;58(3):339–353.
67. Smith SM, Le Beau MM, Huo D, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood*. 2003;102(1):43–52.
68. Larson RA. Cytogenetics, not just previous therapy, determines the course of therapy-related myeloid neoplasms. *J Clin Oncol*. 2012;30(19):2300–2302.
69. Andersen MK, Christiansen DH, Jensen BA, Ernst P, Hauge G, Pedersen-Bjergaard J. Therapy-related acute lymphoblastic leukaemia with *MLL* rearrangements following DNA topoisomerase II inhibitors, an increasing problem: report on two new cases and review of the literature since 1992. *Br J Haematol*. 2001;114(3):539–543.
70. Ganzel C, Devlin S, Douer D, Rowe JM, Stein EM, Tallman MS. Secondary acute lymphoblastic leukaemia is constitutional and probably not related to prior therapy. *Br J Haematol*. 2015;170(1):50–55.
71. Kayser S, Döhner K, Krauter J, et al; German-Austrian AMLSG. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. *Blood*. 2011;117(7):2137–2145.
72. Pedersen-Bjergaard J, Philip P. Two different classes of therapy-related and de-novo acute myeloid leukemia? *Cancer Genet Cytogenet*. 1991;55(1):119–124.
73. Qian Z, Joslin JM, Tennant TR, et al. Cytogenetic and genetic pathways in therapy-related acute myeloid leukemia. *Chem Biol Interact*. 2010;184(1–2):50–57.
74. Allan JM, Wild CP, Rollinson S, et al. Polymorphism in glutathione S-transferase P1 is associated with susceptibility to chemotherapy-induced leukemia [published correction appears in *Proc Natl Acad Sci U S A*. 2001;98(26):15394]. *Proc Natl Acad Sci U S A*. 2001;98(20):11592–11597.
75. Bolufer P, Collado M, Barragan E, et al. Profile of polymorphisms of drug-metabolising enzymes and the risk of therapy-related leukaemia. *Br J Haematol*. 2007;136(4):590–596.
76. Churpek JE, Marquez R, Neistadt B, et al. Inherited mutations in cancer susceptibility genes are common among survivors of breast cancer who develop therapy-related leukemia. *Cancer*. 2016;122(2):304–311.
77. Bizzozero OJ Jr, Johnson KG, Ciocco A, Kawasaki S, Toyoda S. Radiation-related leukemia in Hiroshima and Nagasaki 1946–1964. II. *Ann Intern Med*. 1967;66(3):522–530.
78. Ivanov VK, Tsyb AF, Khait SE, et al. Leukemia incidence in the Russian cohort of Chernobyl emergency workers. *Radiat Environ Biophys*. 2012;51(2):143–149.
79. Levine EG, Bloomfield CD. Leukemias and myelodysplastic syndromes secondary to drug, radiation, and environmental exposure. *Semin Oncol*. 1992;19(1):47–84.
80. Pearce MS, Salotti JA, Little MP, et al. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. *Lancet*. 2012;380(9840):499–505.
81. Darrington DL, Vose JM, Anderson JR, et al. Incidence and characterization of secondary myelodysplastic syndrome and acute myelogenous leukemia following high-dose chemoradiotherapy and autologous stem-cell transplantation for lymphoid malignancies. *J Clin Oncol*. 1994;12(12):2527–2534.
82. Miller JS, Arthur DC, Litz CE, Neglia JP, Miller WJ, Weisdorf DJ. Myelodysplastic syndrome after autologous bone marrow transplantation: an additional late complication of curative cancer therapy. *Blood*. 1994;83(12):3780–3786.
83. Stone RM, Neuberger D, Soiffer R, et al. Myelodysplastic syndrome as a late complication following autologous bone marrow transplantation for non-Hodgkin's lymphoma. *J Clin Oncol*. 1994;12(12):2535–2542.
84. Austin H, Delzell E, Cole P. Benzene and leukemia: a review of the literature and a risk assessment. *Am J Epidemiol*. 1988;127(3):419–439.
85. Khalade A, Jaakkola MS, Pukkala E, Jaakkola JJ. Exposure to benzene at work and the risk of leukemia: a systematic review and meta-analysis. *Environ Health*. 2010;9:31. doi: 10.1186/1476-069X-9-31.
86. Hauptmann M, Stewart PA, Lubin JH, et al. Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. *J Natl Cancer Inst*. 2009;101(24):1696–1708.
87. Bachand AM, Mundt KA, Mundt DJ, Montgomery RR. Epidemiological studies of formaldehyde exposure and risk of leukemia and nasopharyngeal cancer: a meta-analysis. *Crit Rev Toxicol*. 2010;40(2):85–100.
88. Meyerson HJ, Farhi DC, Rosenthal NS. Transient increase in blasts mimicking acute leukemia and progressing myelodysplasia in patients receiving growth factor. *Am J Clin Pathol*. 1998;109(6):675–681.
89. Aitelli C, Wasson L, Page R. Pernicious anemia: presentations mimicking acute leukemia. *South Med J*. 2004;97(3):295–297.
90. Dokal IS, Cox TM, Galton DA. Vitamin B-12 and folate deficiency presenting as leukaemia [published correction appears in *BMJ*. 1990;300(6736):1394]. *BMJ*. 1990;300(6734):1263–1264.
91. Schmiegelow K, Forestier E, Hellebostad M, et al; Nordic Society of Paediatric Haematology and Oncology. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia [published correction appears in *Leukemia*. 2010;24(3):670]. *Leukemia*. 2010;24(2):345–354.
92. Larson RA. Acute lymphoblastic leukemia. In: Lichtman MA, Kaushansky K, Prchal JT, Levi MM, Burns LJ, Armitage JO, eds. *Williams Hematology*. 9th ed. New York, NY: McGraw-Hill; 2016:1505–1526.
93. Onishi Y, Matsuno Y, Tateishi U, et al. Two entities of precursor T-cell lymphoblastic leukemia/lymphoma based on radiologic and immunophenotypic findings. *Int J Hematol*. 2004;80(1):43–51.
94. Kulkarni KP, Marwaha RK. Significance of mediastinal adenopathy in childhood acute lymphoblastic leukemia: prognostic impact and association with clinicodemographic factors. *Pediatr Hematol Oncol*. 2012;29(6):562–564.
95. Bakst RL, Tallman MS, Douer D, Yahalom J. How I treat extramedullary acute myeloid leukemia. *Blood*. 2011;118(14):3785–3793.
96. Cho-Vega JH, Medeiros LJ, Prieto VG, Vega F. Leukemia cutis. *Am J Clin Pathol*. 2008;129(1):130–142.
97. Lee WJ, Moon HR, Won CH, et al. Precursor B- or T-lymphoblastic lymphoma presenting with cutaneous involvement: a series of 13 cases including 7 cases of cutaneous T-lymphoblastic lymphoma. *J Am Acad Dermatol*. 2014;70(2):318–325.
98. Nabhan C, Tolentino A, Meyer A, Tallman MS. Cutaneous involvement with B-lineage acute lymphoblastic leukemia. *Leuk Lymphoma*. 2012;53(5):987–989.
99. Liesveld JL, Lichtman MA. Acute myelogenous leukemia. In: Lichtman MA, Kaushansky K, Prchal JT, Levi MM, Burns LJ, Armitage JO, eds. *Williams Hematology*. 9th ed. New York, NY: McGraw-Hill; 2016:1373–1436.
100. Gaidzik VI, Bullinger L, Schlenk RF, et al. *RUNX1* mutations in acute myeloid leukemia: results from a comprehensive genetic and clinical analysis from the AML study group. *J Clin Oncol*. 2011;29(10):1364–1372.
101. Metzeler KH, Maharry K, Radmacher MD, et al. *TET2* mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2011;29(10):1373–1381.

102. Montesinos P, Rayón C, Vellenga E, et al; PETHEMA; HOVOG Groups. Clinical significance of CD56 expression in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline-based regimens. *Blood*. 2011;117(6):1799–1805.

103. Stölzel F, Pfirrmann M, Aulitzky WE, et al; Study Alliance Leukemia. Risk stratification using a new prognostic score for patients with secondary acute myeloid leukemia: results of the prospective AML96 trial. *Leukemia*. 2011;25(3):420–428.

104. Tallman MS, Kim HT, Montesinos P, et al. Does microgranular variant morphology of acute promyelocytic leukemia independently predict a less favorable outcome compared with classical M3 APL? A joint study of the North American Intergroup and the PETHEMA Group. *Blood*. 2010;116(25):5650–5659.

105. Ho PA, Zeng R, Alonzo TA, et al. Prevalence and prognostic implications of WT1 mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. *Blood*. 2010;116(5):702–710.

106. Moorman AV, Ensor HM, Richards SM, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. *Lancet Oncol*. 2010;11(5):429–438.

107. Metzeler KH, Dufour A, Benthaus T, et al. ERG expression is an independent prognostic factor and allows refined risk stratification in cytogenetically normal acute myeloid leukemia: a comprehensive analysis of ERG, MN1, and BAALC transcript levels using oligonucleotide microarrays. *J Clin Oncol*. 2009;27(30):5031–5038.

108. Karman K, Forestier E, Heyman M, et al; Nordic Society of Pediatric Hematology, Oncology (NOPHO); Swedish Cytogenetic Leukemia Study Group (SCLSG); NOPHO Leukemia Cytogenetic Study Group (NLCSG). Clinical and cytogenetic features of a population-based consecutive series of 285 pediatric T-cell acute lymphoblastic leukemias: rare T-cell receptor gene rearrangements are associated with poor outcome. *Genes Chromosomes Cancer*. 2009;48(9):795–805.

109. Gaidzik VI, Schlenk RF, Moschny S, et al; German-Austrian AML Study Group. Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. *Blood*. 2009;113(19):4505–4511.

110. Virappane P, Gale R, Hills R, et al. Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. *J Clin Oncol*. 2008;26(33):5429–5435.

111. Oudot C, Auclerc MF, Levy V, et al. Prognostic factors for leukemic induction failure in children with acute lymphoblastic leukemia and outcome after salvage therapy: the FRALLE 93 study. *J Clin Oncol*. 2008;26(9):1496–1503.

112. Aricò M, Valsecchi MG, Rizzari C, et al. Long-term results of the AIEOP-ALL-95 Trial for Childhood Acute Lymphoblastic Leukemia: insight on the prognostic value of DNA index in the framework of Berlin-Frankfurt-Muenster based chemotherapy. *J Clin Oncol*. 2008;26(2):283–289.

