

Initial Diagnostic Work-Up of Acute Leukemia: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists and American Society of Hematology Guideline

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PURPOSE The College of American Pathologists (CAP) and the American Society of Hematology (ASH) developed an evidence-based guideline on the initial diagnostic work-up of acute leukemia (AL). Because of the relevance of this topic to the ASCO membership, ASCO reviewed the guideline and applied a set of procedures and policies for endorsing clinical practice guidelines that have been developed by other professional organizations.

METHODS The CAP-ASH guideline on initial diagnostic work-up of AL was reviewed for developmental rigor by methodologists. Then, an ASCO Endorsement Expert Panel updated the literature search and reviewed the content and recommendations.

RESULTS The ASCO Expert Panel determined that the recommendations from the guideline, published in 2016, are clear, thorough, and based on the most relevant scientific evidence. ASCO fully endorsed the CAP-ASH guideline on initial diagnostic work-up of AL and included some discussion points according to clinical practice and updated literature.

CONCLUSION Twenty-seven guideline statements were reviewed. Some discussion points were included to better assess CNS involvement in leukemia and to provide novel insights into molecular diagnosis and potential markers for risk stratification and target therapy. These discussions are categorized into four sections: (1) initial diagnosis focusing on basic diagnostics and determination of risk parameters, (2) molecular markers and minimal residual disease detection, (3) context of referral to another institution with expertise in the management of AL, and (4) reporting and record keeping for better outlining and follow-up discussion. Additional information is available at: www.asco.org/hematologic-malignancies-guidelines.

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INTRODUCTION

The laboratory evaluation of patients suspected of having acute leukemia (AL) is critical though complex and has evolved significantly with the incorporation of advanced laboratory techniques. Aside from the traditional techniques (cytomorphology, cytochemistry, immunophenotyping by multiparameter flow cytometry, or immunohistochemical staining and molecular or cytogenetics study),¹⁻³ emerging advanced molecular diagnostics such as next-generation sequencing (NGS) technology have become more important in the diagnosis and risk stratification of AL.⁴⁻⁷

In general, the aforementioned four traditional techniques are the backbone of the initial diagnostic work-up of AL, leading to risk-group stratification and fine-tuning by molecular signatures. Recent advances in sequencing to define the molecular landscape have brought novel insights into the pathogenesis of AL, have helped identify new genetic subtypes of AL and

additional risk factors, and have led to the development of novel treatment strategies and personalized medicine. However, the appropriate ways in which to introduce molecular tests into the initial work-up and follow-up of AL and to integrate them into the conventional approaches are still under debate,⁴ and this requires guidance.

In 2017, an evidence-based guideline for the initial work-up of AL was published by the College of American Pathologists (CAP) and the American Society of Hematology (ASH).⁸ Since that time, advances in molecular techniques and identification and validation of new molecular markers via large cohorts have contributed to better risk stratification of patients with AL. Second, a revision of the WHO classification of tumors of hematopoietic and lymphoid tissues was made in 2016⁹ and fully published in 2017,¹⁰ also leading to new risk categories and refined subclassification. Therefore, the current CAP-ASH guidelines⁸ were reviewed by ASCO

ASSOCIATED CONTENT

Appendix

Data Supplement/ Methodology Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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THE BOTTOM LINE

Initial Diagnostic Work-Up of Acute Leukemia: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists and American Society of Hematology Guideline

ASCO endorses the Initial Diagnostic Work-up of Acute Leukemia Clinical Practice Guideline by CAP and ASH.

Guideline Questions

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 work-up of a patient with AL?
3. At the time of diagnosis, what tests are required for all patients for the initial evaluation of 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?

Target Population Children and adults with AL.

Target Audience Primary care providers, nurses, medical oncologists, pediatric oncologists, hematologists, pathologists, radiation oncologists, other providers.

Methods An ASCO Expert Panel was convened to consider endorsing the CAP and ASH initial diagnostic work-up of AL clinical practice guideline recommendations that were based on a systematic review of the medical literature. The ASCO Expert Panel considered the methodology used in the 2017 guideline by considering the results from the AGREE II review instrument. The ASCO Expert Panel carefully reviewed the 2017 guideline content to determine its appropriateness for ASCO endorsement.

Key Recommendations

Recommendation 1. The treating clinician should provide relevant clinical data or ensure that these are readily accessible by the pathologist (*Strong recommendation*).

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 AL. The treating clinician should also obtain and provide information regarding any family history of any hematologic disorders or other malignancies.

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

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

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

Recommendation 4. The treating clinician or pathologist should obtain a fresh bone marrow (BM) aspirate for all patients suspected of AL, a portion of which should be used to make BM aspirate smears for morphologic evaluation. The pathologist should evaluate an adequate BM trephine core biopsy, BM trephine touch preparations, and/or marrow clots, if available, in conjunction with the BM aspirates (*Strong recommendation*).

Note: If BM aspirate material is inadequate or if there is a compelling clinical reason to avoid BM examination, PB may 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 smears and the core biopsy specimens, or the interpretations of those specimens should be correlated if performed by different physicians.

Recommendation 5. In addition to performing 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 AML (including APL), including early T-ALL, B-ALL, and AL of ambiguous lineage in all patients diagnosed with AL. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis (*Strong recommendation*).

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THE BOTTOM LINE (CONTINUED)

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 medium, for disaggregation for genetic studies and flow cytometry.

