

Reporting blast percentage for response assessment in acute leukemias: recommendations from an EHA/ELN expert panel

by Sa A. Wang, Leonor Arenillas, Francesco Buccisano, Monika Bruggemann, Wolfgang Kern, Manuel Menes, Adriana Plesa, Louisa Stone, Dominique Wellnitz, David A. Westerman, Brent L. Wood and Sylvie D. Freeman

Received: May 15, 2025.

Accepted: August 8, 2025.

Citation: Sa A. Wang, Leonor Arenillas, Francesco Buccisano, Monika Bruggemann, Wolfgang Kern, Manuel Menes, Adriana Plesa, Louisa Stone, Dominique Wellnitz, David A. Westerman, Brent L. Wood and Sylvie D. Freeman. Reporting blast percentage for response assessment in acute leukemias: recommendations from an EHA/ELN expert panel.

Haematologica. 2025 Sept 4. doi: 10.3324/haematol.2025.288228 [Epub ahead of print]

Publisher's Disclaimer.

E-publishing ahead of print is increasingly important for the rapid dissemination of science.

Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication.

E-publishing of this PDF file has been approved by the authors.

After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in a regular issue of the journal.

All legal disclaimers that apply to the journal also pertain to this production process.

Reporting blast percentage for response assessment in acute leukemias: recommendations from an EHA/ELN expert panel

Sa A. Wang¹, Leonor Arenillas², Francesco Buccisano³, Monika Bruggemann⁴, Wolfgang Kern⁵, Manuel Menes⁶, Adriana Plesa⁷, Louisa Stone⁸, Dominique Wellnitz⁴, David A. Westerman⁹, Brent L. Wood¹⁰, Sylvie D. Freeman¹¹

1. Department of Hematopathology, MD Anderson Cancer Center, Houston, USA
2. Department of Pathology, Hospital del Mar, Barcelona, Spain
3. Department of Biomedicine and Prevention, University Tor Vergata, Rome, Italy
4. Medical Department II, Hematology and Oncology, University Hospital Schleswig-Holstein, Kiel, Germany
5. MLL Munich Leukemia Laboratory, Munich, Germany
6. Department of Pathology, Baptist Health South Florida, Miami, Florida, USA
7. Department of Hematology Laboratory and FlowCytometry, Lyon University Hospital, CHU-HCL, Lyon Sud, Pierre Benite, France
8. Auckland City Hospital, Auckland, New Zealand
9. Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia
10. Department of Pathology and Laboratory Medicine, Children's Hospital of Los Angeles, CA, USA
11. School of Infection, Inflammation and Immunology, University of Birmingham, UK

Corresponding Author

Sylvie D Freeman
s.freeman@ bham.ac.uk

Author Contributions

Sa A. Wang, panel co-chairperson, writer, **Leonor Arenillas**, panel member, writer, **Francesco Buccisano**, panel member, writer, **Monika Bruggemann**, panel member, writer, **Wolfgang Kern**, panel member, writer, **Manuel Menes**, panel member, writer, **Adriana Plesa**, panel member, writer, **Louisa Stone**, panel member, writer, **Dominique Wellnitz**, panel member, writer, **David A. Westerman**, panel member, writer, **Brent L Wood**, panel member, writer, **Sylvie D Freeman**, panel co-chairperson, writer.
All panel members contributed equally.

Disclosures.

SAW, no competing interest, **LA**, no competing interest, **FB**, Consulting/Advisory Boards: Jazz Pharmaceuticals, Laboratoires Delbert, Novartis, Speaker: Astellas, Bristol Myers Squibb, Janssen-Cilag, Servier, **MB**, Speaker, travel support: Amgen, BD, Janssen, Pfizer, Advisory Board: Amgen, AstraZeneca, Hello Healthcare, Incyte, **WK**, part ownership of MLL Munich Leukemia Laboratory, **MM**, no competing interest, **AP**, no competing interest, **LS**, no competing interest, DoW, no competing interest, **DAW**, no competing interest, **BLW**, Advisory Board: Amgen, Kite Pharma, **SF**, Advisory Board: MPAACT, Speaker: Novartis, Jazz Pharmaceuticals, Research Funding: Jazz Pharmaceuticals (Inst), Bristol Myers Squibb/Celgene (Inst), AstraZeneca (Inst), Cytex (Inst).

Abstract

Evaluation of bone marrow blast percentage is paramount to response criteria in acute leukemias. There is an identified need within the framework of updated laboratory practices to reduce inconsistencies in methodologies used by clinical laboratories to report blast values and clarify aspects of reporting.

Representatives from international specialised working groups including the European Hematology Association (EHA) Diagnosis in Hematological Diseases Specialised Working Group and the European LeukemiaNet (ELN) produced consensus guidance for harmonised blast assessment to define response categories in acute leukemic patients. These address sampling best practice, key considerations for generating the most accurate blast enumeration and the limitations across the methodologies in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and acute leukemia of ambiguous lineage. An integrated reporting scheme for deriving blast percentage is provided for ALL and AML. This incorporates results from appropriate measurable residual disease assays with morphological crosscheck.

The practical guide and approach presented herein should facilitate uniform reporting standards both within clinical trials and in broader clinical practice.

Introduction

Evaluation of therapeutic response in acute leukemia requires a documented bone marrow blast percentage. According to the International Working Group (IWG 2003) criteria¹, this percentage is determined based on morphological blast count. Although this method has been widely used in clinical practices over the years, it is recognised as imperfect due to its subjective nature, limited ability to distinguish non-neoplastic from leukemic blasts, and significant interoperator variation, even among experts.

Over the past two decades, clinical laboratory practices have evolved with advancements in integrated reporting, clinical flow cytometry and access to measurable residual disease (MRD) assays. Strong evidence supports MRD assays in measuring treatment response in acute leukemias.²⁻¹⁰ Such assays are intended to directly identify residual leukemia for which morphologic blasts are a surrogate. There remains, however, uncertainty in defining blasts for response categories with concerns in the community regarding a certain lack of standardisation in how blast percentages are derived. The reporting of blast percentage in post-treatment bone marrow (BM) has significant implications, affecting clinical trial endpoints, inclusion eligibility for relapse/ refractory studies, comparison of real world outcomes and international training in hemato-oncology diagnostics.

Following a 2024 FDA mini symposium (“the Methodology of Quantitating Blasts in Acute Leukemias for Response Assessments”), which highlighted inconsistencies and practical challenges in methodologies used by clinical laboratories for reporting blast percentages, a panel of experts in acute leukemia diagnostics was convened. The panel includes representatives from the European LeukemiaNet (ELN), the European Hematology Association (EHA) Diagnosis in Hematological Diseases Specialised Working Group and other large international centers involved in adult and pediatric acute leukemia treatment. This paper presents the panel’s consensus guidance on post treatment BM blast enumeration in acute leukemia that incorporates appropriate ancillary tests, and aligns with updated clinical laboratory practice. The recommendations provide criteria for harmonizing reporting across institutions, facilitating comparability of data, and enhancing the reliability of response assessment in acute leukemia.

Sampling

Information on time from treatment and PB counts are required for best interpretation and is particularly important in the case of BMs taken before count recovery. Although early (day 14-21) BMs remain common practice in some centers to guide the use of a second induction, BMs to categorise remission status should be performed at count recovery or when count recovery is expected (~day 28-35 with intensive chemotherapy).

