GUIDELINE ARTICLE

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

Sa A. Wang,[1] Leonor Arenillas,[2] Francesco Buccisano,[3] Monika Bruggemann,[4] Wolfgang Kern,[5] Manuel Menes,[6] Adriana Plesa,[7] Louisa Stone,[8] Dominique Wellnitz,[4] David A. Westerman,[9] Brent L. Wood[10] and Sylvie D. Freeman[11]

[1]Department of Hematopathology, MD Anderson Cancer Center, Houston, TX, 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, FL, USA; [7]Department of Hematology Laboratory and Flow Cytometry, 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, Los Angeles, CA, USA and [11]School of Infection, Inflammation and Immunology, University of Birmingham, Birmingham, UK

Correspondence: S.D. Freeman
s.freeman@bham.ac.uk

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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 specialized working groups including the European Hematology Association (EHA) Diagnosis in Hematological Diseases Specialized Working Group and the European LeukemiaNet (ELN) produced consensus guidance for harmonized blast assessment to define response categories in patients with acute leukemia. This guidance addresses 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 (BM) blast percentage. According to the International Working Group (IWG 2003) criteria,[1] this percentage is determined based on a morphological blast count. Although this method has been widely used in clinical practice over the years, it is recognized as imperfect because of 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 morphological blasts are a surrogate. There does, however, remain uncertainty in defining blasts for response categories with concerns in the community regarding a certain lack of standardization in how blast percentages are derived. The reporting of blast percentage in post-treatment 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 mini-symposium by the Food and Drug Administration in 2024 ("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 included representatives from the European LeukemiaNet (ELN), the European Hematology Association (EHA) Diagnosis in Hematological Diseases Specialized 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, which 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 peripheral blood counts is required for best interpretation and is particularly important in the case of BM samples taken before count recovery. Although early (day 14-21) BM sampling remains common practice in some centers to guide the use of a second induction, BM samples to categorize remission status should be taken 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 of BM aspirate should be used for BM smears. The remaining volume of the same, single ("first pull") aspiration, up to a maximum of 4 mL, should be reserved for the most appropriate MRD assay(s), divided equally between molecular and flow samples as needed. Two milliliters 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 mL 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 the patient's bedside, not only to preserve cytomorphology but also to examine whether BM particles are present and, if the quality is insufficient, the aspirate can be repeated immediately. The criteria to evaluate whether BM aspirate samples are adequate are listed 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 tre-

Table 1. Criteria for defining aspirate samples as adequate.

• First pull for MRD sample(s) (2-4 mL) and cytomorphology (0.5 mL)

Morphology
• 5+ smears made fresh at bedside
• Consider 1x squash preparations and, if trephine taken, 1-3x touch preparations
• Romanowsky stain with proven efficacy in ALL/AML/MDS cases
• Exclude unassessable morphology, e.g., 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 BM aspirate 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

MRD: measurable/minimal residual disease; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; MDS: myelodysplastic neoplasms; BM, bone marrow.

phine 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 and 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.

Morphological 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" (*Online Supplementary Table S1*), such as regenerating myeloid precursors, reactive monocytes, 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, and granulated blasts may be confused with promyelocytes.

Integrated remission reporting

Acute lymphoblastic leukemia

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 ≥5% does not define persistent disease or relapse unless confirmed as leukemic by an appropriate MRD assay such as flow cytometry, quantitative (q) polymerase chain reaction (PCR) and next-generation sequencing (NGS) or by diagnostic genetic assays (fluorescence *in situ* hybridization or karyotyping). Conversely, a morphological blast count of <5% no longer defines remission if MRD testing is positive. The primary reporting of response blast percentage should be done from 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 *KMT2A* rearrangements (loss of flow cytometry or *IG/TR* leukemic markers), *DUX4*-rearranged, *ZNF384*-rearranged or *PAX5*-P80R-mutated ALL,[16] or after immunotherapy[17,18] (loss of flow cytometry leukemic gating markers such as CD19 and CD22). Additionally, cytomorphology can 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. Recommendations for reporting blast percentage with appropriate sample qualifiers in the integrated response assessment are shown in Figure 1. Importantly, it was agreed that the blast percentage of an MRD-positive BM sample should only be reported by the cytomorphological count if there is a major discrepancy in blast category[3] 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 summarized 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 varia-