Recommendation 6. For patients with suspected or confirmed AL, the pathologist may request and evaluate cytochemical studies to assist in the diagnosis and classification of AML (*Expert consensus opinion*)

Recommendation 7. The treating clinician or pathologist may use cryopreserved cells or nucleic acid, formalin-fixed, nondecalcified paraffin-embedded tissue, or unstained marrow aspirate or PB smears 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 (*Recommendation*).

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

Recommendation 9. For patients with AL other than those with ALL who are 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 and enumeration of blasts on a cytocentrifuge preparation are performed and are reviewed by the pathologist (*Expert consensus opinion*).

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

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

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

Recommendation 12. 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 (*Strong recommendation*).

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); *ETV6-RUNX1*, t(9;22)(q34.1;q11.2); *BCR-ABL1*, *KMT2A (MLL)* translocation, *iAMP21*, and trisomy 4 and 10 is performed (*Strong recommendation*).

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); *BCR-ABL1* is performed. In addition, testing for *KMT2A (MLL)* translocations may be performed. (*Strong recommendation* for testing for t(9;22)(q34.1;q11.2) and *BCR-ABL1*; *Recommendation* for testing for *KMT2A (MLL)* translocations).

Recommendation 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 include, but are 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 (*Recommendation*).

Recommendation 16. 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. (*Strong recommendation* for testing for *FLT3-ITD*; *Recommendation* for testing for other mutational analysis).

Recommendation 17. For adult patients with confirmed core binding factor (CBF) AML (AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* or inv(16)(p13.1q22)/t(16;16)(p13.1;q22); *CBFB-MYH11*), the pathologist or treating clinician should ensure that appropriate mutational analysis for *KIT* is performed. For pediatric patients with confirmed CBF AML; *RUNX1-RUNX1T1* or inv(16)(p13.1q22)/t(16;16)(p13.1;q22); *CBFB-MYH11*, the pathologist or treating clinician may ensure that appropriate mutational analysis for *KIT* is performed. (*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).

Recommendation 18. 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 (*Strong recommendation*).

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THE BOTTOM LINE (CONTINUED)

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

Recommendation 20. For patients with confirmed AL, no recommendation is made for or against the use of global or gene-specific methylation, microRNA expression, or gene expression analysis for diagnosis or prognosis (*No recommendation*).

Recommendation 21. For patients with confirmed mixed-phenotype AL, 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 (*Strong recommendation*).

Recommendation 22. All laboratory testing performed for the initial work-up and diagnosis of a patient with AL must be performed in a laboratory that is in compliance with regulatory and/or accreditation requirements (*Strong recommendation*).

Recommendation 23. If, after examination of a PB smear, 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 (*Strong recommendation*).

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*).

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*).

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 (*Strong recommendation*).

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

Recommendation 27. Treating physicians and pathologists should use the current WHO terminology for the final diagnosis and classification of AL (*Strong recommendation*).

Additional Resources

More information, including a Data Supplement, a Methodology Supplement, slide sets, and clinical tools and resources, is available at www.asco.org/hematologic-malignancies-guidelines. Patient information is available at www.cancer.net

A link to the Initial Diagnostic Work-up of Acute Leukemia Clinical Practice Guideline by CAP and ASH can be found at <http://www.archivesofpathology.org>

ASCO believes that cancer clinical trials are vital to inform medical decisions and improve cancer care, and that all patients should have the opportunity to participate.

Endorsement Expert Panelists, and discussion points are used to summarize issues that were identified from the updated literature.

OVERVIEW OF THE ASCO GUIDELINE ENDORSEMENT PROCESS

ASCO has policies and procedures for endorsing practice guidelines that have been developed by other professional organizations. The goal of guideline endorsement is to

increase the number of high-quality, ASCO-vetted guidelines available to the ASCO membership. The ASCO endorsement process involves an assessment by ASCO staff of candidate guidelines for methodologic quality using the Rigour of Development subscale of the Appraisal of Guidelines for Research and Evaluation II (AGREE II) instrument. (See Methodology Supplement for more detail.)

Disclaimer

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Guideline and Conflicts of Interest

The Expert Panel was assembled in accordance with ASCO’s Conflict of Interest Policy Implementation for Clinical Practice Guidelines (“Policy,” found at <http://www.asco.org/rwc>). All members of the Expert Panel completed ASCO’s disclosure form, which requires disclosure of financial and other interests, including relationships with commercial entities that are reasonably likely to experience a direct regulatory or commercial impact as a result of promulgation of the guideline. Categories for disclosure include employment; leadership; stock or other ownership; honoraria, consulting or advisory role; speaker’s bureau; research funding; patents, royalties, other intellectual property; expert testimony; travel, accommodations, expenses; and other relationships. In accordance with the Policy, the majority of the members of the Expert Panel did not disclose any relationships constituting a conflict under the Policy.

CLINICAL QUESTIONS AND TARGET POPULATION

The CAP-ASH guideline⁸ addressed laboratory testing for the initial work-up for proper diagnosis, determination of

prognostic factors, and possible future monitoring of ALs. The complete set of clinical questions and corresponding recommendations is provided in The Bottom Line box. The target population for the 2017 guideline was children and adults with AL.

SUMMARY OF THE INITIAL DIAGNOSTIC WORK-UP OF AL GUIDELINE DEVELOPMENT METHODOLOGY

An Expert Panel and a Scientific Advisory Panel that included experts in pathology, medical oncology, hematology, hematopathology, pediatric oncology, and cytogenetics, a methodologist, and a patient advocate developed the CAP-ASH guideline. The literature search of OvidSP, PubMed, and Science Direct spanned 2005 to 2015. Details of the search strategies and the study inclusion criteria and outcomes of interest are available at <https://doi.org/10.5858/arpa.2016-0504-CP>.