For post-treatment BM examination, practices vary across different treatment protocols, patient populations, institutions, and geographic regions. Some centers perform only BM

aspiration, with or without clot preparation for histology, while others routinely include trephine biopsy and touch imprints in addition to BM aspirate.

Operators should avoid allocating excessive BM aspirate volume for cytomorphology as this compromises the “first pull” quality of the MRD sample. No more than 0.5 mL BM aspirate should be used for BM smears. The remaining volume of the same, single (“first pull”) aspiration, up to a maximum of 4mL, should be reserved for the most appropriate MRD assay(s), divided equally between molecular and flow samples as needed. Two mL of BM per MRD assessment is usually sufficient when peripheral blood counts are near normal. Hemodilution resulting in potentially significant MRD underestimation occurs after the first 2-4 mLs of BM aspiration.¹¹ EDTA anticoagulant is generally preferred for molecular MRD testing^{7, 12} and is also acceptable for cytomorphology and flow cytometry.¹³ The best practice is to prepare BM aspirate smears at patient bedside, not only to preserve cytomorphology but also to examine whether BM particles are present and, if the quality is insufficient, BM aspirate can be repeated immediately.

Evaluation criteria for adequate aspirate samples are provided in [Table 1](#).

The group acknowledged that the inclusion of a BM trephine biopsy at remission assessment is not routine practice in many countries. However, BM trephine biopsy should be performed when aspirate material is insufficient or at repeat testing when prior BM aspirate is inadequate for differential counting because of insufficient quality, either due to necrosis, fibrosis, hypocellularity, or patchy blast involvement. In these instances touch imprints of the trephine biopsy (ideally 2-3 slides) should be made as they can provide improved cytomorphological assessment in these scenarios.

Morphology

An adequate BM smear prepared by the squash (crush) or wedge method should contain multiple spicules and a well-represented mix of hematopoietic cells, including megakaryocytes, nucleated erythrocytes, granulocytes and their precursors. The presence of histiocytes, plasma cells, and mast cells further confirms proper marrow representation ([Table 1](#)). Smears should be well spread in a thin monolayer, evenly stained using high quality Wright-Giemsa or May-Grünwald-Giemsa, and free of artifacts. Cells should be well-preserved, displaying clear nuclear and cytoplasmic details.

BM smears are considered inadequate ([Table 1](#)) when marrow particles are absent or scarce, as a result of excessive hemodilution (dry tap), or when spicules are present but acellular, necrotic, or degenerative. Inadequacy may also arise from improper air-drying, fixation issues, poor cell preservation, or suboptimal staining quality. The term "suboptimal" refers to smears of intermediate quality between "adequate" and "inadequate," where marrow cells are present and countable but are hemodiluted or have low cellularity. In such cases, a disclaimer should be provided to comment on the quality of the specimens.

Morphologic counting should be performed near BM spicules, where hematopoietic cells are most concentrated. Multiple smears should be assessed, particularly in cases with a patchy blast distribution. BM blast enumeration is based on a 500-cell count^{10, 14}. The group recognized that blast counting is prone to interobserver variability. Distinguishing leukemic blasts from “blast mimics” ([Supplementary Table S1](#)) such as regenerating myeloid precursors, reactive monocytes, or hematogones (normal precursor B cells) or plasmacytoid dendritic cells, can be challenging, and in many instances, impossible. Additionally, dysplastic myelocytes, promyelocytes, and erythroblasts may be misclassified as blasts. Blasts can also be underestimated due to unusual cytomorphology. For example, small blasts may be mistaken for lymphocytes, blasts with basophilic cytoplasm may be misidentified as pronormoblasts, atypical lymphocytes or monocytes, or granulated blasts may be confused with promyelocytes.

Integrated Remission Reporting

Acute lymphoblastic leukemia (ALL)

In acute lymphoblastic leukemia (ALL), MRD performed by an appropriate validated assay is recommended to replace morphology as the gold standard to assess BM remission.^{2-6, 15} A morphological blast count $\geq 5\%$ does not define persistent disease or relapse unless confirmed as leukemic by an appropriate MRD assay such as flow cytometry, quantitative (q)PCR and next-generation sequencing (NGS) or by diagnostic genetic assays (fluorescence in situ hybridization (FISH) or karyotyping). Conversely, a morphologic blast count of $<5\%$ no longer defines remission if MRD testing is positive. The primary reporting of response blast percentage should be done by an MRD assay having a minimum validated sensitivity of 0.01%, such as flow cytometry, allele-specific qPCR or amplicon NGS to quantify clonal immunoglobulin (IG) and/or T-cell receptor (TR) gene rearrangements.

It is important to note that BM sample quality and hemodilution cannot be assessed by molecular MRD assays. This limitation also applies to flow cytometry ALL MRD assays, unless appropriate myeloid markers are incorporated to evaluate hemodilution by the percentage of granulocytes or mast cells.¹¹

Cytomorphological assessment of smears can serve as an indicator of MRD sample adequacy if both originate from the same (first pull) BM aspirate source. We recommend cytomorphology for this purpose and to identify any major discrepancy between the blast count and MRD results. The latter is particularly important for identifying potential MRD target loss, such as from lineage switch, especially in the case of ALL with *KMT2Ar* (loss of flow cytometry or IG/TR leukemic markers), *DUX4r*, *ZNF384r*- or *PAX5-P80R*- ALL¹⁶ or post immunotherapy^{17, 18} (loss of flow cytometry leukemic gating markers like CD19 and CD22).

Additionally, cytomorphology can also identify any significant underestimation or overestimation of aspirate leukemic blast percentage by the MRD assay due to issues with MRD sample quality, such as delayed transit or overlysis of nucleated red blood cells (RBCs).

Recommendations for reporting blast percentage with appropriate sample qualifiers in the integrated response assessment are shown in [Figure 1](#). Importantly, it is agreed that the blast percentage of a MRD positive BM sample should only be reported by the cytomorphological count if there is a major discrepancy in blast category³ between cytomorphology and the MRD assay and, critically, if there is a high probability that cytomorphology is more accurate due to MRD target loss or a non-representative MRD sample.

Key considerations for generating the most accurate blast enumeration in ALL during remission assessments are summarised below with recommended blast detection methods.

Flow Cytometry

The group noted variation in the cell denominators used to calculate the flow cytometric ALL MRD percentage in published clinical validation studies (total nucleated cells or non-erythroid cells or mononuclear cells). This variation extends to the current assays used by the different international trial networks. The denominator of total nucleated cells aligns with cytomorphological blast counting and many molecular methods. Incorporating a nuclear dye improves accuracy, particularly when erythrocyte lysis is suboptimal. Preservation of nucleated erythroid cells through use of fixative containing lysing reagents, optimizing cell recovery during wash steps, and use of low forward-scatter acquisition thresholds/discriminators is important for accurate enumeration. If preservation of nucleated erythroid cells may be suboptimal, integrated reporting with cytomorphology is necessary to identify cases with erythroid hyperplasia. In an MRD positive sample, the MRD percentage values reported using non-erythroid cells as the denominator may be significantly higher than MRD values derived from total nucleated cells when there is pronounced erythroid hyperplasia. The use of mononuclear cells as denominator is not recommended for assessing BM remission.