113. Lange BJ, Smith FO, Feusner J, et al. Outcomes in CCG-2961, a Children's Oncology Group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children's oncology group. *Blood*. 2008;111(3):1044–1053.

114. Yanada M, Matsushita T, Asou N, et al. Severe hemorrhagic complications during remission induction therapy for acute promyelocytic leukemia: incidence, risk factors, and influence on outcome. *Eur J Haematol*. 2007;78(3):213–219.

115. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with *CEBPA* mutations; only *CEBPA* double mutations, but not single *CEBPA* mutations are associated with favourable prognosis. *Br J Cancer*. 2009;100(8):1343–1346.

116. Marcucci G, Maharry K, Radmacher MD, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, *CEBPA* mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study [published correction appears in *J Clin Oncol*. 2008;26(36):6021]. *J Clin Oncol*. 2008;26(31):5078–5087.

117. Schneider F, Hoster E, Unterhalt M, et al. *NPM1* but not *FLT3*-ITD mutations predict early blast cell clearance and CR rate in patients with normal karyotype AML (NK-AML) or high-risk myelodysplastic syndrome (MDS). *Blood*. 2009;113(21):5250–5253.

118. Paschka P, Marcucci G, Ruppert AS, et al. Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. *J Clin Oncol*. 2008;26(28):4595–4602.

119. Damm F, Heuser M, Morgan M, et al. Integrative prognostic risk score in acute myeloid leukemia with normal karyotype. *Blood*. 2011;117(17):4561–4568.

120. Becker H, Marcucci G, Maharry K, et al. Favorable prognostic impact of *NPM1* mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2010;28(4):596–604.

121. Rubnitz JE, Razzouk BI, Lensing S, Pounds S, Pui C-H, Ribeiro RC. Prognostic factors and outcome of recurrence in childhood acute myeloid leukemia. *Cancer*. 2007;109(1):157–163.

122. Jiao B, Wu CF, Liang Y, et al. *AML1-ETO9a* is correlated with *C-KIT* overexpression/mutations and indicates poor disease outcome in t(8;21) acute myeloid leukemia-M2. *Leukemia*. 2009;23(9):1598–1604.

123. Johnston DL, Alonzo TA, Gerbing RB, Lange BJ, Woods WG. The presence of central nervous system disease at diagnosis in pediatric acute

myeloid leukemia does not affect survival: a Children's Oncology Group study. *Pediatr Blood Cancer*. 2010;55(3):414–420.

124. Moorman AV, Richards SM, Robinson HM, et al; UK Medical Research Council (MRC)/National Cancer Research Institute (NCRI) Childhood Leukaemia Working Party (CLWP). Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). *Blood*. 2007;109(6):2327–2330.

125. Prébet T, Boissel N, Reutenauer S, et al; Acute Leukemia French Association; Groupe Ouest-Est des Leucémies et Autres Maladies du Sang (GOELAMS); Core Binding Factor Acute Myeloid Leukemia (CBF AML) intergroup. Acute myeloid leukemia with translocation (8;21) or inversion (16) in elderly patients treated with conventional chemotherapy: a collaborative study of the French CBF-AML intergroup. *J Clin Oncol*. 2009;27(28):4747–4753.

126. Santamaria CM, Chillón MC, Garcia-Sanz R, et al. High *FOXO3a* expression is associated with a poorer prognosis in AML with normal cytogenetics. *Leuk Res*. 2009;33(12):1706–1709.

127. Schwind S, Marcucci G, Maharry K, et al. *BAALC* and *ERG* expression levels are associated with outcome and distinct gene and microRNA expression profiles in older patients with de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Blood*. 2010;116(25):5660–5669.

128. Zachariadis V, Gauffin F, Kuchinskaya E, et al. The frequency and prognostic impact of dic(9;20)(p13.2;q11.2) in childhood B-cell precursor acute lymphoblastic leukemia: results from the NOPHO ALL-2000 trial. *Leukemia*. 2011;25(4):622–628.

129. Wheatley K, Brookes CL, Howman AJ, et al; Nordic Society of Pediatric Hematology, Oncology (NOPHO); Swedish Cytogenetic Leukemia Study Group (SCLSG). Prognostic factor analysis of the survival of elderly patients with AML in the MRC AML11 and LRF AML14 trials. *Br J Haematol*. 2009;145(5):598–605.

130. Renneville A, Boissel N, Nibourel O, et al. Prognostic significance of DNA methyltransferase 3A mutations in cytogenetically normal acute myeloid leukemia: a study by the Acute Leukemia French Association. *Leukemia*. 2012;26(6):1247–1254.

131. Schwind S, Marcucci G, Kohlschmidt J, et al. Low expression of MN1 associates with better treatment response in older patients with de novo cytogenetically normal acute myeloid leukemia. *Blood*. 2011;118(15):4188–4198.

132. Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008. *World Health Organization Classification of Tumours*; vol 2.

133. Lee SH, Erber WN, Porwit A, Tomonaga M, Peterson LC; International Council for Standardization in Hematology. ICSH guidelines for the standardization of bone marrow specimens and reports. *Int J Lab Hematol*. 2008;30(5):349–364.

134. Lynch DW, Stauffer SL, Rosenthal NS. Adequacy of powered vs manual bone marrow biopsy specimens: a retrospective review of sequential marrow aspirates and biopsies in 68 patients. *Am J Clin Pathol*. 2015;143(4):535–539.

135. Odejide OO, Cronin AM, DeAngelo DJ, et al. Improving the quality of bone marrow assessment: impact of operator techniques and use of a specimen preparation checklist. *Cancer*. 2013;119(19):3472–3478.

136. Peterson LC, Agosti SJ, Hoyer JD, et al; Hematology Clinical Microscopy Resource Committee; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with hematopoietic neoplasms of the bone marrow: a basis for checklists. *Arch Pathol Lab Med*. 2002;126(9):1050–1056.

137. Wilkins BS. Pitfalls in bone marrow pathology: avoiding errors in bone marrow trephine biopsy diagnosis. *J Clin Pathol*. 2011;64(5):380–386.

138. Bennett JM, Orazi A. Diagnostic criteria to distinguish hypocellular acute myeloid leukemia from hypocellular myelodysplastic syndromes and aplastic anemia: recommendations for a standardized approach. *Haematologica*. 2009;94(2):264–268.

139. Riley RS, Williams D, Ross M, et al. Bone marrow aspirate and biopsy: a pathologist's perspective, II: interpretation of the bone marrow aspirate and biopsy. *J Clin Lab Anal*. 2009;23(5):259–307.

140. Matutes E, Pickl WF, Van't Veer M, et al. Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. *Blood*. 2011;117(11):3163–3171.

141. van den Anker W, Westers TM, de Leeuw DC, et al. A threshold of 10% for myeloperoxidase by flow cytometry is valid to classify acute leukemia of ambiguous and myeloid origin. *Cytometry B Clin Cytom*. 2013;84(2):114–118.

142. Horna P, Zhang L, Sotomayor EM, Lancet JE, Moscinski LC. Diagnostic immunophenotype of acute promyelocytic leukemia before and early during therapy with all-trans retinoic acid. *Am J Clin Pathol*. 2014;142(4):546–552.

143. Paietta E, Goloubeva O, Neuberg D, et al; Eastern Cooperative Oncology Group. A surrogate marker profile for PML/RAR alpha expressing acute promyelocytic leukemia and the association of immunophenotypic markers with morphologic and molecular subtypes. *Cytometry B Clin Cytom*. 2004;59(1):1–9.

144. Zhou Y, Jorgensen JL, Wang SA, et al. Usefulness of CD11a and CD18 in flow cytometric immunophenotypic analysis for diagnosis of acute promyelocytic leukemia. *Am J Clin Pathol*. 2012;138(5):744–750.

145. Liu W, Hasserjian RP, Hu Y, et al. Pure erythroid leukemia: a reassessment of the entity using the 2008 World Health Organization classification. *Mod Pathol*. 2011;24(3):375–383.