The searches identified 124 studies for inclusion in the guideline’s qualitative synthesis of the literature. The Expert Panel reviewed data from systematic reviews, randomized controlled trials, nonrandomized controlled trials, and prospective comparative studies.

RESULTS OF THE ASCO METHODOLOGY REVIEW

The methodology review of the CAP-ASH guideline was completed independently by two ASCO guideline staff members using the Rigor of Development subscale of the AGREE II instrument. Detailed results of the scoring for this guideline are available on request from guidelines@asco.org. Overall, the guideline scored 91% (Methodology Supplement). The preliminary ASCO content reviewers of the CAP-ASH guideline and the ASCO Expert Panel found the recommendations well supported in the original guideline. Each section, including an introduction, methods, results, and guideline statements, was clear and well referenced from the systematic review.

This is the most recent information as of the publication date. For updates, the most recent information, and to submit new evidence, please visit www.asco.org/hematologic-malignancies-guidelines.

METHODS AND RESULTS OF THE ASCO UPDATED LITERATURE REVIEW

This systematic review-based guideline product was developed by a multidisciplinary Expert Panel, which included a patient representative and an ASCO guidelines staff member with health research methodology expertise (Table A1). MEDLINE data from 2015 to 2018 were searched. The search was designed to update the CAP-ASH guideline literature search and was restricted to articles published in English, and to systematic reviews, meta-analyses, and randomly assigned controlled trials. The updated search was guided by the signals¹¹ approach, which is designed to identify only new, potentially practice-changing data—

TABLE 1. Initial Work-Up of Acute Leukemia: Demographics and Physical Examination

Patent Demographic Data and History
Age
Sex
Ethnicity
Medical history (hereditary or acquired hematologic disorder or nonhematologic malignancy)
Prior or current treatment (cytotoxic, radiation, or immunotherapy)
Prior exposure to toxin
History of transfusion, growth factor use, and medications that may lead to marrow suppression or AL or that may obscure the features of AL
Family history (hematologic disorder or nonhematologic malignancy, germline predisposing condition or syndromes)
Physical examination
Organomegaly
Lymphadenopathy
Cutaneous lesion
Neurologic findings

Abbreviation: AL, acute leukemia.

signals—that might translate into revised practice recommendations. The approach relies on targeted routine literature searching and the expertise of ASCO Expert Panel members to help identify potential signals. All funding for the administration of the project was provided by ASCO. The Methodology Supplement (available at www.asco.org/hematologic-malignancies-guidelines) provides additional information about the signals approach.

The updated search yielded 34 records. A review of these results revealed no new evidence that would warrant substantive modification of the initial diagnostic work-up for AL guideline practice recommendations.

RESULTS OF THE ASCO CONTENT REVIEW

The ASCO Expert Panel reviewed the CAP-ASH guideline on initial diagnostic work-up for AL and concurs that the recommendations are clear, thorough, and based on the most relevant scientific evidence in this content area, and that they present options that will be acceptable to patients. Overall, the ASCO Expert Panel agrees with the recommendations as stated in the guideline.

DISCUSSION

The CAP-ASH guideline recommendations were reviewed and endorsed, and the following issues are discussed based on the updated literature.

Initial Diagnostics Focusing on Basic Diagnostics and Determination of Risk Parameters

Eleven of the guideline recommendations addressed this topic (Recommendations 1 to 11), and some of these

recommendations required additional discussion on the basis of different experiences of the Expert Panel in the diagnostic work-up of AL, the recently published WHO classification of hematopoietic and lymphoid tissue,¹⁰ and other updated guidelines.¹² Basic diagnostics consists of collecting relevant clinical data, physical examination results, and laboratory and imaging findings. In terms of clinical data, ethnicity or race has been slightly underestimated as a prognostic factor in the past. Hispanics have now been recognized to have the worst outcome in acute lymphoblastic leukemia (ALL), to have a higher incidence of Philadelphia (Ph)/*BCR-ABL1*-like ALL, and to have an increased risk of many adverse effects, including pancreatitis.^{13,14}

It is also recommended to document environmental and/or occupational exposure to reassess certain potential risk factors for AL, such as infection agents or exposure to formaldehyde, butadiene, or small doses of ionizing radiation.¹⁵ The study of environmental risk for pediatric AL is limited. One study showed an association between childhood AL and exposure to benzene and hydrocarbons from dwellings neighboring auto repair garages and petrol stations.¹⁶

As far as initial laboratory study is concerned, in addition to the already mentioned laboratory data, there is expert consensus (based on the National Comprehensive Cancer Network guideline¹⁷) that lactate dehydrogenase, a comprehensive metabolic panel, and phosphate and uric acid levels should be included because they are important representations of tumor lysis syndrome, which can be found particularly in patients with B-lymphoblastic lymphoma¹⁷; in addition, a coagulation panel including prothrombin time, partial thromboplastin time, and fibrinogen activity needs to be initially evaluated to detect early disseminated intravascular coagulation in patients with acute promyelocytic leukemia (APL).¹⁸