Following targeted therapies, such as anti-CD19 therapy in B-ALL, alternative primary gating strategies should be applied for leukemic cell identification.¹⁹⁻²¹

Molecular Methods

IG/TR MRD assessment, either using qPCR or NGS, is considered the molecular gold standard to quantify MRD in ALL²²⁻²⁴. In *KMT2A*-rearranged ALL, IG/TR rearrangements may be absent or display clonal evolution during the disease course, compromising MRD monitoring. In

contrast, the *KMT2A* rearrangement is a highly stable marker and therefore the recommended MRD marker in this molecular subgroup as measured by qPCR.^{25, 26} In Philadelphia chromosome (Ph)+ ALL, reverse transcriptase qPCR (RT-qPCR) of *BCR::ABL1* is commonly used to monitor MRD. RT-qPCR quantifies gene expression at the cDNA level, with the transcript level normalized against a housekeeping gene (typically *ABL* or *GUS*). As a result, MRD values by RT-qPCR cannot be directly compared with MRD measured by IG/TR or flow cytometry. In addition, the *BCR::ABL1* translocation is not restricted to the ALL compartment in about 40% of Ph+ ALLs²⁷⁻²⁹, leading to significant discrepancy between *BCR::ABL1* by RT-qPCR and MRD by IG/TR or flow cytometry in a considerable fraction of patients. *BCR::ABL1* transcripts can be found in different lineages including mature myeloid cells. We therefore emphasize that percentage leukemic blasts should not be reported from positive *BCR::ABL1* MRD values. However, monitoring *BCR::ABL1* remains relevant for guiding TKI therapy in Ph+ ALL^{5, 6}.

Discordance between molecular MRD and flow cytometry or cytomorphology is also possible in other subtypes of B-ALL when small monocytic subpopulations derived from the leukemic clone persist after therapy (e.g. Ph-like B-ALL) or undergo monocytic transdifferentiation (e.g. DUX4 rearranged ALL). In T-ALL, discrepancies between molecular MRD and cytomorphology or flow cytometric values may result from post therapy leukemic differentiation to a more mature immunophenotype akin to mature T cells.

Cytomorphology

Blast mimics ([Supplementary Table S1](#)) and aspirate adequacy should be considered. Assessing blasts based on BM trephine biopsy will address the concern for BM aplasia, necrosis or fibrosis but does not reach the level of accuracy and sensitivity of flow cytometry and IG/TR NGS for MRD.

Acute Myeloid Leukemia (AML)

Recommendations for reporting blast percentage with appropriate sample qualifiers in the integrated response assessment of AML are shown in [Figure 2](#).

In AML, it is strongly recommended to perform MRD analysis using an appropriate assay (flow cytometry, qPCR or NGS) having a minimum validated threshold of 0.1% in accordance with the ELN recommendations.^{7, 10} This recommendation ensures uniform response reporting for all AML patients. An MRD negative result by the appropriate residual disease assay(s) should be considered as remission at a level of blasts <0.1% (or < limit of detection of assay) if the criteria for an adequate MRD sample with low probability of MRD target loss are met. A morphological count of blasts ≥5% does not define persistent disease or relapse if

the appropriate MRD assay or other diagnostic tests for genetic abnormalities in AML and high risk MDS are negative.³⁰⁻³³

Response assessment in an adequate, representative BM aspirate is more accurate and reproducible by flow cytometry measuring myeloid blasts than morphology due to the inherent subjectivity of cytomorphology and the challenges in distinguishing myeloid blasts from regenerating myeloid precursors and other myeloid blast mimics ([Supplementary Table S1](#)). However, there are exceptions, particularly in certain subtypes of AML, such as Acute Megakaryocytic Leukemia (AMKL), erythroid (acute erythroid leukemia, AEL/pure erythroid leukemia, PEL) and acute monocytic AML. AMKL is often associated with significant BM fibrosis resulting in a dry tap and frequently requires trephine biopsy with immunohistochemistry studies for residual AML assessment. Flow cytometry may underestimate residual AEL/PEL depending on the red cell lysis procedure used during sample preparation or if a limited set of markers is used to define leukemic pronormoblasts. Acute monocytic leukemia may show an immunophenotype similar to mature monocytes, which limits the ability to accurately identify leukemic monocytes. Due to these reasons, the consensus is that blast percentage should continue to be reported by cytomorphological count for these AML subtypes if BM cytomorphology shows $\geq 5\%$ blasts together with a positive MRD result. Otherwise, we recommend that the primary reporting for the blast percentage in an MRD positive aspirate is by flow cytometry unless the flow cytometric sample is of non-representative quality and there is a major discrepant excess of blasts by morphology.

Key considerations for generating more accurate enumeration of AML blasts at remission assessments are summarised below by blast detection methods, AML subtypes and sample quality.

Flow Cytometry

The recommendation for enumerating blasts at response assessment of AMLs is by flow cytometry MRD assay. The guidance below also applies to standard flow blast enumeration, required for example when patients with CBF or *NPM1* mutated AML are monitored by an ELN PCR MRD assay without parallel flow MRD testing.

The denominator for blast percentage is CD45 expressing cells (excluding CD45-negative RBCs, platelet clumps and debris) in accordance with the ELN MRD recommendations.^{7, 13} Defining the total myeloid blast compartment requires CD34, CD117 (immaturity) together with HLA-DR (immaturity for granulocytic lineage), CD33, CD13 (myeloid lineage), CD45 and light scatter parameters (Figure 3). Total myeloid blasts are most consistently quantified as CD34+ and/or CD117+ cells within the CD45/SSC “blast region” (intermediate/low CD45 and SSC) after exclusion of technical artefact, CD34+ hematogones and CD117+ non-blast cells. The latter consist of immature erythroid precursors, immature NK cells (usually CD117^{weak}

with higher CD45 expression, CD7+, CD56+), promyelocytes, subset of basophils, neoplastic plasma cells (CD38^{high}) and mast cells (CD117^{high}). AML blasts are rarely negative for CD45 but this possibility should be considered and the CD45 gating should be adjusted accordingly. HLA-DR can sometimes serve as the primary marker of immaturity in AML blasts that only express dim levels of CD34 and/or CD117. This approach provides a blast % but often with insufficient specificity for MRD detection unless major immunophenotypic aberrancies allow discrete gating of the leukemic progenitors.

Residual blasts/promonocytes of acute monocytic leukemia may fall outside this CD45/SSC blast gate due to a higher CD45 expression and/or side scatter characteristics (monocytic region) and often lack expression of CD34 and/or CD117. Aberrant expressions of asynchronous monocytic markers (e.g., CD14, CD11b, HLA-DR, CD35, CD300e) can help discriminate leukemic monocytic blasts from normal monocytic cells but with less certainty regarding immaturity and with lower specificity compared to MRD detection of CD34+ and/or CD117+ AML blasts.

Myeloid cells displaying immaturity markers eg CD117, but with higher SSC that fall outside the CD45/SSC blast region should be quantified as AML blasts if they are abnormal based on an immunophenotypic profile that deviates from normal myeloid precursors.

A patient's leukemic progenitors can consist of several subpopulations with varied CD34 and CD117 expression plus heterogeneity in aberrancy, reminiscent of disordered normal maturational patterns. Occasionally leukemic cells have insufficient aberrancy to be distinguished from normal progenitors. A discrepant excess of % total myeloid blasts by flow cytometry compared to the % MRD in an MRD positive aspirate may be due to this in a leukemic subpopulation - in which case the % total myeloid blasts is an estimate of the upper boundary of potential involvement-, or, to transient normal haematopoietic rebound/regeneration. If the latter is suspected, for example in a pediatric patient, this should be noted in the integrated report but not misinterpreted as residual leukemia.