146. Oki Y, Kantarjian HM, Zhou X, et al. Adult acute megakaryocytic leukemia: an analysis of 37 patients treated at M.D. Anderson Cancer Center. *Blood*. 2006;107(3):880–884.
147. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol*. 2009;10(2):147–156.
148. Thomas DA, O'Brien S, Faderl S, et al. Chemoimmunotherapy with a modified hyper-CVAD and rituximab regimen improves outcome in de novo Philadelphia chromosome-negative precursor B-lineage acute lymphoblastic leukemia. *J Clin Oncol*. 2010;28(24):3880–3889.
149. van Dongen JJ, Lhermitte L, Böttcher S, et al; EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708). EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia*. 2012;26(9):1908–1975.
150. Johansson U, Bloxham D, Couzens S, et al; British Committee for Standards in Haematology. Guidelines on the use of multicolour flow cytometry in the diagnosis of haematological neoplasms. British Committee for Standards in Haematology. *Br J Haematol*. 2014;165(4):455–488.
151. Davis BH, Holden JT, Bene MC, et al. 2006 Bethesda International Consensus recommendations on the flow cytometric immunophenotypic analysis of hematolymphoid neoplasia: medical indications. *Cytometry B Clin Cytom*. 2007;72(suppl 1):S5–13.
152. Wood BL, Arroz M, Barnett D, et al. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. *Cytometry B Clin Cytom*. 2007;72(suppl 1):S14–22.
153. Rowley JD. The role of cytogenetics in hematology [editorial review]. *Blood*. 1976;48(1):1–7.
154. Rowley JD, Vignon C, Gollin SM, Rosenberg CL, Wyandt HE, Milunsky A. Chromosomal translocations in secondary acute myeloid leukemia. *N Engl J Med*. 1996;334(9):601–603.
155. Harrison CJ. The detection and significance of chromosomal abnormalities in childhood acute lymphoblastic leukaemia. *Blood Rev*. 2001;15(1):49–59.
156. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. *Blood Rev*. 2004;18(2):115–136.
157. Mrózek K, Harper DP, Apland PD. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. *Hematol Oncol Clin North Am*. 2009;23(5):991–1010.
158. Harrison CJ, Hills RK, Moorman AV, et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. *J Clin Oncol*. 2010;28(16):2674–2681.
159. Grimwade D, Hills RK, Moorman AV, et al; National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116(3):354–365.
160. Byrd JC, Mrózek K, Dodge RK, et al; Cancer and Leukemia Group B (CALGB 8461). Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100(13):4325–4336.
161. Frohling S, Schlenk RF, Kayser S, et al. Cytogenetics and age are major determinants of outcome in intensively treated acute myeloid leukemia patients older than 60 years: results from AMLSG trial AML HD98-B. *Blood*. 2006;108(10):3280–3288.
162. Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial: the Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood*. 1998;92(7):2322–2333.
163. Haferlach T, Kern W, Schoch C, et al; German AML Cooperative Group. A new prognostic score for patients with acute myeloid leukemia based on cytogenetics and early blast clearance in trials of the German AML Cooperative Group. *Haematologica*. 2004;89(4):408–418.
164. Lazarevic V, Hörstedt AS, Johansson B, et al. Incidence and prognostic significance of karyotypic subgroups in older patients with acute myeloid leukemia: the Swedish population-based experience. *Blood Cancer J*. 2014;4:e188. doi: 10.1038/bcj.2014.10.
165. Mrózek K, Marcucci G, Nicolet D, et al. Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. *J Clin Oncol*. 2012;30(36):4515–4523.
166. Slovak ML, Kopecky KJ, Cassileth PA, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood*. 2000;96(13):4075–4083.
167. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. *J Clin Oncol*. 1996;14(1):18–24.
168. Armand P, Kim HT, Zhang MJ, et al. Classifying cytogenetics in patients with acute myelogenous leukemia in complete remission undergoing allogeneic transplantation: a Center for International Blood and Marrow Transplant Research study. *Biol Blood Marrow Transplant*. 2012;18(2):280–288.
169. Pullarkat V, Slovak ML, Kopecky KJ, Forman SJ, Appelbaum FR. Impact of cytogenetics on the outcome of adult acute lymphoblastic leukemia: results of Southwest Oncology Group 9400 study. *Blood*. 2008;111(5):2563–2572.
170. Schultz KR, Pullen DJ, Sather HN, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). *Blood*. 2007;109(3):926–935.
171. Moorman AV, Harrison CJ, Buck GA, et al; Adult Leukaemia Working Party, Medical Research Council/National Cancer Research Institute. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. *Blood*. 2007;109(8):3189–3197.
172. Mancini M, Scappaticci D, Cimino G, et al. A comprehensive genetic classification of adult acute lymphoblastic leukemia (ALL): analysis of the GIMEMA 0496 protocol. *Blood*. 2005;105(9):3434–3441.
173. Wetzler M, Dodge RK, Mrózek K, et al. Prospective karyotype analysis in adult acute lymphoblastic leukemia: the cancer and leukemia Group B experience. *Blood*. 1999;93(11):3983–3993.
174. Betz BL, Hess JL. Acute myeloid leukemia diagnosis in the 21st century. *Arch Pathol Lab Med*. 2010;134(10):1427–1433.
175. Kolitz JE, George SL, Dodge RK, et al; Cancer and Leukemia Group B. Dose escalation studies of cytarabine, daunorubicin, and etoposide with and without multidrug resistance modulation with PSC-833 in untreated adults with acute myeloid leukemia younger than 60 years: final induction results of Cancer and Leukemia Group B Study 9621. *J Clin Oncol*. 2004;22(21):4290–4301.
176. Rowe JM, Buck G, Burnett AK, et al; MRC/NCRI Adult Leukemia Working Party. Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. *Blood*. 2005;106(12):3760–3767.
177. Schlenk RF, Benner A, Hartmann F, et al; AML Study Group Ulm (AMLSG ULM). Risk-adapted postremission therapy in acute myeloid leukemia: results of the German multicenter AML HD93 treatment trial. *Leukemia*. 2003;17(8):1521–1528.
178. Petersdorf SH, Rankin C, Head DR, et al. Phase II evaluation of an intensified induction therapy with standard daunorubicin and cytarabine followed by high dose cytarabine for adults with previously untreated acute myeloid leukemia: a Southwest Oncology Group study (SWOG-9500). *Am J Hematol*. 2007;82(12):1056–1062.
179. Mrózek K, Carroll AJ, Maharry K, et al. Central review of cytogenetics is necessary for cooperative group correlative and clinical studies of adult acute leukemia: the Cancer and Leukemia Group B experience. *Int J Oncol*. 2008;33(2):239–244.
180. Walker A, Mrózek K, Kohlschmidt J, et al; Alliance for Clinical Trials in Oncology. New recurrent balanced translocations in acute myeloid leukemia and myelodysplastic syndromes: cancer and leukemia group B 8461. *Genes Chromosomes Cancer*. 2013;52(4):385–401.
181. National Comprehensive Cancer Network. NCCN guidelines for treatment of cancer by site: acute lymphoblastic leukemia. http://www.nccn.org/professionals/physician_gls/f_guidelines_nojava.asp#site. Accessed October 10, 2016.
182. Milligan DW, Grimwade D, Cullis JO, et al; British Committee for Standards in Haematology. Guidelines on the management of acute myeloid leukaemia in adults. *Br J Haematol*. 2006;135(4):450–474.
183. Avet-Loiseau H. Fish analysis at diagnosis in acute lymphoblastic leukemia. *Leuk Lymphoma*. 1999;33(5–6):441–449.
184. Fröhling S, Kayser S, Mayer C, et al; AML Study Group Ulm. Diagnostic value of fluorescence in situ hybridization for the detection of genomic aberrations in older patients with acute myeloid leukemia. *Haematologica*. 2005;90(2):194–199.
185. Kwon WK, Lee JY, Mun YC, Seong CM, Chung WS, Huh J. Clinical utility of FISH analysis in addition to G-banded karyotype in hematologic malignancies and proposal of a practical approach. *Korean J Hematol*. 2010;45(3):171–176.
186. Robinson HM, Harrison CJ, Moorman AV, Chudoba I, Strefford JC. Intrachromosomal amplification of chromosome 21 (*iAMP21*) may arise from a breakage-fusion-bridge cycle. *Genes Chromosomes Cancer*. 2007;46(4):318–326.
187. Harrison CJ. The management of patients with leukaemia: the role of cytogenetics in this molecular era. *Br J Haematol*. 2000;108(1):19–30.
188. He R, Wiktor AE, Hanson CA, et al. Conventional karyotyping and fluorescence in situ hybridization: an effective utilization strategy in diagnostic adult acute myeloid leukemia. *Am J Clin Pathol*. 2015;143(6):873–878.
189. Haferlach C, Rieder H, Lillington DM, et al. Proposals for standardized protocols for cytogenetic analyses of acute leukemias, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myeloproliferative disorders, and myelodysplastic syndromes. *Genes Chromosomes Cancer*. 2007;46(5):494–499.
190. Foucar K, Anastasi J. Acute myeloid leukemia with recurrent cytogenetic abnormalities. *Am J Clin Pathol*. 2015;144(1):6–18.
191. Tallman MS, Altman JK. How I treat acute promyelocytic leukemia. *Blood*. 2009;114(25):5126–5135.
192. Vardiman JW, Brunning RD, Arber DA, Le Beau MM, Porwit A, Tefferi A. Introduction and overview of the classification of the myeloid neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, eds. *WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues*. 4th ed.

Lyon, France: IARC Press; 2008:18–30. *World Health Organization Classification of Tumours*; vol 2.

193. Marcucci G, Radmacher MD, Maharry K, et al. MicroRNA expression in cytogenetically normal acute myeloid leukemia. *N Engl J Med*. 2008;358(18):1919–1928.

194. Whitman SP, Ruppert AS, Radmacher MD, et al. *FLT3* D835/I836 mutations are associated with poor disease-free survival and a distinct gene-expression signature among younger adults with de novo cytogenetically normal acute myeloid leukemia lacking *FLT3* internal tandem duplications. *Blood*. 2008;111(3):1552–1559.

195. Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, et al. Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. *Blood*. 2009;113(23):5951–5960.

196. Milani L, Lundmark A, Kivilainen A, et al. DNA methylation for subtype classification and prediction of treatment outcome in patients with childhood acute lymphoblastic leukemia. *Blood*. 2010;115(6):1214–1225.

197. Baust JM, Corwin WL, VanBuskirk R, Baust JG. Biobanking: the future of cell preservation strategies. *Adv Exp Med Biol*. 2015;864:37–53.

198. Barbaric D, Dalla-Pozza L, Byrne JA. A reliable method for total RNA extraction from frozen human bone marrow samples taken at diagnosis of acute leukaemia. *J Clin Pathol*. 2002;55(11):865–867.

199. Jrgensen H, Hokland P, Petersen EL, Hokland M. DNA preparation from cryopreserved bone marrow cell samples. *Anal Biochem*. 1995;225(2):346–348.

200. Payton JE, Grieselhuber NR, Chang LW, et al. High throughput digital quantification of mRNA abundance in primary human acute myeloid leukemia samples. *J Clin Invest*. 2009;119(6):1714–1726.

201. Bosga-Bouwer AG, Hendriks D, Vellenga E, Zorgdrager H, van den Berg E. Cytogenetic analysis of cryopreserved bone marrow cells. *Cancer Genet Cytogenet*. 2001;124(2):165–168.

202. McConnell TS, Cordova LM, Baczek NA, Foucar K, Dewald GW. Chromosome analysis of cryopreserved cells. *Cancer Genet Cytogenet*. 1990;45(2):179–191.

203. Abruzzese E, Radford JE, Miller JS, et al. Detection of abnormal pretransplant clones in progenitor cells of patients who developed myelodysplasia after autologous transplantation. *Blood*. 1999;94(5):1814–1819.

204. Min HJ, Lee J, Choi JE, Shin S, Lee DS. Fluorescence in situ hybridization (FISH) using snap frozen buffy coat. *Ann Clin Lab Sci*. 2007;37(1):85–88.

205. Campos L, Guyotat D, Larese A, et al. Expression of immunological markers on leukemic cells before and after cryopreservation and thawing. *Cryobiology*. 1988;25(1):18–22.

206. Donmez A, Yilmaz F, Soyer N, Cagiran S, Arik B, Tombuloglu M. The loss of CD34⁺ cells in peripheral hematopoietic stem cell products cryopreserved by non-controlled rate freezing and stored at –80 degrees C after overnight storage. *Transfus Apher Sci*. 2014;51(2):188–192.

207. Flores AI, McKenna DH, Montalbán MA, De la Cruz J, Wagner JE, Bornstein R. Consistency of the initial cell acquisition procedure is critical to the standardization of CD34⁺ cell enumeration by flow cytometry: results of a pairwise analysis of umbilical cord blood units and cryopreserved aliquots. *Transfusion*. 2009;49(4):636–647.