Subsequently, specific diagnostics on peripheral blood (PB) and bone marrow (BM) regarding cytomorphology and flow cytometry immunophenotyping are imperative for the final diagnosis of AL. Additional flow cytometry performed on BM aspirate is unnecessary when a flow study has been performed in the patient's PB before BM biopsy and is diagnostic. Manual differential count, flow cytometry, fluorescent in situ hybridization (FISH), and NGS are recommended to be performed on the patient's PB specimen if the PB shows sufficient blasts. (See Recommendation 4, because in some cases it is not possible to perform a BM aspiration as a result of the unstable clinical condition of the patient at diagnosis, or because of a dry tap). When a complete panel of ancillary studies has been performed on a positive PB or BM sample, no additional biopsy of extramedullary tumor is necessary, and we agree with the recommendation that a repeat biopsy is required only when PB and BM are not involved. A recent large multicenter study including 3,522 patients with acute

TABLE 2. Summary of Initial Work-Up of Acute Leukemia According to Age Group and Subclassification
Clinical and Laboratory Parameters

Patient Group	Test	Preferred Sample or Preparation	Initial Required Tests	Recommended But Not Required Tests	Monitoring Test
All patients with AL	Radiologic tests			Mediastinum, liver, spleen, nodal, extranodal, or cutaneous mass(es)	
	Routine laboratory tests	PB*	CBC data PB differential count PB smear review		
	Other laboratory tests	PB	Comprehensive metabolism panel to monitor eventual tumor lysis syndrome at start and during induction treatment†		
	BM biopsy	BM*	Morphologic examination of BM aspirate, touch imprint, cell clots, and core biopsy Examine PB for cell count of blasts and render a diagnosis if BM materials are inadequate or if BM biopsy is contraindicated or not permitted	Perform cell count and review morphology on touch imprint preparation if BM aspirate is unobtainable or there is a dry tap	
Immunophenotyping		BM*	Multicolor comprehensive flow cytometry panel to cover a diagnosis of B- or T-ALL, AML, or MPAL		Monitoring for MRD
			Perform on BM aspirate or PB if no BM aspirate is available, or perform on core biopsy tissue (unfixed, in culture media) only if BM aspirate is unobtainable		
			IHC study when necessary		
Cytogenetics		BM	Conventional karyotyping (must be performed on BM)	FISH according to the subclassification of AL	
			Use PB specimen if no BM is obtained or if there is a contraindication for BM biopsy		
Molecular study (PCR, RT-PCR, immunoglobulin, or TCR, fusion transcript, or NGS)		BM	Selective, based on the subtype of AL (BM)		Monitoring for MDS
			Use PB if no BM is obtained or there is a contraindication for BM biopsy		
Adult ALL	FISH study	BM	t(9;22)(q34.1;q11.2)/BCR-ABL1	KMT2A(MLL) gene translocation	
	Molecular study and/or NGS mutation profile‡	BM or PB	RT-PCR	PAX-5, JAK1, JAK2, and/or IKZF1 for B-ALL	Monitoring for MRD
			BCR-ABL1 fusion products	Overexpression of CRLF2 for B-ALL	
			NOTCH1 and/or FBXW7 for T-ALL		
LP and/or CSF		Fluid and cytospin	Perform cell count	Flow cytometry	Monitoring for post-treatment status
			Examine CSF cytology		
			Enumeration of blasts		
			IHC study with TdT stain†		
(continued on following page)					

TABLE 2. Summary of Initial Work-Up of Acute Leukemia According to Age Group and Subclassification (continued)
Clinical and Laboratory Parameters

Patient Group	Test	Preferred Sample or Preparation	Initial Required Tests	Recommended But Not Required Tests	Monitoring Test	
Childhood ALL	FISH study	BM	t(12;21)(p13.2;q22.1)		Monitoring for MRD	
			<i>ETV6-RUNX1</i>			
			<i>iAMP21</i>			
			Trisomy 4 and 10			
			t(9;22)(q34.1;q11.2)/ <i>BCR-ABL1</i>			
			<i>KMT2A(MLL)</i> gene translocation			
Adult AML	Molecular study and/or NGS mutation profile†	BM or PB	RT-PCR:	<i>PAX-5</i> , <i>JAK1</i> , <i>JAK2</i> , and/or <i>IKZF1</i> for B-ALL	Monitoring for MRD	
			<i>BCR-ABL1</i> fusion products	Overexpression of <i>CRLF2</i> for B-ALL		
	LP and/or CSF	Fluid and cytospin		<i>ETV6-RUNX1</i> fusion products	<i>NOTCH1</i> and/or <i>FBXW7</i> for T-ALL	Monitoring for post-treatment status
				Perform cell count	Flow cytometry	
				Examine CSF cytology		
				Enumeration of blasts		
		IHC study with TdT stain†				
	Cytochemical study		BM or PB		MPO and NSE for diagnosis and subclassification of AML‡	
	FISH study		BM	Rapid FISH for <i>PML-RARA</i> if APL is suspected		
	Molecular genetics and/or NGS mutation profile†		BM or PB	<i>FLT3-ITD</i>	<i>IDH1</i> , <i>IDH2</i> , <i>TET2</i> , <i>WT1</i> , <i>DNMT3A</i> , and/or <i>TP53</i> for prognosis and/or targeted therapy	Monitoring for MRD
<i>NPM1</i>						
<i>CEBPA</i>						
<i>RUNX1</i>						
<i>KIT</i> (when CBF AML is diagnosed)						
<i>PML-RARA</i> if APL is suspected or diagnosed†						
<i>RUNX1-RUNX1T1</i> or <i>CBFB-MYH11</i> if the CBF AML is diagnosed†						
LP and/or CSF		Fluid and cytospin	Perform cell count			
Other laboratory tests		PB		Examine CSF cytology		
				Enumeration of blasts by a pathologist		
				for patients who are receiving IT therapy or patients with AML without contraindication		
			Flow cytometry			
			DIC profile only if APL is suspected			