.

Molecular Methods

Most AML-defining genetic abnormalities involve fusions or rearrangements that require RNA based assays. It is important to note that in AML MRD positive BM aspirates, blast percentage cannot be derived from RT-qPCR MRD assays measuring AML-defining gene fusions or *NPM1* mutations due to variability in RNA transcript numbers per leukemic cell, RNA transcripts in differentiating cells and non-dividing cells. Currently mutated *NPM1* is the only AML defining genetic abnormality suitable for quantifying percentage blasts by DNA based VAF frequencies in MRD positive samples. Other target gene mutations for DNA based MRD assays are either potentially subclonal and unstable (*FLT3* mutations) or potentially pre-leukemic (e.g. *IDH2*, *SRSF2*) or insufficiently validated (*CEBPA*).

In the context of differentiation therapies, such as FLT3 inhibitors, IDH inhibitors and the emerging menin inhibitors, non-blast cells may retain the AML genetic abnormality during maturation. This is similar to what is observed with acute promyelocytic leukemia (APML) after ATRA and arsenic trioxide induction. Delayed clearance of these cells could result in a discrepancy between molecular MRD frequencies and conventional blast enumeration by cytomorphology and flow cytometry. Therefore, blast reporting should be restricted to cells that are defined as blasts by cytomorphology and flow cytometry, excluding differentiating cells. In APML, the most important timepoint for BM response assessment is at the end of consolidation rather than after induction and must include RT-qPCR MRD testing for molecular remission.

Cytomorphology

Similar to cytomorphology evaluation of ALL, blast mimics ([Supplementary Table S1](#)) and aspirate adequacy should be considered. For promonocytes that are considered as blast equivalent, mimics include reactive/regenerating monocytes and dysplastic promyelocytes. With differentiation therapy incomplete leukemic blast differentiation may result in retention of blast-like features such as the persistence of Auer rods. This is common in APML BMs after ATRA and arsenic trioxide induction.

Assessing BM trephine biopsy will address the concern for BM aplasia, necrosis or fibrosis. BM fibrosis is common in AMKL, AML progressed from myeloproliferative neoplasms (MPN) or MDS with fibrosis, and certain subtypes of acute leukemia such as AML with *MECOM* rearrangement. When appropriate, CD34 and/or CD117 immunohistochemistry can be performed on fibrotic BM to estimate blast percentage. AMKL often requires immunohistochemistry studies (e.g., CD31, CD41, CD42b and CD61) on trephine biopsy for blast enumeration. E-cadherin immunohistochemistry may be of value in the diagnosis of PEL.^{34, 35}

In patients with significant BM fibrosis, circulating blasts may be present even in the absence of excess BM blasts, which should be taken into consideration for integrated reporting.

Acute leukemias of mixed or ambiguous lineage (ALAL)

ALAL includes acute undifferentiated leukemia (AUL) and mixed phenotype acute leukemias (MPAL).³⁶ While AUL lack lineage-defining markers, blasts in MPAL express lineage-specific markers of more than one lineage. MPAL may present with a single blast population expressing both myeloid and lymphoid lineage-defining markers (biphenotypic or mixed phenotype) or with separate myeloid and lymphoid blast populations (bilineal or mixed lineages). However, it is fairly common in MPAL to observe a combination of mixed lineage and mixed phenotype blasts in a given case where some markers are shared by all blasts while other markers are differentially expressed.

Reporting percentage blasts during response assessment in ALAL requires a flow cytometry panel that includes essential markers for all involved lineages. Accurate enumeration can be challenging, particularly in MPAL where core blast markers such as CD34 may show heterogeneous expression among blast subpopulations and distinct aberrancies may not be fully captured by a single lineage MRD panel.

Emphasis is given to the leukemia associated immunophenotype (LAIP) in selecting the appropriate panels; however, these leukemias are particularly prone to change in phenotype and lineage switch since initial therapy is usually selected to target one lineage so a more comprehensive panel that assesses all relevant lineages is often required. For AUL or MPAL with one blast population, either an AML MRD or ALL MRD panel might be adequate, provided the necessary markers are included in the panels. In bilineal MPAL, simultaneous assessment by AML MRD and ALL MRD panels is generally required. Finally, flow cytometry assessment of MRD in ALAL and MPAL poses unique challenges, as certain markers critical for lineage assignment (i.e. myeloperoxidase, cytoplasmic CD3) may not be part of recommended and/or validated AML MRD panels.

As genetic rearrangements are frequent in MPAL, MRD negativity can potentially be defined by the appropriate RNA or DNA based PCR test (*BCR::ABL1*, rearrangements involving *KMT2A*,) but assays for rarer rearrangements (*ZNF384* and others) require further validation.

Summary and Outlook

Establishing standardised criteria for reporting blast percentage is crucial for ensuring uniform response and relapse assessment in acute leukemias. Such criteria should reflect the common practices and experience of laboratory networks associated with national study groups and international consortia in the management of acute leukemias. This convened international expert panel reached consensus on all aspects of the recommendations that are presented here. The implementation of internationally harmonized procedures for blast percentage reporting is anticipated to improve the consistency and quality of this data entry across trials and centers. Although access to appropriate MRD technologies may be restricted in certain centers and countries, these assays are the basis for the accurate and sensitive quantification of blasts. MRD assays are intended to directly identify residual leukemia whilst morphologic blasts are a mixture of both non-neoplastic and leukemic progenitors. Consequently, the linkage between morphologic blast counts and leukemic treatment response is inherently imperfect. Nevertheless, morphology continues to play a key role in the comprehensive assessment of treatment response. Within the framework of our recommendations, morphology should be used to provide a qualitative evaluation that incorporates response category for all BM samples and for quantitative reporting where there are recognised limitations in flow cytometric or NGS blast enumeration, as outlined in

our expert panel recommendations. Discrepancies between morphology and the other methodologies frequently correlate with treatment context ([Supplementary Table S1](#)). This underlines the importance of ensuring appropriate clinical information (leukemia subtype, treatment / timepoint / PB counts and use of GCSF) is included at request. The application of artificial intelligence with digitalised morphology slides and flow cytometric data may further enhance the role of morphology and improve standardised blast enumeration in the future. Additionally, international external quality assurance programs for MRD assays should play a crucial role in cross-validating blast percentage results across laboratories, particularly those involved in clinical trial reporting. Finally we note that longer assay turnaround times can impede timely integrated response reporting and therefore rapid treatment decisions. This is particularly relevant to molecular MRD, partly due to the need for batching of these tests. We therefore strongly encourage regional efforts to reduce variation by improving these assay turnaround times.