208. Hensley TR, Easter AB, Gerds SE, et al. Enumeration of major peripheral blood leukocyte populations for multicenter clinical trials using a whole blood phenotyping assay. *J Vis Exp*. 2012;(67):e4302. doi: 10.3791/4302.

209. Lanza F, Moretti S, Castagnari B, et al. Assessment of distribution of CD34 epitope classes in fresh and cryopreserved peripheral blood progenitor cells and acute myeloid leukemic blasts. *Hematologica*. 1999;84(11):969–977.

210. Nemes E, Kagina BM, Smit E, et al. Differential leukocyte counting and immunophenotyping in cryopreserved ex vivo whole blood. *Cytometry A*. 2015;87(2):157–165.

211. Ojeda-Urbe M, Sovalat H, Bourderont D, et al. Peripheral blood and BM CD34⁺ CD38⁻ cells show better resistance to cryopreservation than CD34⁺ CD38⁺ cells in autologous stem cell transplantation. *Cytotherapy*. 2004;6(6):571–583.

212. Pinto LA, Trivett MT, Wallace D, et al. Fixation and cryopreservation of whole blood and isolated mononuclear cells: influence of different procedures on lymphocyte subset analysis by flow cytometry. *Cytometry B Clin Cytom*. 2005;63(1):47–55.

213. Rasmussen SM, Bilgrau AE, Schmitz A, et al. Stable phenotype of B-cell subsets following cryopreservation and thawing of normal human lymphocytes stored in a tissue biobank. *Cytometry B Clin Cytom*. 2015;88(1):40–49.

214. Rosillo MC, Ortuno F, Rivera J, Moraleda JM, Vicente V. Cryopreservation modifies flow-cytometric analysis of hemopoietic cells. *Vox Sang*. 1995;68(4):210–214.

215. Sattui S, de la Flor C, Sanchez C, et al. Cryopreservation modulates the detection of regulatory T cell markers. *Cytometry B Clin Cytom*. 2012;82(1):54–58.

216. Alsayed H, Owaidah T, Al Rawas F. Validation of a modified cryopreservation method for leukemic blasts for flow cytometry assessment. *Hematol Oncol Stem Cell Ther*. 2008;1(2):94–97.

217. Carrick DM, Mehaffey MG, Sachs MC, et al. Robustness of next generation sequencing on older formalin-fixed paraffin-embedded tissue. *PLoS One*. 2015;10(7):e0127353. doi: 10.1371/journal.pone.0127353.

218. Lin J, Kennedy SH, Svarovsky T, et al. High-quality genomic DNA extraction from formalin-fixed and paraffin-embedded samples deparaffinized using mineral oil. *Anal Biochem*. 2009;395(2):265–267.

219. Pikor LA, Enfield KS, Cameron H, Lam WL. DNA extraction from paraffin embedded material for genetic and epigenetic analyses. *J Vis Exp*. 2011;(49):2763. doi: 10.3791/2763.

220. Potluri K, Mahas A, Kent MN, Naik S, Markey M. Genomic DNA extraction methods using formalin-fixed paraffin-embedded tissue. *Anal Biochem*. 2015;486:17–23.

221. Prince ME, Ubell ML, Castro J, et al. Tissue-preserving approach to extracting DNA from paraffin-embedded specimens using tissue microarray technology. *Head Neck*. 2007;29(5):465–471.

222. Hedegaard J, Thorsen K, Lund MK, et al. Next-generation sequencing of RNA and DNA isolated from paired fresh-frozen and formalin-fixed paraffin-embedded samples of human cancer and normal tissue. *PLoS One*. 2014;9(5):e98187. doi: 10.1371/journal.pone.0098187.

223. Kashofer K, Viertler C, Pichler M, Zatloukal K. Quality control of RNA preservation and extraction from paraffin-embedded tissue: implications for RT-PCR and microarray analysis. *PLoS One*. 2013;8(7):e70714. doi: 10.1371/journal.pone.0070714.

224. Li J, Smyth P, Flavin R, et al. Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells. *BMC Biotechnol*. 2007;7:36. doi: 10.1186/1472-6750-7-36.

225. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol*. 2002;161(6):1961–1971.

226. Arzt L, Kothmaier H, Quehenberger F, et al. Evaluation of formalin-free tissue fixation for RNA and microRNA studies. *Exp Mol Pathol*. 2011;91(2):490–495.

227. Zhang X, Chen J, Radcliffe T, Lebrun DP, Tron VA, Feilolter H. An array-based analysis of microRNA expression comparing matched frozen and formalin-fixed paraffin-embedded human tissue samples. *J Mol Diagn*. 2008;10(6):513–519.

228. Alkan S, Lehman C, Sarago C, Sidawy MK, Karcher DS, Garrett CT. Polymerase chain reaction detection of immunoglobulin gene rearrangement and bcl-2 translocation in archival glass slides of cytologic material. *Diagn Mol Pathol*. 1995;4(1):25–31.

229. Nanassy OZ, Haydock PV, Reed MW. Capture of genomic DNA on glass microscope slides. *Anal Biochem*. 2007;365(2):240–245.

230. Roy-Chowdhuri S, Chow CW, Kane MK, et al. Optimizing the DNA yield for molecular analysis from cytologic preparations. *Cancer Cytopathol*. 2016;124(4):254–260.

231. Mastrangelo R, Poplack D, Bleyer A, Riccardi R, Sather H, D'Angio G. Report and recommendations of the Rome workshop concerning poor-prognosis acute lymphoblastic leukemia in children: biologic bases for staging, stratification, and treatment. *Med Pediatr Oncol*. 1986;14(3):191–194.

232. van der Does-van den Berg A, Bartram CR, Basso G, et al. Minimal requirements for the diagnosis, classification, and evaluation of the treatment of childhood acute lymphoblastic leukemia (ALL) in the “BFM Family” Cooperative Group. *Med Pediatr Oncol*. 1992;20(6):497–505.

233. Bürger B, Zimmermann M, Mann G, et al. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blasts or traumatic lumbar puncture. *J Clin Oncol*. 2003;21(2):184–188.

234. Cherlow JM, Sather H, Steinherz P, et al. Craniospinal irradiation for acute lymphoblastic leukemia with central nervous system disease at diagnosis: a report from the Children's Cancer Group. *Int J Radiat Oncol Biol Phys*. 1996;36(1):19–27.

235. te Loo DM, Kamps WA, van der Does-van den Berg A, van Wering ER, de Graaf SS; Dutch Childhood Oncology Group. Prognostic significance of blasts in the cerebrospinal fluid without pleocytosis or a traumatic lumbar puncture in children with acute lymphoblastic leukemia: experience of the Dutch Childhood Oncology Group. *J Clin Oncol*. 2006;24(15):2332–2336.

236. Mahmoud HH, Rivera GK, Hancock ML, et al. Low leukocyte counts with blast cells in cerebrospinal fluid of children with newly diagnosed acute lymphoblastic leukemia. *N Engl J Med*. 1993;329(5):314–319.

237. Ko SY, Chi HS, Jang S, Park CJ. Morphologic detection of blast cells in the cerebrospinal fluid at diagnosis of adult acute lymphoblastic leukemia appears to be associated with adverse prognosis. *Int J Lab Hematol*. 2014;36(4):451–458.

238. Pui CH, Dahl GV, Kalwinsky DK, et al. Central nervous system leukemia in children with acute nonlymphoblastic leukemia. *Blood*. 1985;66(5):1062–1067.

239. Stewart DJ, Keating MJ, McCredie KB, et al. Natural history of central nervous system acute leukemia in adults. *Cancer*. 1981;47(1):184–196.

240. Cheng CL, Li CC, Hou HA, et al. Risk factors and clinical outcomes of acute myeloid leukaemia with central nervous system involvement in adults. *BMC Cancer*. 2015;15:344. doi: 10.1186/s12885-015-1376-9.

241. Rozovski U, Ohanian M, Ravandi F, et al. Incidence of and risk factors for involvement of the central nervous system in acute myeloid leukemia. *Leuk Lymphoma*. 2015;56(5):1392–1397.

242. Abbott BL, Rubnitz JE, Tong X, et al. Clinical significance of central nervous system involvement at diagnosis of pediatric acute myeloid leukemia: a single institution's experience. *Leukemia*. 2003;17(11):2090–2096.

243. Webb DK, Harrison G, Stevens RF, et al. Relationships between age at diagnosis, clinical features, and outcome of therapy in children treated in the Medical Research Council AML 10 and 12 trials for acute myeloid leukemia. *Blood*. 2001;98(6):1714–1720.