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TABLE 2. Summary of Initial Work-Up of Acute Leukemia According to Age Group and Subclassification (continued)
Clinical and Laboratory Parameters

Patient Group	Test	Preferred Sample or Preparation	Initial Required Tests	Recommended But Not Required Tests	Monitoring Test
Childhood AML	Cytochemical study	BM or PB		MPO and NSE for diagnosis and subclassification of AML§	
	Molecular study and/or NGS mutation profile†	BM or PB	<i>FLT-ITD</i>	<i>IDH1</i> , <i>IDH2</i> , <i>TET2</i> , <i>WT1</i> , <i>DNMT3A</i> , and/or <i>TP53</i> for prognosis and/or targeted therapy	Monitoring for MRD
			<i>NPM1</i> <i>CEBPA</i> <i>RUNX1</i>	KIT (when CBF AML is diagnosed)	
			PML-RARA if APL is suspected or diagnosed† <i>RUNX1-RUNX1T1</i> or <i>CBFB-MYH11</i> if the CBF AML is diagnosed†		
	LP and/or CSF	Fluid and cytospin		Perform cell count Examine CSF cytology Enumeration of blasts by a pathologist for patients who are receiving IT therapy or patients with AML without contraindication Flow cytometry	
	Other laboratory tests	PB		DIC profile only if APL is suspected	
Adult or childhood MPAL	FISH	BM	t(9;22)(q34.1;q11.2)/ <i>BCR-ABL1</i> <i>KMT2A(MLL)</i> gene translocation		
	Molecular study	BM or PB	Real-time quantitative RT-PCR for <i>BCR-ABL1</i> fusion product		Monitoring for MRD
AL with extramedullary manifestation but without BM or PB involvement	Biopsy	Tissue*	Morphologic examination		
	Immunophenotyping	Tissue*	IHC		
	Cytogenetics	Tissue	Karyotyping (fresh tissue) or FISH (cytospin or paraffin-embedded tissue) according to the subclassification of AL	Fresh tissue for flow cytometry	
		Tissue	PCR and/or NGS mutation tests according to the subclassification of AL		
Molecular study and/or NGS mutation profile‡	Tissue			Monitoring MRD	

Abbreviations: AL, acute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; B-ALL, B-lymphoblastic leukemia; BM, bone marrow; CBF, core-binding factor; CBF AML, core-binding factor acute myeloid leukemias, including AML with t(8;21)(q22;q22.1)/*RUNX1-RUNX1T1*, or inv(16)(p13.1q22)/*CBFB-MYH11*; CSF, cerebrospinal fluid; DIC, disseminated intravascular coagulation; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; IT, intrathecal; LP, lumbar puncture; MDS, myelodysplastic syndromes; MPAL, mixed phenotypic acute leukemia; MPO, myeloperoxidase; MRD, minimal residual disease; NGS, next-generation sequencing; NSE, nonspecific esterase; PB, peripheral blood; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-quantitative polymerase chain reaction; T-ALL, T-lymphoblastic leukemia; TCR, T-cell receptor; TdI, terminal deoxynucleotidyl transferase.

*It is recommended that the harvested specimens (fresh, frozen, or paraffin embedded) and their products (ie, nucleic acid) should be properly identified and stored under appropriate conditions in a laboratory for molecular or genetic study (for additional diagnostic, prognostic, or therapeutic purpose), in case no sufficient blasts yielded in post-treatment specimen.

†Not included in College of American Pathologists–American Society of Hematology guideline but suggested by ASCO Endorsement Expert Panel.

‡Molecular studies do not include global or gene-specific methylation, microRNA expression, or gene expression analysis, which is not recommended for diagnostic purpose.

§Sudan Black B, periodic acid-Schiff, or acid phosphatase is not recommended as a routine test for AL.

myeloid leukemia (AML) showed that the presence of an extramedullary myeloid disorder (EMD) or the number of specific sites of EMD did not have independent prognostic value.¹⁹ Thus, the treatment plan for these patients is based on identified AML prognostic factors such as WBC count or cytogenetics. NGS study in the patients with simultaneous EMD and AML showed no differences in the two specimens, suggesting that the molecular profile obtained from leukemic BM is sufficient and representative of the molecular signature in EMD.²⁰

According to a recent CAP survey study, flow cytometric immunophenotyping (FCI) and karyotype analysis have now been implemented routinely in most diagnostic laboratories in the United States (99% and 96%, respectively), whereas cytochemical studies are less common (24.8% for AML and 14.2% for ALL).²¹ In current practice, these tests have been largely replaced by FCI and have little use in the subclassification of AL. Therefore, cytochemical studies are proposed as supplemental tools, only for those cases where limited PB or BM is available for FCI or where FCI results are ambiguous. In certain low- and middle-income countries, these techniques may still have their value when other diagnostic tools have not been established.