References

1. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol*. 2003;21(24):4642-4649.
2. Gokbuget N, Boissel N, Chiaretti S, et al. Diagnosis, prognostic factors, and assessment of ALL in adults: 2024 ELN recommendations from a European expert panel. *Blood*. 2024;143(19):1891-1902.
3. Buchmann S, Schrappe M, Baruchel A, et al. Remission, treatment failure, and relapse in pediatric ALL: an international consensus of the Ponte-di-Legno Consortium. *Blood*. 2022;139(12):1785-1793.
4. Bassan R, Bruggemann M, Radcliffe HS, Hartfield E, Kreuzbauer G, Wetten S. A systematic literature review and meta-analysis of minimal residual disease as a prognostic indicator in adult B-cell acute lymphoblastic leukemia. *Haematologica*. 2019;104(10):2028-2039.
5. Saygin C, Cannova J, Stock W, Muffly L. Measurable residual disease in acute lymphoblastic leukemia: methods and clinical context in adult patients. *Haematologica*. 2022;107(12):2783-2793.
6. Short NJ, Aldoss I, DeAngelo DJ, et al. Clinical use of measurable residual disease in adult ALL: recommendations from a panel of US experts. *Blood Adv*. 2025;9(6):1442-1451.
7. Heuser M, Freeman SD, Ossenkoppele GJ, et al. 2021 Update on Measurable Residual Disease (MRD) in Acute Myeloid Leukemia (AML): A Consensus Document from the European LeukemiaNet MRD Working Party. *Blood*. 2021;138(26):2753-2767.
8. Short NJ, Zhou S, Fu C, et al. Association of Measurable Residual Disease With Survival Outcomes in Patients With Acute Myeloid Leukemia: A Systematic Review and Meta-analysis. *JAMA Oncol*. 2020;6(12):1890-1899.
9. Freeman SD, Hills RK, Virgo P, et al. Measurable Residual Disease at Induction Redefines Partial Response in Acute Myeloid Leukemia and Stratifies Outcomes in Patients at Standard Risk Without NPM1 Mutations. *J Clin Oncol*. 2018;36(15):1486-1497.
10. Dohner H, Wei AH, Appelbaum FR, et al. Diagnosis and Management of AML in Adults: 2022 ELN Recommendations from an International Expert Panel. *Blood*. 2022;140(12):1345-1377.
11. Tettero JM, Heidinga ME, Mocking TR, et al. Impact of hemodilution on flow cytometry based measurable residual disease assessment in acute myeloid leukemia. *Leukemia*. 2024;38(3):630-639.
12. Bruggemann M, Schrauder A, Raff T, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. *Leukemia*. 2010;24(3):521-535.
13. Tettero JM, Freeman S, Buecklein V, et al. Technical Aspects of Flow Cytometry-based Measurable Residual Disease Quantification in Acute Myeloid Leukemia: Experience of the European LeukemiaNet MRD Working Party. *Hemasphere*. 2022;6(1):e676.
14. Lee SH, Erber WN, Porwit A, Tomonaga M, Peterson LC, International Council for Standardization In H. ICSH guidelines for the standardization of bone marrow specimens and reports. *Int J Lab Hematol*. 2008;30(5):349-364.

15. Gupta S, Devidas M, Loh ML, et al. Flow-cytometric vs. -morphologic assessment of remission in childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group (COG). *Leukemia*. 2018;32(6):1370-1379.
16. Novakova M, Zaliova M, Fiser K, et al. DUX4r, ZNF384r and PAX5-P80R mutated B-cell precursor acute lymphoblastic leukemia frequently undergo monocytic switch. *Haematologica*. 2021;106(8):2066-2075.
17. Gokbuget N, Boissel N, Chiaretti S, et al. Management of ALL in adults: 2024 ELN recommendations from a European expert panel. *Blood*. 2024;143(19):1903-1930.
18. Silbert SK, Rankin AW, Hoang CN, et al. Project EVOLVE: An international analysis of postimmunotherapy lineage switch, an emergent form of relapse in leukemia. *Blood*. 2025;146(4):437-455.
19. Rolf N, Al-Attar A, Bunting S, Oak J. Pitfalls and Strategies for Flow Cytometry-based Post-Immunotherapy Disease Monitoring of Hematological Malignancies. *International Clinical Cytometry Society*. https://www.cytometry.org/web/modules/Module_28.pdf. Accessed on April 16 2024.
20. Chen X, Gao Q, Roshal M, Cherian S. Flow cytometric assessment for minimal/measurable residual disease in B lymphoblastic leukemia/lymphoma in the era of immunotherapy. *Cytometry B Clin Cytom*. 2023;104(3):205-223.
21. Cherian S, Miller V, McCullouch V, Dougherty K, Fromm JR, Wood BL. A novel flow cytometric assay for detection of residual disease in patients with B-lymphoblastic leukemia/lymphoma post anti-CD19 therapy. *Cytometry B Clin Cytom*. 2018;94(1):112-120.
22. Garand R, Beldjord K, Cave H, et al. Flow cytometry and IG/TCR quantitative PCR for minimal residual disease quantitation in acute lymphoblastic leukemia: a French multicenter prospective study on behalf of the FRALLE, EORTC and GRAALL. *Leukemia*. 2013;27(2):370-376.
23. van der Velden VHJ, Dombrink I, Alten J, et al. Analysis of measurable residual disease by IG/TR gene rearrangements: quality assurance and updated EuroMRD guidelines. *Leukemia*. 2024;38(6):1315-1322.
24. Bruggemann M, Kotrova M. Minimal residual disease in adult ALL: technical aspects and implications for correct clinical interpretation. *Blood Adv*. 2017;1(25):2456-2466.
25. Kim R, Bergugnat H, Pastoret C, et al. Genetic alterations and MRD refine risk assessment for KMT2A-rearranged B-cell precursor ALL in adults: a GRAALL study. *Blood*. 2023;142(21):1806-1817.
26. Burmeister T, Stroh AS, Kehden B, et al. Measurable residual disease quantification in adult patients with KMT2A-rearranged acute lymphoblastic leukemia. *Leukemia*. 2024;38(7):1600-1603.
27. Nagel I, Bartels M, Duell J, et al. Hematopoietic stem cell involvement in BCR-ABL1-positive ALL as a potential mechanism of resistance to blinatumomab therapy. *Blood*. 2017;130(18):2027-2031.
28. Arber DA, Hasserjian RP, Orazi A, et al. Classification of myeloid neoplasms/acute leukemia: Global perspectives and the international consensus classification approach. *Am J Hematol*. 2022;97(5):514-518.
29. Hovorkova L, Zaliova M, Venn NC, et al. Monitoring of childhood ALL using BCR-ABL1 genomic breakpoints identifies a subgroup with CML-like biology. *Blood*. 2017;129(20):2771-2781.

30. Inaba H, Coustan-Smith E, Cao X, et al. Comparative analysis of different approaches to measure treatment response in acute myeloid leukemia. *J Clin Oncol*. 2012;30(29):3625-3632.
31. Ouyang J, Goswami M, Tang G, et al. The clinical significance of negative flow cytometry immunophenotypic results in a morphologically scored positive bone marrow in patients following treatment for acute myeloid leukemia. *Am J Hematol*. 2015;90(6):504-510.
32. Freeman SD, Hills RK, Russell NH, et al. Induction response criteria in acute myeloid leukaemia: implications of a flow cytometric measurable residual disease negative test in refractory adults. *Br J Haematol*. 2019;186(1):130-133.
33. Brodersen LE, Gerbing RB, Pardo ML, et al. Morphologic remission status is limited compared to DeltaN flow cytometry: a Children's Oncology Group AAML0531 report. *Blood Adv*. 2020;4(20):5050-5061.
34. Wang W, Wang SA, Medeiros LJ, Khoury JD. Pure erythroid leukemia. *Am J Hematol*. 2017;92(3):292-296.
35. Caldwell I, Ruskova A, Royle G, Liang J, Bain BJ. Pure erythroid leukemia: The value of E-cadherin in making the diagnosis. *Am J Hematol*. 2019;94(6):726-727.
36. Weinberg OK, Arber DA, Dohner H, et al. The International Consensus Classification of acute leukemias of ambiguous lineage. *Blood*. 2023;141(18):2275-2277.