244. Collins C, Knoderer H. Central nervous system involvement at the time of presentation in acute promyelocytic leukemia. *Pediatr Blood Cancer*. 2010;54(4):603–605.
245. Johnston DL, Alonzo TA, Gerbing RB, Lange BJ, Woods WG. Superior outcome of pediatric acute myeloid leukemia patients with orbital and CNS myeloid sarcoma: a report from the Children's Oncology Group. *Pediatr Blood Cancer*. 2012;58(4):519–524.
246. Cheng H, Yang Y, Dai W, et al. Acute leukemia presenting with blasts first found in the cerebrospinal fluid but not in the peripheral blood. *J Clin Neurosci*. 2010;17(10):1252–1255.
247. Howard SC, Gajjar AJ, Cheng C, et al. Risk factors for traumatic and bloody lumbar puncture in children with acute lymphoblastic leukemia. *JAMA*. 2002;288(16):2001–2007.
248. Gajjar A, Harrison PL, Sandlund JT, et al. Traumatic lumbar puncture at diagnosis adversely affects outcome in childhood acute lymphoblastic leukemia. *Blood*. 2000;96(10):3381–3384.
249. Huppman AR, Rheingold SR, Bailey LC, Helfrich M, Choi JK. Detection of leukemic lymphoblasts in CSF is instrument-dependent. *Am J Clin Pathol*. 2012;137(5):795–799.
250. Amadori S, Ceci A, Comelli A, et al. Treatment of acute myelogenous leukemia in children: results of the Italian Cooperative Study AIEOP/LAM 8204. *J Clin Oncol*. 1987;5(9):1356–1363.
251. Ravindranath Y, Steuber CP, Krischer J, et al. High-dose cytarabine for intensification of early therapy of childhood acute myeloid leukemia: a Pediatric Oncology Group study. *J Clin Oncol*. 1991;9(4):572–580.
252. Woods WG, Koblinsky N, Buckley J, et al. Intensively timed induction therapy followed by autologous or allogeneic bone marrow transplantation for children with acute myeloid leukemia or myelodysplastic syndrome: a Children's Cancer Group pilot study. *J Clin Oncol*. 1993;11(8):1448–1457.
253. Creutzig U, Ritter J, Zimmermann M, Schellong G. Does cranial irradiation reduce the risk for bone marrow relapse in acute myelogenous leukemia? Unexpected results of the Childhood Acute Myelogenous Leukemia Study BFM-87. *J Clin Oncol*. 1993;11(2):279–286.
254. Kobayashi R, Tawa A, Hanada R, et al; Japanese Childhood AML Cooperative Study Group. Extramedullary infiltration at diagnosis and prognosis in children with acute myelogenous leukemia. *Pediatr Blood Cancer*. 2007;48(4):393–398.
255. Chang H, Brandwein J, Yi QL, Chun K, Patterson B, Brien B. Extramedullary infiltrates of AML are associated with CD56 expression, 11q23 abnormalities and inferior clinical outcome. *Leuk Res*. 2004;28(10):1007–1011.
256. Bisschop MM, Révész T, Bierings M, et al. Extramedullary infiltrates at diagnosis have no prognostic significance in children with acute myeloid leukaemia. *Leukemia*. 2001;15(1):46–49.
257. Johnston DL, Alonzo TA, Gerbing RB, Lange BJ, Woods WG. Risk factors and therapy for isolated central nervous system relapse of pediatric acute myeloid leukemia. *J Clin Oncol*. 2005;23(36):9172–9178.
258. Peterson BA, Brunning RD, Bloomfield CD, et al. Central nervous system involvement in acute nonlymphocytic leukemia: a prospective study of adults in remission. *Am J Med*. 1987;83(3):464–470.
259. Slevin ML, Pfall EM, Aherne GW, Harvey VJ, Johnston A, Lister TA. Effect of dose and schedule on pharmacokinetics of high-dose cytosine arabinoside in plasma and cerebrospinal fluid. *J Clin Oncol*. 1983;1(9):546–551.
260. Martínez-Cuadrón D, Montesinos P, Pérez-Sirvent M, et al. Central nervous system involvement at first relapse in patients with acute myeloid leukemia. *Haematologica*. 2011;96(9):1375–1379.
261. Ranta S, Nilsson F, Harila-Saari A, et al. Detection of central nervous system involvement in childhood acute lymphoblastic leukemia by cytomorphology and flow cytometry of the cerebrospinal fluid. *Pediatr Blood Cancer*. 2015;62(6):951–956.
262. Mitri Z, Siddiqui MT, El Rassi F, et al. Sensitivity and specificity of cerebrospinal fluid flow cytometry for the diagnosis of leukemic meningitis in acute lymphoblastic leukemia/lymphoma. *Leuk Lymphoma*. 2014;55(7):1498–1500.
263. Subirá D, Castañón S, Román A, et al. Flow cytometry and the study of central nervous disease in patients with acute leukaemia. *Br J Haematol*. 2001;112(2):381–384.
264. Sayed D, Badrawy H, Ali AM, Shaker S. Immunophenotyping and immunoglobulin heavy chain gene rearrangement analysis in cerebrospinal fluid of pediatric patients with acute lymphoblastic leukemia. *Leuk Res*. 2009;33(5):655–661.
265. Del Principe MI, Buccisano F, Cefalo M, et al. High sensitivity of flow cytometry improves detection of occult leptomeningeal disease in acute lymphoblastic leukemia and lymphoblastic lymphoma. *Ann Hematol*. 2014;93(9):1509–1513.
266. Loeb KR, Cherian S, Becker PS, et al. Comparative analysis of flow cytometry and morphology for the detection of acute myeloid leukaemia cells in cerebrospinal fluid. *Br J Haematol*. 2016;172(1):134–136.
267. Seifert RP, Bulkeley W III, Zhang L, Menes M, Bui MM. A practical approach to diagnose soft tissue myeloid sarcoma preceding or coinciding with acute myeloid leukemia. *Ann Diagn Pathol*. 2014;18(4):253–260.
268. Paydas S, Zorludemir S, Ergin M. Granulocytic sarcoma: 32 cases and review of the literature. *Leuk Lymphoma*. 2006;47(12):2527–2541.
269. Neiman RS, Barcos M, Berard C, et al. Granulocytic sarcoma: a clinicopathologic study of 61 biopsied cases. *Cancer*. 1981;48(6):1426–1437.
270. Peker D, Parekh V, Paluri R, et al. Clinicopathological and molecular features of myeloid sarcoma as initial presentation of therapy-related myeloid neoplasms: a single institution experience. *Int J Hematol*. 2014;100(5):457–463.
271. Wilson CS, Medeiros LJ. Extramedullary manifestations of myeloid neoplasms. *Am J Clin Pathol*. 2015;144(2):219–239.
272. Byrd JC, Edenfield WJ, Shields DJ, Dawson NA. Extramedullary myeloid cell tumors in acute nonlymphocytic leukemia: a clinical review. *J Clin Oncol*. 1995;13(7):1800–1816.
273. Dusenbery KE, Howells WB, Arthur DC, et al. Extramedullary leukemia in children with newly diagnosed acute myeloid leukemia: a report from the Children's Cancer Group. *J Pediatr Hematol Oncol*. 2003;25(10):760–768.
274. Jenkin RD, Al-Shabanah M, Al-Nasser A, et al. Extramedullary myeloid tumors in children: the limited value of local treatment. *J Pediatr Hematol Oncol*. 2000;22(1):34–40.
275. Yamauchi K, Yasuda M. Comparison in treatments of nonleukemic granulocytic sarcoma: report of two cases and a review of 72 cases in the literature. *Cancer*. 2002;94(6):1739–1746.
276. Chiaretti S, Vitale A, Cazzaniga G, et al. Clinico-biological features of 5202 patients with acute lymphoblastic leukemia enrolled in the Italian AIEOP and GIMEMA protocols and stratified in age cohorts. *Haematologica*. 2013;98(11):1702–1710.
277. Streuli RA, Kaneko Y, Variakojis D, Kinnealey A, Golomb HM, Rowley JD. Lymphoblastic lymphoma in adults. *Cancer*. 1981;47(10):2510–2516.
278. Maitra A, McKenna RW, Weinberg AG, Schneider NR, Kroft SH. Precursor B-cell lymphoblastic lymphoma: a study of nine cases lacking blood and bone marrow involvement and review of the literature. *Am J Clin Pathol*. 2001;115(6):868–875.
279. Millot F, Robert A, Bertrand Y, et al. Cutaneous involvement in children with acute lymphoblastic leukemia or lymphoblastic lymphoma: the Children's Leukemia Cooperative Group of the European Organization of Research and Treatment of Cancer (EORTC). *Pediatrics*. 1997;100(1):60–64.
280. Lin P, Jones D, Dorfman DM, Medeiros LJ. Precursor B-cell lymphoblastic lymphoma: a predominantly extranodal tumor with low propensity for leukemic involvement. *Am J Surg Pathol*. 2000;24(11):1480–1490.
281. Alexiev BA, Wang W, Ning Y, et al. Myeloid sarcomas: a histologic, immunohistochemical, and cytogenetic study. *Diagn Pathol*. 2007;2:42. doi: 10.1186/1746-1596-2-42.
282. Sangle NA, Schmidt RL, Patel JL, et al. Optimized immunohistochemical panel to differentiate myeloid sarcoma from blastic plasmacytoid dendritic cell neoplasm. *Mod Pathol*. 2014;27(8):1137–1143.
283. Suh YK, Shin HJ. Fine-needle aspiration biopsy of granulocytic sarcoma: a clinicopathologic study of 27 cases. *Cancer*. 2000;90(6):364–372.
284. Park KU, Lee DS, Lee HS, Kim CJ, Cho HI. Granulocytic sarcoma in MLL-positive infant acute myelogenous leukemia: fluorescence in situ hybridization study of childhood acute myelogenous leukemia for detecting MLL rearrangement. *Am J Pathol*. 2001;159(6):2011–2016.
285. Bhaker P, Das A, Rajwanshi A, et al. Precursor T-lymphoblastic lymphoma: speedy diagnosis in FNA and effusion cytology by morphology, immunochemistry, and flow cytometry. *Cancer Cytopathol*. 2015;123(9):557–565.
286. Pileri SA, Ascani S, Cox MC, et al. Myeloid sarcoma: clinico-pathologic, phenotypic and cytogenetic analysis of 92 adult patients. *Leukemia*. 2007;21(2):340–350.
287. Cervantes GM, Cayci Z. Intracranial CNS manifestations of myeloid sarcoma in patients with acute myeloid leukemia: a review of the literature and three case reports from the author's institution. *J Clin Med*. 2015;4(5):1102–1112.
288. Deeb G, Baer MR, Gaile DP, et al. Genomic profiling of myeloid sarcoma by array comparative genomic hybridization. *Genes Chromosomes Cancer*. 2005;44(4):373–383.
289. Falini B, Lenze D, Hasserjian R, et al. Cytoplasmic mutated nucleophosmin (NPM) defines the molecular status of a significant fraction of myeloid sarcomas. *Leukemia*. 2007;21(7):1566–1570.
290. Ansari-Lari MA, Yang CF, Tinawi-Aljundi R, et al. *FLT3* mutations in myeloid sarcoma. *Br J Haematol*. 2004;126(6):785–791.
291. Menasce LP, Banerjee SS, Beckett E, Harris M. Extra-medullary myeloid tumour (granulocytic sarcoma) is often misdiagnosed: a study of 26 cases. *Histopathology*. 1999;34(5):391–398.
292. Chang CC, Eshoa C, Kampalath B, Shidham VB, Perkins S. Immunophenotypic profile of myeloid cells in granulocytic sarcoma by immunohistochemistry: correlation with blast differentiation in bone marrow. *Am J Clin Pathol*. 2000;114(5):807–811.
293. Traweek ST, Arber DA, Rappaport H, Brynes RK. Extramedullary myeloid cell tumors. An immunohistochemical and morphologic study of 28 cases. *Am J Surg Pathol*. 1993;17(10):1011–1019.
294. Buccisano F, Maurillo L, Spagnoli A, et al. Cytogenetic and molecular diagnostic characterization combined to postconsolidation minimal residual disease assessment by flow cytometry improves risk stratification in adult acute myeloid leukemia. *Blood*. 2010;116(13):2295–2303.
295. Maloney KW, Carroll WL, Carroll AJ, et al. Down syndrome childhood acute lymphoblastic leukemia has a unique spectrum of sentinel cytogenetic lesions that influences treatment outcome: a report from the Children's Oncology Group. *Blood*. 2010;116(7):1045–1050.
296. Patel B, Rai L, Buck G, et al. Minimal residual disease is a significant predictor of treatment failure in non T-lineage adult acute lymphoblastic