The ASCO Expert Panel recommends that, besides BM and PB, cerebrospinal fluid (CSF) be collected for pathologist evaluation for all patients with ALL at diagnosis, after treatment follow-up, and when a CNS relapse is suspected. CAP-ASH expert consensus opinion is to collect and examine CSF from patients with AML who are receiving intrathecal therapy, although the incidence of CNS involvement in AML is lower than in ALL.^{22,23} The Expert Panel recommends immunophenotyping studies as an additional detection technique next to cytomorphologic examination of cytopins, particularly for those with a low-level involvement of leukemic cells in the CSF that cannot be assessed well by morphologic examination only.^{24,25} Terminal deoxynucleotidyl transferase immunohistochemistry staining on cytopins has been used alternatively for detection of CNS disease in ALL,^{26,27} and evaluation of CSF by multicolor FCI has been adopted recently in some centers.²⁸ FCI, using at least six, but nowadays in some laboratories eight to 10 colors, has led to more specific and sensitive diagnostics.^{24,29} This has allowed for improved detection of CNS involvement. The use of molecular tools (ie, polymerase chain reaction [PCR] and NGS) for low-level CSF involvement is still under study. However, this is not feasible in every laboratory, and cost efficiency is questionable.

Molecular Markers and Minimal Residual Disease Detection

This section was addressed by 10 of the recommendations (Recommendations 12 to 21). The discussion here was mainly based on the results of translational research, supported by better molecular detection techniques.

Molecular diagnostics have been developing in the past few years, and many molecular markers have been included as one of the key diagnostic criteria in the revised 2017 WHO classification.¹⁰ Substantial changes concerning molecular diagnostics have been made in the CAP-ASH guidelines.⁸

Cytogenetic characterization (including ploidy status) and molecular studies can be performed by karyotyping, FISH, PCR, and new mutation detection techniques such as NGS, RNA sequencing, and whole-genome sequencing. FISH and PCR studies should be conducted for additional molecular evaluation at initial diagnosis of AL. An increasing number of molecular aberrations have been discovered by mutation analysis using PCR and/or NGS.

These mutation tests should be performed simultaneously when karyotyping or a FISH study is performed. Although molecular techniques such as FISH and PCR might be replaced by NGS in the not too distant future, NGS has not been widely implemented in current laboratories. The Expert Panel considers that this and other new detection techniques will be adopted in the near future and soon will become an integral part of modern work-up. Besides cytogenetic characterization and detection of molecular targets, determination of signatures for minimal residual disease (MRD) monitoring is an important part of the diagnostic work-up.

Molecular Characterization of ALL

For pediatric B-lymphoblastic leukemia (B-ALL), the traditional targets as described in Recommendations 13 and 14 (such as t(12;21)(p13.2;q22.1)/*ETV6-RUNX1*, t(9;22)(q34.1;q11.2)/*BCRABL1*, *KMT2A (MLL)* translocation, *iAMP21*, and trisomy 4 and 10) should be determined. In adult patients, the pathologist or treating clinician should ensure that at least testing for t(9;22)(q34.1;q11.2)/*BCR-ABL1* is performed. In order not to miss some cryptic or atypical translocation by karyotyping in ALL cases, FISH or PCR for *BCR-ABL1* should be performed in concordance with NGS studies of ALL that may be adopted to identify cryptic or submicroscopic genetic alterations that define all B-ALL subtypes including *BCR-ABL1*-like variant.³⁰

In addition, testing for *KMT2A (MLL)* translocations is recommended. Furthermore, *NOTCH1* and *FBXW7* mutations should be evaluated in patients with T-lymphoblastic leukemia (T-ALL).^{1,31} A recent study of 131 patients with T-ALL demonstrated that the *NOTCH1/FBXW7/RAS/P TEN* status and lactate dehydrogenase level are independent prognostic factors for outcome in T-ALL.³²

Because the *IKZF1* (Ikaros family zinc finger) gene is frequently deleted in adults and children with B-ALL and was shown to have independent prognostic significance and to be associated with poor clinical outcome in the context of current risk-adapted protocols, *IKZF1* should be regularly included in the screening panel for all patients with ALL.^{33,34} In addition, a recent study showed that

altered *IKZF1* predicted a high risk in pediatric patients with ALL with high hyperdiploidy and in those without classifying genetic abnormalities.³³ Thus, the presence of *IKZF1* alteration will change the risk stratification for the two karyotyping groups, emphasizing its prognostic importance. The determination of the pattern of mutation and the variant allele frequency of *IKZF1* is also critical. Dominant-negative *IKZF1* alterations or low variant allele frequency did not have a prognostic value, whereas a loss-of-function mutation with a high deletion load was associated with an inferior outcome (overall survival at 5 years, 28% v 59%; $P < .0001$ in *BCR-ABL1*-like cases).³⁴ Although deep gene sequencing is useful for the detection of *IKZF1*, the cost of the test may limit its use as a routine laboratory test. A breakpoint-specific multiplex PCR assay has been introduced in some laboratories to detect *IKZF1* intragenic deletions and to monitor residual disease.³⁵ Patients who are diagnosed with B-ALL and are *BCR-ABL1* negative may be analyzed by NGS or other molecular study for *PAX-5*, *JAK*, *IKZF1*, and *CRLF2* gene alterations, other evidence of a *BCR-ABL1*-like signature, *MEF2D* fusion, *ZNF 384* fusion, *TCF3-PBX1*, and *DUX4/ERG* fusions.^{36,37} Regarding these new targets, it is more important to look for *ABL*-class fusion genes or those involving the JAK-STAT pathway, which are more actionable than other genes recommended.^{30,38,39} Of note, *BCR-ABL1*-like ALLs are mutually exclusive with B-ALL with sentinel translocations (*BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, and *KMT2A MLL*). Patients with *BCR-ABL1*-like ALL and *ABL*-class fusions have the promising prospect of being sensitive to tyrosine kinase inhibitors, similar to those harboring the *BCR-ABL1* gene rearrangement.^{40,41}