Table 1 Criteria for adequate aspirate samples

- First pull for MRD sample(s) (2 up to 4 mLs) and cytomorphology (0.5 mLs)

Morphology

- 5+ smears made fresh at bedside,
- Consider 1x squash prep & if trephine taken 1-3x touch preps
- Romanowsky stain with proven efficacy in ALL/AML/MDS cases
- Exclude unassessable morphology eg due to insufficiently dried smears
- Assess particle numbers - smear should be particulate
- Assess cellularity of trails
- Differential counting nearest to particles, 500 nucleated cells in MRD setting
- Assess aspirate representative of BM
 - includes erythropoiesis, granulopoiesis with maturation sequence, megakaryopoiesis
 - includes plasma cells, histiocytes and mast cells
- Correlate BMA differential with peripheral blood counts if considering hemodilution

Flow cytometry

- 500,000+ nucleated cells per antibody tube
- Assess hemodilution by appropriate markers or smear

Molecular MRD

- Minimum number of nucleated cells according to specification of MRD assay
- Sample-specific sensitivity matches sensitivity as specified for the MRD assay
- Assess hemodilution by smear

MDS, myelodysplastic neoplasms; BM, bone marrow; MRD, measurable/minimal residual disease; BMA, bone marrow aspirate.

Figures

Figure 1. Response Assessment in ACUTE LYMPHOBLASTIC LEUKEMIA

Notes.

1. In adequate MRD sample. If MRD positive $<0.01\%$ or MRD low level, this should be specified with LOD in MRD report.
2. Consider Marker loss: post immunotherapy, sequence loss for IH/TR, lineage switch including in KMT2Ar patients.
3. Cytomorphology may include trephine biopsy and touch-prep if aspirate quality is inadequate or suboptimal
4. If only one MRD method is used, consider additional MRD technology, request repeat at an interval that may vary by protocols and clinical scenarios

Abbreviations: MRD, measurable /minimal residual disease, Ig/TR, immunoglobuline (IG) and/or T-cell receptor (TR) gene rearrangements

Figure 2. Response Assessment in ACUTE MYELOID LEUKEMIA

Notes.

1. In adequate MRD sample, using ELN appropriate molecular or flow (that includes DfN) MRD assay.
2. If molecular MRD assay, consider potential wild type evolution (*FLT3*, *NPM1*).
3. Cytomorphology may include trephine biopsy and touch-prep if aspirate quality is inadequate or suboptimal
4. If adequate sample, representative of morphology
5. Report the entire abnormal myeloid blast population defined by 'deviation from normal' (= 'refractory/relapse by flow' if $\geq 5\%$ abnormal blasts)

Abbreviations: AML, acute myeloid leukemia, MDS, myelodysplastic neoplasms, MRD, measurable /minimal residual disease, LOD, limit of detection, DfN, Different from normal analysis.

Figure 3. Flow cytometric blast gating at response assessment with an AML MRD panel

Analysis starts with singlet gate, followed by removing CD45-negative debris, unlysed RBCs and platelets. CD45/SSC "blast" gate should include all CD34+ cells (highlighted in red) and most of CD117+ cells (in dark purple) and may be extended to include monocytic cells as appropriate. In this response assessment BM, further analysis shows that among the CD34+ cells, there are many stage I hematogones (pink), normal myeloid precursors (red) and plasmacytoid dendritic cell precursors (CD123bright+HLADR+). AML blasts are highlighted in black, identified by aberrant expressions of bright CD33, loss of CD13 and HLA-DR, positive for CD56 with markedly decreased CD45 and CD38, at 0.25% of CD45 expressing cells.

Figure 4. Flow cytometric blast gating in a AML response assessment bone marrow with significant background dysplasia.

In bone marrow with marked erythroid hyperplasia, removing RBCs is more effective using the SSC/FSC plot, since nucleated erythrocytes may fall into CD45 very low region. The

CD45/SSC "blasts" gate is ill defined due to dysgranulopoiesis/granulocyte hypogranulation, and only a subset of cells expressing immature markers (CD117+CD34subset+). The CD117+ cells (9.8%) do not represent blasts, in fact, they include erythroid precursors (CD36+CD123-CD13-HLADRdim/negative); basophils (CD123bright+HLADR-), and promyelocytes (CD33+CD13+CD34-). The AML blast are highlighted in black, CD34+CD117+CD7+CD123uniformly+, 1.3% of the CD45 expressing cells.

Figure 1 Response Assessment in ACUTE LYMPHOBLASTIC LEUKEMIA

Response Assessments	Report
MRD <0.01% ^{1, 2}	Report Remission, blasts <0.01% + Cytomorphology Quality ³
Cytomorphology <5% blasts + MRD positive ≥ 0.01% ¹	Report % blasts by Flow Cytometry or IG/TR MRD + Cytomorphology Quality ³
Cytomorphology ≥5% blasts + MRD positive ≥ 0.01% ¹	Report % blasts by Cytomorphology if major discrepant excess blasts by Cytomorphology ⁴ with potential MRD target loss ²
	Otherwise, report % blasts by Flow cytometry or IG/TR MRD + Cytomorphology Quality ³

Figure 2 Response Assessment in ACUTE MYELOID LEUKEMIA (INCLUDING MDS/AML)

Response Assessments	Report
MRD negative^{1,2}	Report Remission, blasts <0.1% or <LOD + Cytomorphology Quality³
Cytomorphology <5% blasts + MRD positive	Report % blasts by Flow Cytometry (MRD) assay⁵ + Cytomorphology Quality³
Cytomorphology ≥5% blasts + MRD positive	Report % blasts by Cytomorphology for Erythroid, Monocytic or Megakaryocytic AML
	Otherwise report % blasts by Flow Cytometry (MRD) assay^{4, 5} + Cytomorphology Quality³