- leukaemia: final results of the international trial UKALL XII/ECOG2993. *Br J Haematol*. 2010;148(1):80–89.
297. Basso G, Veltroni M, Valsecchi MG, et al. Risk of relapse of childhood acute lymphoblastic leukemia is predicted by flow cytometric measurement of residual disease on day 15 bone marrow. *J Clin Oncol*. 2009;27(31):5168–5174.
298. Markova J, Trnkova Z, Michkova P, et al. Monitoring of minimal residual disease in patients with core binding factor acute myeloid leukemia and the impact of C-KIT, FLT3, and JAK2 mutations on clinical outcome. *Leuk Lymphoma*. 2009;50(9):1448–1460.
299. Zhou J, Goldwasser MA, Li A, et al; Dana-Farber Cancer Institute ALL Consortium. Quantitative analysis of minimal residual disease predicts relapse in children with B-lineage acute lymphoblastic leukemia in DFCl ALL Consortium Protocol 95-01. *Blood*. 2007;110(5):1607–1611.
300. Jeha S, Pei D, Raimondi SC, et al. Increased risk for CNS relapse in pre-B cell leukemia with the t(1;19)/TCF3-PBX1. *Leukemia*. 2009;23(8):1406–1409.
301. Maurillo L, Buccisano F, Del Principe ML, et al; Children's Oncology Group. Toward optimization of postremission therapy for residual disease-positive patients with acute myeloid leukemia. *J Clin Oncol*. 2008;26(30):4944–4951.
302. Mullighan CG, Su X, Zhang J, et al. Deletion of *IKZF1* and prognosis in acute lymphoblastic leukemia. *N Engl J Med*. 2009;360(5):470–480.
303. Waanders E, van der Velden VHJ, van der Schoot CE, et al. Integrated use of minimal residual disease classification and *IKZF1* alteration status accurately predicts 79% of relapses in pediatric acute lymphoblastic leukemia. *Leukemia*. 2011;25(2):254–258.
304. Yin JAL, O'Brien MA, Hills RK, Daly SB, Wheatley K, Burnett AK. Minimal residual disease monitoring by quantitative RT-PCR in core binding factor AML allows risk stratification and predicts relapse: results of the United Kingdom MRC AML-15 trial. *Blood*. 2012;120(14):2826–2835.
305. Feller N, van der Velden VH, Brooimans RA, et al. Defining consensus leukemia-associated immunophenotypes for detection of minimal residual disease in acute myeloid leukemia in a multicenter setting. *Blood Cancer J*. 2013;3:e129. doi: 10.1038/bcj.2013.27.
306. Olaru D, Campos L, Flandrin P, et al. Multiparametric analysis of normal and postchemotherapy bone marrow: implication for the detection of leukemia-associated immunophenotypes. *Cytometry B Clin Cytom*. 2008;74(1):17–24.
307. Chen W, Karandikar NJ, McKenna RW, Kroft SH. Stability of leukemia-associated immunophenotypes in precursor B-lymphoblastic leukemia/lymphoma: a single institution experience. *Am J Clin Pathol*. 2007;127(1):39–46.
308. Voskova D, Schoch C, Schnittger S, Hiddemann W, Haeflrich T, Kern W. Stability of leukemia-associated aberrant immunophenotypes in patients with acute myeloid leukemia between diagnosis and relapse: comparison with cytomorphologic, cytogenetic, and molecular genetic findings. *Cytometry B Clin Cytom*. 2004;62(1):25–38.
309. Gaipa G, Basso G, Biondi A, Campana D. Detection of minimal residual disease in pediatric acute lymphoblastic leukemia. *Cytometry B Clin Cytom*. 2013;84(6):359–369.
310. Weir EG, Cowan K, LeBeau P, Borowitz MJ. A limited antibody panel can distinguish B-precursor acute lymphoblastic leukemia from normal B precursors with four color flow cytometry: implications for residual disease detection. *Leukemia*. 1999;13(4):558–567.
311. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia*. 2003;17(6):1013–1034.
312. Kreyenberg H, Eckert C, Yarkin Y, et al. Immunoglobulin and T-cell receptor gene rearrangements as PCR-based targets are stable markers for monitoring minimal residual disease in acute lymphoblastic leukemia after stem cell transplantation. *Leukemia*. 2009;23(7):1355–1358.
313. Hokland P, Ommen HB, Nyvold CG, Roug AS. Sensitivity of minimal residual disease in acute myeloid leukaemia in first remission—methodologies in relation to their clinical situation. *Br J Haematol*. 2012;158(5):569–580.
314. Bibault JE, Figeac M, Hélevaut N, et al. Next-generation sequencing of FLT3 internal tandem duplications for minimal residual disease monitoring in acute myeloid leukemia. *Oncotarget*. 2015;6(26):22812–22821.
315. Wu D, Emerson RO, Sherwood A, et al. Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IGH. *Clin Cancer Res*. 2014;20(17):4540–4548.
316. Rubnitz JE, Wichlan D, Devidas M, et al; Children's Oncology Group. Prospective analysis of TEL gene rearrangements in childhood acute lymphoblastic leukemia: a Children's Oncology Group study. *J Clin Oncol*. 2008;26(13):2186–2191.
317. Schultz KR, Carroll A, Heerema NA, et al; Children's Oncology Group. Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children's Oncology Group study AALL0031. *Leukemia*. 2014;28(7):1467–1471.
318. Heerema NA, Carroll AJ, Devidas M, et al. Intrachromosomal amplification of chromosome 21 is associated with inferior outcomes in children with acute lymphoblastic leukemia treated in contemporary standard-risk children's oncology group studies: a report from the Children's Oncology Group. *J Clin Oncol*. 2013;31(27):3397–3402.
319. Moorman AV, Robinson H, Schwab C, et al. Risk-directed treatment intensification significantly reduces the risk of relapse among children and adolescents with acute lymphoblastic leukemia and intrachromosomal amplification of chromosome 21: a comparison of the MRC ALL97/99 and UKALL2003 trials. *J Clin Oncol*. 2013;31(27):3389–3396.
320. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood*. 2004;103(12):4396–4407.
321. Yanada M, Takeuchi J, Sugiura I, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol*. 2006;24(3):460–466.
322. Tanguy-Schmidt A, Rousselot P, Chalandon Y, et al. Long-term follow-up of the imatinib GRAAPH-2003 study in newly diagnosed patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: a GRAALL study. *Biol Blood Marrow Transplant*. 2013;19(1):150–155.
323. de Labarthe A, Rousselot P, Huguët-Rigal F, et al; Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL). Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. *Blood*. 2007;109(4):1408–1413.
324. Clappier E, Collette S, Grardel N, et al. *NOTCH1* and *FBXW7* mutations have a favorable impact on early response to treatment, but not on outcome, in children with T-cell acute lymphoblastic leukemia (T-ALL) treated on EORTC trials 58881 and 58951. *Leukemia*. 2010;24(12):2023–2031.
325. Familiades J, Bousquet M, Lafage-Pochitaloff M, et al. *PAX5* mutations occur frequently in adult B-cell progenitor acute lymphoblastic leukemia and *PAX5* haploinsufficiency is associated with BCR-ABL1 and TCF3-PBX1 fusion genes: a GRAALL study. *Leukemia*. 2009;23(11):1989–1998.
326. Asnafi V, Buzyn A, Le Noir S, et al. *NOTCH1/FBXW7* mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. *Blood*. 2009;113(17):3918–3924.
327. Kuiper RP, Waanders E, van der Velden VH, et al. *IKZF1* deletions predict relapse in uniformly treated pediatric precursor B-ALL. *Leukemia*. 2010;24(7):1258–1264.
328. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *Lancet Oncol*. 2009;10(2):125–134.
329. Baldus CD, Thibaut J, Goekbuget N, et al. Prognostic implications of *NOTCH1* and *FBXW7* mutations in adult acute T-lymphoblastic leukemia. *Haematologica*. 2009;94(10):1383–1390.
330. Cario G, Zimmermann M, Romey R, et al. Presence of the *P2RY8-CRLF2* rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. *Blood*. 2010;115(26):5393–5397.
331. Flex E, Petrangeli V, Stella L, et al. Somatic acquired *JAK1* mutations in adult acute lymphoblastic leukemia. *J Exp Med*. 2008;205(4):751–758.
332. Harvey RC, Mullighan CG, Wang X, et al. Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. *Blood*. 2010;116(23):4874–4884.
333. Heesch S, Goekbuget N, Stroux A, et al. Prognostic implications of mutations and expression of the Wilms tumor 1 (*WT1*) gene in adult acute T-lymphoblastic leukemia. *Haematologica*. 2010;95(6):942–949.
334. Kox C, Zimmermann M, Stanulla M, et al. The favorable effect of activating *NOTCH1* receptor mutations on long-term outcome in T-ALL patients treated on the ALL-BFM 2000 protocol can be separated from *FBXW7* loss of function. *Leukemia*. 2010;24(12):2005–2013.
335. Iacobucci I, Lonetti A, Messa F, et al. Expression of spliced oncogenic Ikaros isoforms in Philadelphia-positive acute lymphoblastic leukemia patients treated with tyrosine kinase inhibitors: implications for a new mechanism of resistance. *Blood*. 2008;112(9):3847–3855.
336. Harvey RC, Mullighan CG, Chen IM, et al. Rearrangement of *CRLF2* is associated with mutation of *JAK* kinases, alteration of *IKZF1*, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood*. 2010;115(26):5312–5321.
337. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med*. 2014;371(11):1005–1015.
338. Ho PA, Alonzo TA, Kopecky KJ, et al. Molecular alterations of the *IDH1* gene in AML: a Children's Oncology Group and Southwest Oncology Group study. *Leukemia*. 2010;24(5):909–913.
339. Pollard JA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic significance of *KIT* mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood*. 2010;115(12):2372–2379.
340. Abbas S, Lugthart S, Kavelaars FG, et al. Acquired mutations in the genes encoding *IDH1* and *IDH2* both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. *Blood*. 2010;116(12):2122–2126.
341. Kayser S, Schlenk RF, Londono MC, et al. Insertion of *FLT3* internal tandem duplication in the tyrosine kinase domain-1 is associated with resistance to chemotherapy and inferior outcome. *Blood*. 2009;114(12):2386–2392.
342. Schlenk RF, Kayser S, Bullinger L, et al. Differential impact of allelic ratio and insertion site in *FLT3*-ITD-positive AML with respect to allogeneic transplantation. *Blood*. 2014;124(23):3441–3449.