Unlike studies for *CRLF2* expression, which can be detected by a flow cytometry study in B-ALL, alternative studies for the detection of *IKZF1* or *PAX-5* alterations are limited. A recent study using array comparative genomic hybridization was able to identify alterations of *IKZF1*, *PAX5*, and *CDKN2A/B* in 43%, 52%, and 57% of cases, respectively. A FISH study using probes to detect these three alterations led to false negativity in 10%, 40%, and 28% of the cases, respectively.⁴²

Molecular Characterization of AML

In the absence of a full karyotype, FISH studies or PCR assays for recurring cytogenetic abnormalities in AML are indicated. FISH or PCR for *PML-RARA* should be performed for all cases of suspected APL.⁷

An increasing number of additional cytogenetic aberrations have been identified as having a prognostic role in patients with AML.⁵ Based on these findings, the pathologist or treating clinician should ensure that testing for *FLT3-ITD* is performed because this mutation is associated with poor outcome.⁴³⁻⁴⁵ 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.⁴⁶⁻⁴⁹ Not limited in

adult AML, *IDH* and *IDH2* have been described to be related to poor prognosis but appear in limited numbers in children. Similarly, *TP53* mutation is also an independent prognostic factor that should be tested; it is associated with dismal clinical outcome in childhood leukemia.⁵⁰

For both adult and pediatric patients with confirmed core-binding factor (CBF) AML (AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* or inv(16)(p13.1q22)t(16;16)(p13.1;q22); *CBFB-MYH11*), the pathologist or treating clinician should ensure that appropriate mutational analysis for *KIT* is performed. This recommendation is strong in adults, whereas in pediatric patients this advice is an expert consensus opinion.⁵¹ In pediatric AML, the prognostic impact of *KIT* in CBF AML remains unclear, because several publications refer to similar,⁵²⁻⁵⁴ and others to poor, prognosis compared with nonmutated cases, in contrast to a strong association with a poorer prognosis in adult AML with this mutation.⁵⁵

For patients other than those with confirmed CBF AML, APL, or AML with myelodysplasia-related cytogenetic abnormalities, the pathologist or treating clinician should ensure that mutational analysis for *NPM1*, *CEBPA*, and *RUNX1* is also performed. Because most of these parameters are unknown at the time of the initial work-up of a patient with AL, it may be more practical for these mutation tests to be performed simultaneously with karyotyping or FISH study, when they are performed.

For patients with confirmed mixed-phenotype AL, 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. In some cases, detection of an ALL-specific translocation such as t(12;21)(p13.2;q22.1) *ETV6-RUNX1* may help clarify the diagnosis.⁵⁶

Selected Molecular Markers in Preparation of Detection of MRD

Emerging evidence supports molecular studies as principle tests for monitoring MRD of AL. The key molecular markers included in the initial work-up will be carried on for monitoring MRD (eg, *PML-RARA*, *RUNX1-RUNX1T1*, *CBFB-MYH11*, *NPM1*, *CEBPA*, *RUNX1*, and *KIT*).¹² However, it is unclear whether a large screening panel should be applied for MRD detection. A recent multicenter study cohort of 346 *NPM1*-mutated patients with AML (17 clinical trials) shed light on this issue.⁵⁷ The study showed that *NPM1*-mutated transcripts were identifiable in the blood of a subset of patients (15%) after the second cycle of chemotherapy. The mutated subgroup of patients had a greater risk of relapse and an inferior survival rate when compared with the patients with wild-type *NPM1* (82% v 30%; hazard ratio [HR], 4.80 [95% CI, 2.95 to 7.80]; $P < .001$; and 24% v 75%; HR for death, 4.38 [95% CI, 2.57 to 7.47]; $P < .001$, respectively). Multivariate analysis showed additional detectable MRD to be the only independent prognostic factor for survival (HR, 4.84 [95% CI, 2.57 to 9.15]; $P < .001$).⁵⁷ Prior studies have shown that certain preleukemic genes (frequently *DNMT3A*,

ASXL1, and *TET2*) can be persistent during therapy. However, they are not reliable markers for MRD.⁵⁸⁻⁶⁰ Ivey et al,⁵⁷ using a 51-target gene sequencing panel, also concluded that these preleukemic clones, although lasting, were less critical than *NPM1* in the patient with gene-mutated AML, indicating that real-time quantitative PCR *NPM1* study is the better diagnostic tool in monitoring for MRD in this setting. Besides the aforementioned markers, it is of importance to screen other molecular markers that have predictive or prognostic value in the individual and to monitor them for MRD. A recent consensus from the European LeukemiaNet MRD working group proposed that for detection of molecular MRD, the real-time quantitative PCR platform is preferred to NGS and digital PCR platforms. The latter must be further validated. As well, tests with less sensitivity (eg, Genescan-based fragment analysis for *FLT3* aberrations) are not encouraged for the detection of MRD.⁵⁸

Context of Referral to Another Institution With Expertise in the Management of AL

This topic was addressed in Recommendations 22 to 24. The Expert Panel emphasizes the point that referral to an institution with specific expertise is of major importance to the central work-up of AL.