Figure 3

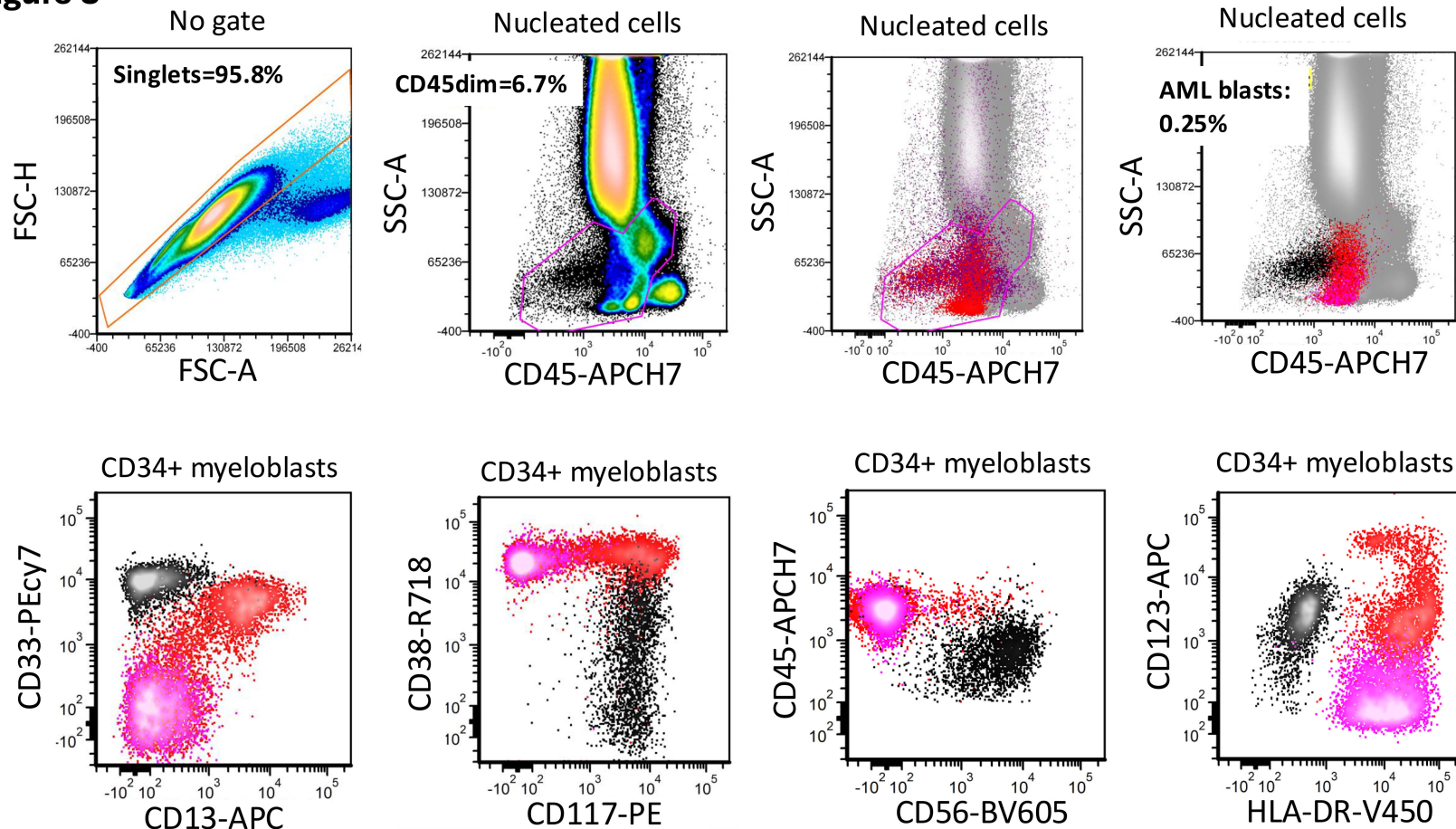


Figure 4

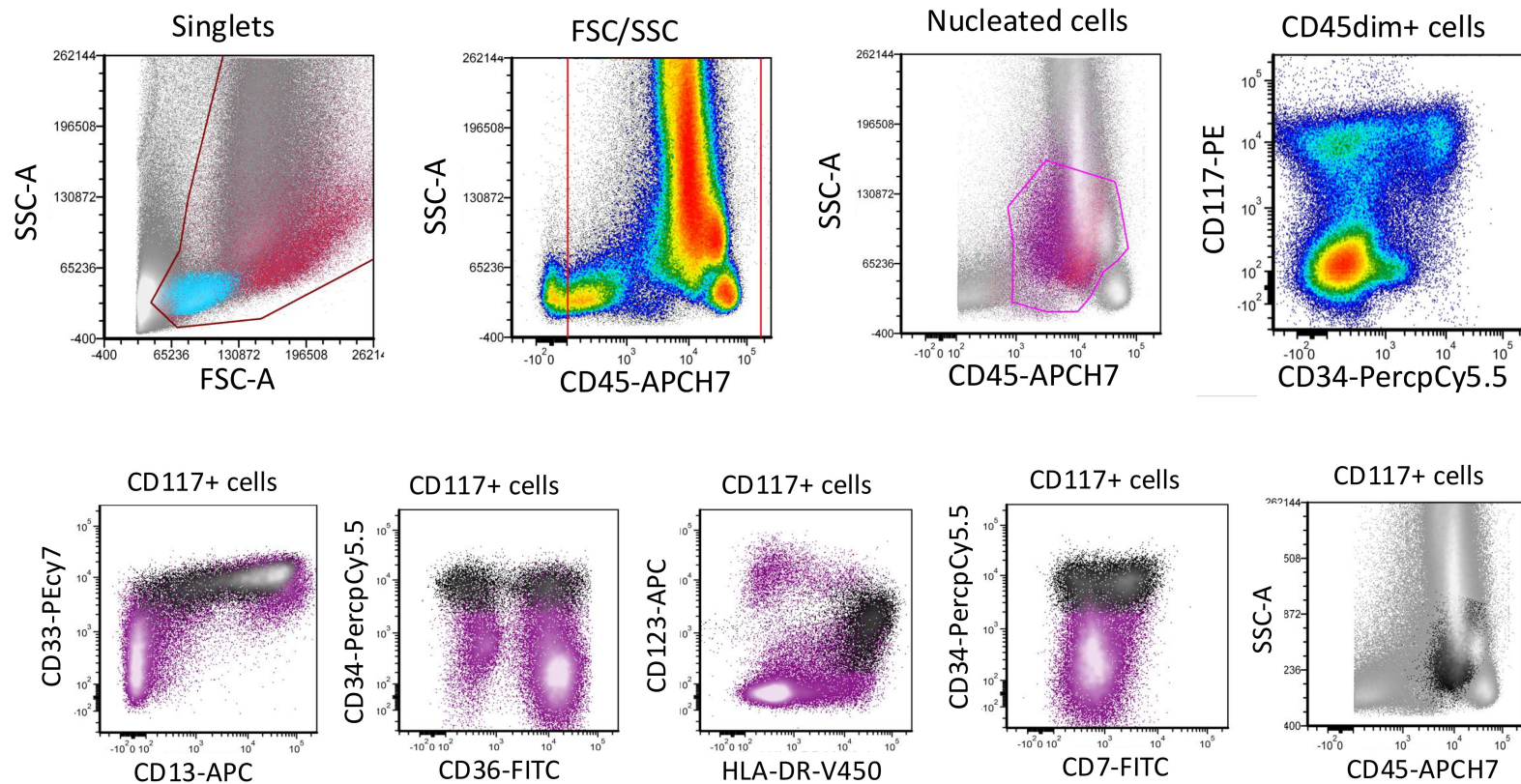
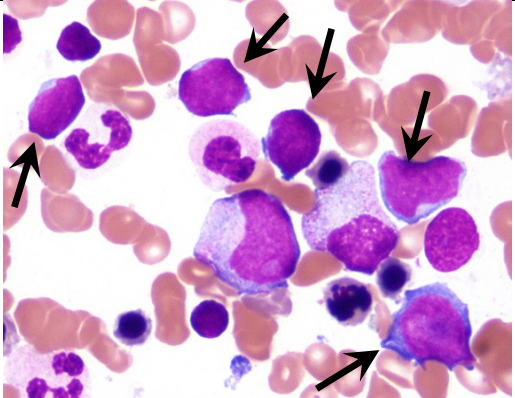
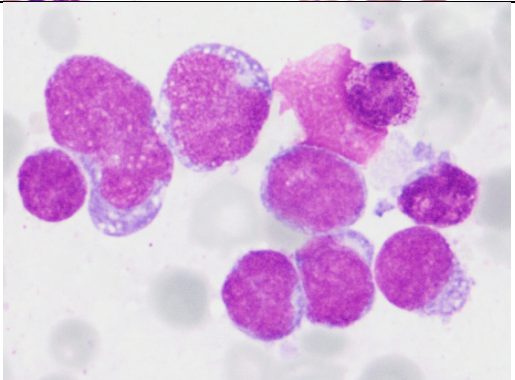
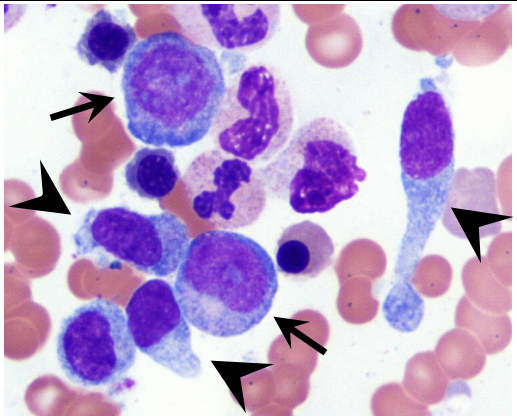
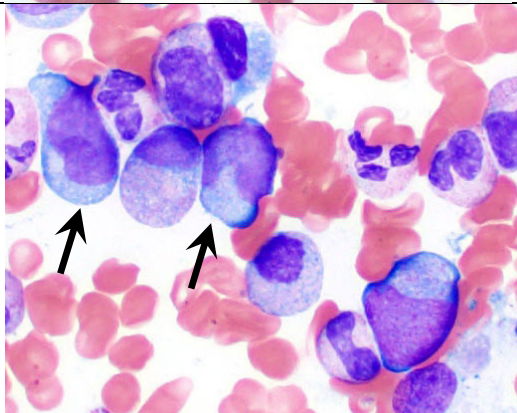
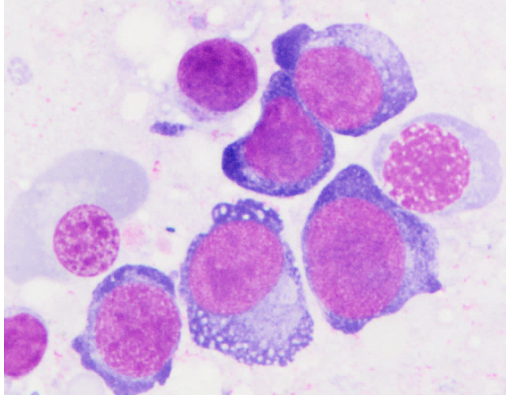
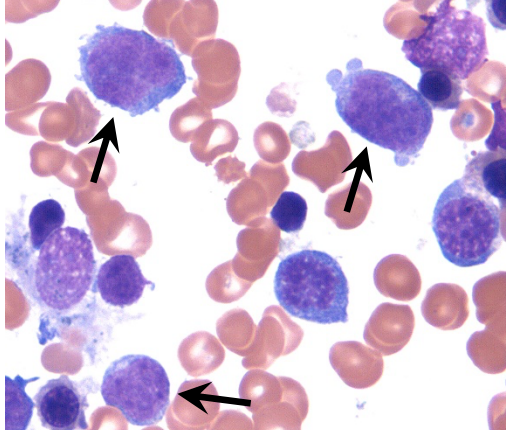