343. Linch DC, Hills RK, Burnett AK, Khwaja A, Gale RE. Impact of *FLT3*(ITD) mutant allele level on relapse risk in intermediate-risk acute myeloid leukemia. *Blood*. 2014;124(2):273–276.
344. Whitman SP, Archer KJ, Feng L, et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of *FLT3*: a cancer and leukemia group B study. *Cancer Res*. 2001;61(19):7233–7239.
345. Frohling S, Schlenk RF, Breitnick J, et al; AML Study Group Ulm Acute Myeloid Leukemia. Prognostic significance of activating *FLT3* mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood*. 2002;100(13):4372–4380.
346. Beran M, Luthra R, Kantarjian H, Estey E. *FLT3* mutation and response to intensive chemotherapy in young adult and elderly patients with normal karyotype. *Leuk Res*. 2004;28(6):547–550.
347. Bienz M, Ludwig M, Leibundgut EO, et al. Risk assessment in patients with acute myeloid leukemia and a normal karyotype [published correction appears in *Clin Cancer Res*. 2005;11(15):5659]. *Clin Cancer Res*. 2005;11(4):1416–1424.
348. Whitman SP, Maharry K, Radmacher MD, et al. *FLT3* internal tandem duplication associates with adverse outcome and gene- and microRNA-expression signatures in patients 60 years of age or older with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Blood*. 2010;116(18):3622–3626.
349. Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079–1089.
350. Becker H, Marcucci G, Maharry K, et al. Mutations of the Wilms tumor 1 gene (*WT1*) in older patients with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Blood*. 2010;116(5):788–792.
351. Paschka P, Schlenk RF, Gaidzik VI, et al. *IDH1* and *IDH2* mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with *NPM1* mutation without *FLT3* internal tandem duplication. *J Clin Oncol*. 2010;28(22):3636–3643.
352. Marcucci G, Maharry K, Wu YZ, et al. *IDH1* and *IDH2* gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2010;28(14):2348–2355.
353. Zhou K-G, Jiang LJ, Shang Z, Wang J, Huang L, Zhou JF. Potential application of *IDH1* and *IDH2* mutations as prognostic indicators in non-promyelocytic acute myeloid leukemia: a meta-analysis. *Leuk Lymphoma*. 2012;53(12):2423–2429.
354. Nomdedéu J, Hoyos M, Carricondo M, et al. Adverse impact of *IDH1* and *IDH2* mutations in primary AML: experience of the Spanish CETLAM group. *Leuk Res*. 2012;36(8):990–997.
355. Devillier R, Mansat-De Mas V, Gelsi-Boyer V, et al. Role of *ASXL1* and *TP53* mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. *Oncotarget*. 2015;6(10):8388–8396.
356. Marcucci G, Metzeler KH, Schwind S, et al. Age-related prognostic impact of different types of *DNMT3A* mutations in adults with primary cytogenetically normal acute myeloid leukemia. *J Clin Oncol*. 2012;30(7):742–750.
357. Thol F, Damm F, Lüdeking A, et al. Incidence and prognostic influence of *DNMT3A* mutations in acute myeloid leukemia. *J Clin Oncol*. 2011;29(21):2889–2896.
358. Gaidzik VI, Schlenk RF, Paschka P, et al. Clinical impact of *DNMT3A* mutations in younger adult patients with acute myeloid leukemia: results of the AML Study Group (AMLSG). *Blood*. 2013;121(23):4769–4777.
359. Rucker FG, Schlenk RF, Bullinger L, et al. *TP53* alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood*. 2012;119(9):2114–2121.
360. Bowen D, Groves MJ, Burnett AK, et al. *TP53* gene mutation is frequent in patients with acute myeloid leukemia and complex karyotype, and is associated with very poor prognosis. *Leukemia*. 2009;23(1):203–206.
361. Ohgami RS, Ma L, Merker JD, et al. Next-generation sequencing of acute myeloid leukemia identifies the significance of *TP53*, *U2AF1*, *ASXL1*, and *TET2* mutations. *Mod Pathol*. 2015;28(5):706–714.
362. Allen C, Hills RK, Lamb K, et al. The importance of relative mutant level for evaluating impact on outcome of *KIT*, *FLT3* and *CBL* mutations in core-binding factor acute myeloid leukemia. *Leukemia*. 2013;27(9):1891–1901.
363. Paschka P, Marcucci G, Ruppert AS, et al; Cancer and Leukemia Group B. Adverse prognostic significance of *KIT* mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. *J Clin Oncol*. 2006;24(24):3904–3911.
364. Schnittger S, Kohl TM, Haferlach T, et al. *KIT*-D816 mutations in *AML1*-ETO-positive AML are associated with impaired event-free and overall survival. *Blood*. 2006;107(5):1791–1799.
365. Shimada A, Taki T, Tabuchi K, et al. *KIT* mutations, and not *FLT3* internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. *Blood*. 2006;107(5):1806–1809.
366. Goemans BF, Zwaan CM, Miller M, et al. Mutations in *KIT* and *RAS* are frequent events in pediatric core-binding factor acute myeloid leukemia. *Leukemia*. 2005;19(9):1536–1542.
367. Grimwade D, Gorman P, Duprez E, et al. Characterization of cryptic rearrangements and variant translocations in acute promyelocytic leukemia. *Blood*. 1997;90(12):4876–4885.
368. Sanz MA, Grimwade D, Tallman MS, et al. Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*. 2009;113(9):1875–1891.
369. Miller WH Jr, Kakizuka A, Frankel SR, et al. Reverse transcription polymerase chain reaction for the rearranged retinoic acid receptor alpha clarifies diagnosis and detects minimal residual disease in acute promyelocytic leukemia. *Proc Natl Acad Sci U S A*. 1992;89(7):2694–2698.
370. Schad CR, Hanson CA, Paietta E, Casper J, Jalal SM, Dewald GW. Efficacy of fluorescence in situ hybridization for detecting *PML/RARA* gene fusion in treated and untreated acute promyelocytic leukemia. *Mayo Clin Proc*. 1994;69(11):1047–1053.
371. Dimov ND, Medeiros LJ, Kantarjian HM, et al. Rapid and reliable confirmation of acute promyelocytic leukemia by immunofluorescence staining with an antipromyelocytic leukemia antibody: the M. D. Anderson Cancer Center experience of 349 patients. *Cancer*. 2010;116(2):369–376.
372. Falini B, Flenghi L, Fagioli M, et al. Immunocytochemical diagnosis of acute promyelocytic leukemia (M3) with the monoclonal antibody PG-M3 (anti-PML). *Blood*. 1997;90(10):4046–4053.
373. Lee HJ, Park HJ, Kim HW, Park SG. Comparison of laboratory characteristics between acute promyelocytic leukemia and other subtypes of acute myeloid leukemia with disseminated intravascular coagulation. *Blood Res*. 2013;48(4):250–253.
374. Chang H, Kuo MC, Shih LY, et al. Clinical bleeding events and laboratory coagulation profiles in acute promyelocytic leukemia. *Eur J Haematol*. 2012;88(4):321–328.
375. Taylor FB Jr, Toh CH, Hoots WK, et al; Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. *Thromb Haemost*. 2001;86(5):1327–1330.
376. Mitrovic M, Suvajdzic N, Bogdanovic A, et al. International Society of Thrombosis and Hemostasis Scoring System for disseminated intravascular coagulation ≥ 6 : a new predictor of hemorrhagic early death in acute promyelocytic leukemia. *Med Oncol*. 2013;30(1):478. doi: 10.1007/s12032-013-0478-y
377. Falini B, Mecucci C, Tiacci E, et al; GIMEMA Acute Leukemia Working Party. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352(3):254–266.
378. Thiede C, Koch S, Creutzig E, et al. Prevalence and prognostic impact of *NPM1* mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood*. 2006;107(10):4011–4020.
379. Döhner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (*NPM1*) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood*. 2005;106(12):3740–3746.
380. Schnittger S, Schoch C, Kern W, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood*. 2005;106(12):3733–3739.
381. Falini B, Mecucci C, Saglio G, et al. *NPM1* mutations and cytoplasmic nucleophosmin are mutually exclusive of recurrent genetic abnormalities: a comparative analysis of 2562 patients with acute myeloid leukemia. *Haematologica*. 2008;93(3):439–442.
382. Haferlach C, Mecucci C, Schnittger S, et al. AML with mutated *NPM1* carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. *Blood*. 2009;114(14):3024–3032.
383. Brown P, McIntyre E, Rau R, et al. The incidence and clinical significance of nucleophosmin mutations in childhood AML. *Blood*. 2007;110(3):979–985.
384. Cazzaniga G, Dell’Oro MG, Mecucci C, et al. Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. *Blood*. 2005;106(4):1419–1422.
385. Hollink IH, Zwaan CM, Zimmermann M, et al. Favorable prognostic impact of *NPM1* gene mutations in childhood acute myeloid leukemia, with emphasis on cytogenetically normal AML. *Leukemia*. 2009;23(2):262–270.
386. Falini B, Maciejewski K, Weiss T, et al. Multilineage dysplasia has no impact on biologic, clinicopathologic, and prognostic features of AML with mutated nucleophosmin (*NPM1*) [published correction appears in *Blood*. 2010;116(6):1017]. *Blood*. 2010;115(18):3776–3786.
387. Koschmieder S, Halmos B, Levantini E, Tenen DG. Dysregulation of the *C/EBP α* differentiation pathway in human cancer. *J Clin Oncol*. 2009;27(4):619–628.
388. Pabst T, Mueller BU, Zhang P, et al. Dominant-negative mutations of *CEBPA*, encoding CCAAT/enhancer binding protein-alpha (*C/EBP α*), in acute myeloid leukemia. *Nat Genet*. 2001;27(3):263–270.
389. Nerlov C. *C/EBP α* mutations in acute myeloid leukaemias. *Nat Rev Cancer*. 2004;4(5):394–400.
390. Wouters BJ, Löwenberg B, Eerpelink-Verschueren CA, van Putten WL, Valk PJ, Delwel R. Double *CEBPA* mutations, but not single *CEBPA* mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression

profile that is uniquely associated with a favorable outcome. *Blood*. 2009; 113(13):3088–3091.