Final Reporting and Record Keeping

This has been described in Recommendations 25 to 27. The Expert Panel also recommends generating a complete report, with basic diagnostics (at least cytomorphology and flow cytometry) within 48 to 72 hours after preliminary diagnosis. This should be followed by a complete final comprehensive report, including available risk factors when all requested test results are available, ideally within 1 week, or extended to 2 weeks. If any result from ordered ancillary studies would change the original diagnosis or subclassification, an amended report should be issued to reflect the change. The treating physician should be informed.

A summary of the initial required tests, recommended but not required tests, and alternative tests for the initial work-up of AL, including general, age-, and disease-specific approaches according to CAP-ASH guidelines with suggestions from the ASCO Endorsement Expert Panel is presented in [Tables 1 and 2](#).

CONCLUSION

These guideline recommendations have been reviewed on the basis of available and mostly updated literature between 2015 and 2018 and on the expertise of the Expert Panel. The discussion points included mostly address issues regarding diagnostics that involve flow cytometry and molecular techniques.

Cytomorphologic assessment is essential for the initial diagnosis of AL. Multicolor (eight to 10) FCI has led to better

distinction among myeloid, lymphoid, or mixed lineage blast origin, even when the number of cells is limited (ie, CNS involvement, fine-needle aspirate of extramedullary leukemic infiltration, or skin biopsy for leukemic cutis). New targets identified by advanced molecular techniques offer the possibility of better risk stratification. Although molecular techniques have been developed quickly, and it is tempting to use them for initial diagnostics, not all laboratories will have all techniques currently available.

The Expert Panel strongly advises clinicians to distinguish between diagnostics that are needed in the first phase to start treatment (by available karyotyping, FISH, and PCR techniques, or if possible, NGS) and subsequently treatment stratification, in contrast to the use of the findings in broader research (ie, whole-exome sequencing, whole-genome sequencing, RNA sequencing, and epigenome study). Central work-up should be performed in a cancer center or a university-based hospital when possible. Finally, complete reporting, including notification of the major risk and stratification factors, should be included in one final report, preferably available within 2 weeks of diagnosis.

ENDORSEMENT RECOMMENDATION

ASCO endorses the initial diagnostic work-up for AL guideline from CAP and ASH.

ADDITIONAL RESOURCES

More information, including a Data Supplement with a reprint of all the CAP-ASH initial diagnostic work-ups for AL guideline recommendations, a Methodology Supplement, slide sets, and clinical tools and resources, is available at www.asco.org/hematologic-malignancies-guidelines. Patient information is available at www.cancer.net.

RELATED ASCO GUIDELINES

- Integration of Palliative Care Into Standard Oncology Care⁶¹ (<http://ascopubs.org/doi/10.1200/JCO.2016.70.1474>)
- Patient-Clinician Communication⁶² (<http://ascopubs.org/doi/10.1200/JCO.2017.75.2311>)
- Antimicrobial Prophylaxis for Adult Patients With Cancer-Related Immunosuppression⁶³ (<http://ascopubs.org/doi/10.1200/JCO.18.00374>)
- Outpatient Management of Fever and Neutropenia in Adults Treated for Malignancy⁶⁴ (<http://ascopubs.org/doi/10.1200/JCO.2017.77.6211>)
- Platelet Transfusion for Patients With Cancer⁶⁵ (<http://ascopubs.org/doi/10.1200/JCO.2017.76.1734>)

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Editor's note: This American Society of Clinical Oncology (ASCO) Clinical Practice Guideline provides recommendations, with comprehensive review and analyses of the relevant literature for each recommendation. Additional information, including a Data Supplement with additional evidence tables, a Methodology Supplement, slide sets, clinical tools and resources, and links to patient information at www.cancer.net, is available at www.asco.org/hematologic-malignancies-guidelines.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT AND DATA AVAILABILITY STATEMENT

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**Initial Diagnostic Work-Up of Acute Leukemia: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists and American Society of Hematology Guideline**

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APPENDIX

TABLE A1. Initial Diagnostic Work-Up of Acute Leukemia: ASCO Clinical Practice Guideline Endorsement of the CAP and ASH Guideline Expert Panel Membership

Name (Designation)	Affiliation or Institution	Role or Area of Expertise
Valérie de Haas, MD (co-chair)	Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands	Pediatric oncologist
Ling Zhang, MD (co-chair)	Moffitt Cancer Center, Tampa, FL	Hematopathologist
Rob Pieters, MD	Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands	Pediatric oncologist
Dipti Patel Donnelly, MD	Virginia Cancer Specialists, Fairfax, VA	Hematology or medical oncologist
Anjali Advani, MD	Cleveland Clinic, Cleveland, OH	Hematology or medical oncologist
Kendra Sweet, MD	Moffitt Cancer Center, Tampa, FL	Hematology or medical oncologist
Ching-Hon Pui, MD	St Jude Children's Research Hospital, Memphis, TN	Hematology or medical oncologist
Daniel A. Arber MD	University of Chicago Medical Center, Chicago, IL	Hematopathologist
Raetasha S. Dabney, MD	Keesler Medical Center, Ocean Springs, MS	PGIN representative
Elizabeth Kitlas	The Leukemia and Lymphoma Society, Rye Brook, NY	Patient representative
Nofisat Ismaila, MD	American Society of Clinical Oncology, Alexandria, VA	Staff or health research methodologist

Abbreviations: ASH, American Society of Hematology; CAP, College of American Pathologists; PGIN, practice guidelines implementation network.