Table S1. Blast mimics in Remission Bone Marrow Post Acute Leukemia Treatment

Cell Type	Clinical Context	Morphological Characteristics	Morphologic illustration
Hematogones (Normal B-precursors)	<ul style="list-style-type: none"> • Infants and young patients • Patients recovering from chemotherapy • Post-stem cell transplantation (SCT) 	<ul style="list-style-type: none"> • Small to medium-sized cells • High nuclear/cytoplasmic ratio • Round to oval nuclei • Condensed, homogeneous chromatin • Scant, basophilic cytoplasm with no granules or Auer Rods <p><i>Arrows: hematogones</i></p>	
Plasmacytoid dendritic cells	<ul style="list-style-type: none"> • Bone marrow recovery after chemotherapy and post SCT • Viral infection, autoimmune conditions 	<ul style="list-style-type: none"> • Medium-sized cells • Eccentric nuclei • Moderately condensed chromatin • Abundant light basophilic cytoplasm • Cytoplasmic microvacuoles in a pearl necklace pattern and pseudopodia-like extensions 	

Promyelocytes¹/myelocytes¹	<ul style="list-style-type: none"> • Bone marrow recovery after treatment with G-CSF • Bone marrow recovery after chemotherapy • Infection 	<ul style="list-style-type: none"> • Rapidly increasing cellularity usually with patchy distribution • Myeloid predominance • Promyelocytes: large, with round to oval nucleus, primary (azurophilic) granules, with paranuclear hof • Myelocytes contain mostly secondary granules, and no nucleoli <p><i>Arrows: promyelocytes; arrow heads: pDCs</i></p>	
Reactive/regenerating monocytes	<ul style="list-style-type: none"> • Bone marrow recovery after chemotherapy • Early post SCT • Recovery from severe infection • G-CSF or GM-CSF administration 	<ul style="list-style-type: none"> • Increase in monocytes, usually with a left shift and asynchronous maturation • Slightly larger, with less condensed chromatin, may contain faintly visible nucleoli, moderate to abundant blue-grey cytoplasm, scattered azurophilic granules <p><i>Arrows: regenerating monocytes</i></p>	

Proerythroblasts¹	<ul style="list-style-type: none"> • Bone marrow recovery after chemotherapy and SCT • VitB12/folate deficiency, hemolysis, erythropoietin administration 	<ul style="list-style-type: none"> • Large, round to oval nuclei with fine and evenly distributed chromatin, 1-3 prominent nucleoli, deeply basophilic cytoplasm • Compensatory hyperplasia of erythroid elements • Mild dysplastic features (e.g. nuclear irregularities, asynchronous maturation, or cytoplasmic vacuolization) 	
Activated lymphocytes	<ul style="list-style-type: none"> • Viral infections • Autoimmune conditions • Drug reactions 	<ul style="list-style-type: none"> • Heterogeneous population with variable size and appearance • Nucleus may show clefts or indentations • Abundant cytoplasm, deeply basophilic with occasional azurophilic granules <p><i>Arrows: reactive lymphocytes</i></p>	

Legend

1. Note that, in addition to regenerative atypia, these cells may exhibit dysplastic changes due to underlying myelodysplasia, making differentiation from leukemic blasts extremely challenging by cytomorphologic evaluation.

Morphologic features of leukemic blasts vary significantly by leukemia subtype. Lymphoblasts are generally small to medium in size, with immature (blastic) chromatin, visible nucleoli, and scant cytoplasm. Some lymphoblasts may be larger, with folded/irregular nuclei, or cytoplasmic vacuoles. AML myeloblasts range from small to large, nucleoli, immature chromatin, round to highly irregular, convoluted/folded nuclei, variable amounts of cytoplasm and cytoplasmic granules. Some can closely mimic normal myeloid precursors. Promonocytes differ from reactive/atypical monocytes by their immature chromatin and delicate nuclear folds.