391. Wouters BJ, Sanders MA, Lugthart S, et al. Segmental uniparental disomy as a recurrent mechanism for homozygous CEBPA mutations in acute myeloid leukemia. *Leukemia*. 2007;21(11):2382–2384.

392. Fasan A, Haferlach C, Alpermann T, et al. The role of different genetic subtypes of CEBPA mutated AML. *Leukemia*. 2014;28(4):794–803.

393. Preudhomme C, Sagot C, Boissel N, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). *Blood*. 2002;100(8):2717–2723.

394. Alvarez S, Suela J, Valencia A, et al. DNA methylation profiles and their relationship with cytogenetic status in adult acute myeloid leukemia. *PLoS One*. 2010;5(8):e12197. doi: 10.1371/journal.pone.0012197.

395. Chuang MK, Chiu YC, Chou WC, Hou HA, Chuang EY, Tien HF. A 3-microRNA scoring system for prognostication in de novo acute myeloid leukemia patients. *Leukemia*. 2015;29(5):1051–1059.

396. Gröschel S, Schlenk RF, Engelmann J, et al. Deregulated expression of EVI1 defines a poor prognostic subset of MLL-rearranged acute myeloid leukemias: a study of the German-Austrian Acute Myeloid Leukemia Study Group and the Dutch-Belgian-Swiss HOVON/SAKK Cooperative Group. *J Clin Oncol*. 2013;31(1):95–103.

397. Li Z, Herold T, He C, et al. Identification of a 24-gene prognostic signature that improves the European LeukemiaNet risk classification of acute myeloid leukemia: an international collaborative study. *J Clin Oncol*. 2013;31(9):1172–1181.

398. Deneberg S, Grövdal M, Karimi M, et al. Gene-specific and global methylation patterns predict outcome in patients with acute myeloid leukemia. *Leukemia*. 2010;24(5):932–941.

399. Deneberg S, Guardiola P, Lennartsson A, et al. Prognostic DNA methylation patterns in cytogenetically normal acute myeloid leukemia are predefined by stem cell chromatin marks. *Blood*. 2011;118(20):5573–5582.

400. Wang Y, Gu M, Mi Y, Qiu L, Bian S, Wang J. Clinical characteristics and outcomes of mixed phenotype acute leukemia with Philadelphia chromosome positive and/or *bcr-abl* positive in adult. *Int J Hematol*. 2011;94(6):552–555.

401. Shimizu H, Yokohama A, Hatsumi N, et al. Philadelphia chromosome-positive mixed phenotype acute leukemia in the imatinib era. *Eur J Haematol*. 2014;93(4):297–301.

402. Manola KN. Cytogenetic abnormalities in acute leukaemia of ambiguous lineage: an overview. *Br J Haematol*. 2013;163(1):24–39.

403. Yan L, Ping N, Zhu M, et al. Clinical, immunophenotypic, cytogenetic, and molecular genetic features in 117 adult patients with mixed-phenotype acute leukemia defined by WHO-2008 classification. *Haematologica*. 2012;97(11):1708–1712.

404. Zhang Y, Wu D, Sun A, et al. Clinical characteristics, biological profile, and outcome of biphenotypic acute leukemia: a case series. *Acta Haematol*. 2011;125(4):210–218.

405. Centers for Medicare & Medicaid Services, Department of Health and Human Services. Medicare, Medicaid and CLIA programs, regulations implementing the Clinical Laboratory Improvement Amendments of 1988 (CLIA)—HCFA: final rule with comment period. *Fed Regist*. 1992;57(40):7002–7186. Codified at 42 CFR §493.

406. Centers for Medicare & Medicaid Services, Department of Health and Human Services. Medicare, Medicaid, and CLIA programs, laboratory requirements relating to quality systems and certain personnel qualifications: final rule. *Fed Regist*. 2003;68(16):3640–3714. Codified at 42 CFR §493.

407. Wagar EA. Laboratory laws and regulations. In: Wagar EA, Horowitz RE, Siegal GP, eds. *Laboratory Administration for Pathologists*. Northfield, IL: CAP Press; 2011:215–233.

408. Krauter J, Wagner K, Schäfer I, et al. Prognostic factors in adult patients up to 60 years old with acute myeloid leukemia and translocations of chromosome band 11q23: individual patient data-based meta-analysis of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol*. 2009;27(18):3000–3006.

409. Forestier E, Heyman M, Andersen MK, et al; Nordic Society of Paediatric Haematology, Oncology (NOPHO); Swedish Cytogenetic Leukaemia Study Group (SCLSG); NOPHO Leukaemia Cytogenetic Study Group (NLCSG). Outcome of *ETV6/RUNX1*-positive childhood acute lymphoblastic leukaemia in the NOPHO-ALL-1992 protocol: frequent late relapses but good overall survival. *Br J Haematol*. 2008;140(6):665–672.

410. Schaich M, Schlenk RF, Al-Ali HK, et al. Prognosis of acute myeloid leukemia patients up to 60 years of age exhibiting trisomy 8 within a non-

complex karyotype: individual patient data-based meta-analysis of the German Acute Myeloid Leukemia Intergroup. *Haematologica*. 2007;92(6):763–770.

411. Barbaric D, Alonzo TA, Gerbing RB, et al. Minimally differentiated acute myeloid leukemia (FAB AML-M0) is associated with an adverse outcome in children: a report from the Children's Oncology Group, studies CCG-2891 and CCG-2961. *Blood*. 2007;109(6):2314–2321.

412. Chiaretti S, Tavolaro S, Ghia EM, et al. Characterization of *ABL1* expression in adult T-cell acute lymphoblastic leukemia by oligonucleotide array analysis. *Haematologica*. 2007;92(5):619–626.

413. Fischer L, Gökbuget N, Schwartz S, et al. CD56 expression in T-cell acute lymphoblastic leukemia is associated with non-thymic phenotype and resistance to induction therapy but no inferior survival after risk-adapted therapy. *Haematologica*. 2009;94(2):224–229.

414. Vance GH, Kim H, Hicks GA, et al. Utility of interphase FISH to stratify patients into cytogenetic risk categories at diagnosis of AML in an Eastern Cooperative Oncology Group (ECOG) clinical trial (E1900). *Leuk Res*. 2007; 31(5):605–609.

415. Patel JL, Smith LM, Anderson J, et al. The immunophenotype of T-lymphoblastic lymphoma in children and adolescents: a Children's Oncology Group report. *Br J Haematol*. 2012;159(4):454–461.

416. Ohgami RS, Arber DA. Challenges in consolidated reporting of hematopoietic neoplasms. *Surg Pathol Clin*. 2013;6(4):795–806.

417. Santamaria C, Chillón MC, Garcia-Sanz R, et al. The relevance of preferentially expressed antigen of melanoma (PRAME) as a marker of disease activity and prognosis in acute promyelocytic leukemia. *Haematologica*. 2008; 93(12):1797–1805.

418. Busse A, Gökbuget N, Siehl JM, et al. Wilms' tumor gene 1 (*WT1*) expression in subtypes of acute lymphoblastic leukemia (ALL) of adults and impact on clinical outcome. *Ann Hematol*. 2009;88(12):1199–1205.

419. de Jonge HJ, Valk PJ, Veeger NJ, et al. High *VEGFC* expression is associated with unique gene expression profiles and predicts adverse prognosis in pediatric and adult acute myeloid leukemia. *Blood*. 2010;116(10):1747–1754.

420. Csinady E, van der Velden VHJ, Joas R, et al. Chromosome 14 copy number-dependent IGH gene rearrangement patterns in high hyperdiploid childhood B-cell precursor ALL: implications for leukemia biology and minimal residual disease analysis. *Leukemia*. 2009;23(5):870–876.

421. Meshinchi S, Stirewalt DL, Alonzo TA, et al. Structural and numerical variation of FLT3/ITD in pediatric AML. *Blood*. 2008;111(10):4930–4933.

422. Ongaro A, De Mattei M, Della Porta MG, et al. Gene polymorphisms in folate metabolizing enzymes in adult acute lymphoblastic leukemia: effects on methotrexate-related toxicity and survival. *Haematologica*. 2009;94(10):1391–1398.

423. Suela J, Alvarez S, Cifuentes F, et al. DNA profiling analysis of 100 consecutive de novo acute myeloid leukemia cases reveals patterns of genomic instability that affect all cytogenetic risk groups. *Leukemia*. 2007;21(6):1224–1231.

424. Gönen M, Sun Z, Figueroa ME, et al. CD25 expression status improves prognostic risk classification in AML independent of established biomarkers: ECOG phase 3 trial, E1900. *Blood*. 2012;120(11):2297–2306.

425. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November, 1997. *Ann Oncol*. 1999;10(12):1419–1432.

426. Ratei R, Schabath R, Karawajew L, et al. Lineage classification of childhood acute lymphoblastic leukemia according to the EGIL recommendations: results of the ALL-BFM 2000 trial. *Klin Padiatr*. 2013;225(suppl 1):S34–39.

427. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias: European Group for the Immunological Characterization of Leukemias (EGIL). *Leukemia*. 1995;9(10):1783–1786.

428. Walter RB, Othus M, Burnett AK, et al. Significance of FAB subclassification of "acute myeloid leukemia, NOS" in the 2008 WHO classification: analysis of 5848 newly diagnosed patients. *Blood*. 2013;121(13):2424–2431.

429. Sandahl JD, Kjeldsen E, Abrahamsson J, et al. The applicability of the WHO classification in paediatric AML: a NOPHO-AML study. *Br J Haematol*. 2015;169(6):859–867.

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

431. Andrews J, Guyatt G, Oxman AD, et al. GRADE guidelines, 14: going from evidence to recommendations: the significance and presentation of recommendations. *J Clin Epidemiol*. 2013;66(7):719–725.

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 |
| | | Elected or appointed positions in other national/international medical organizations | Children's Oncology Group, Philadelphia, Pennsylvania |
| | 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 |
| J. Douglas Rizzo, MD | Leadership in other associations Grants | Society for Hematopathology, Chicago, Illinois USCAP, Palm Springs, California 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.