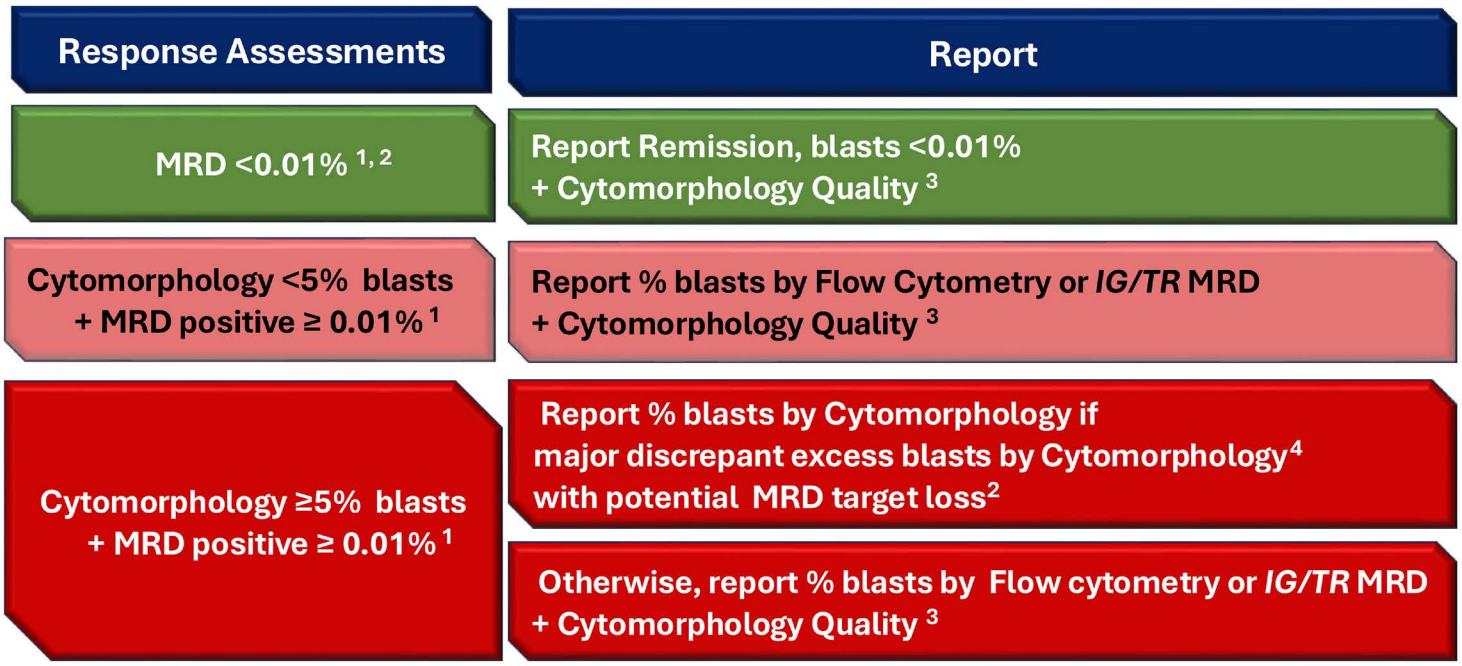

Figure 1. Response assessment in acute lymphoblastic leukemia. [1]In an adequate measurable/minimal residual disease (MRD) sample. If MRD-positive <0.01% or MRD low level, this should be specified with limit of detection in the MRD report. [2]Consider marker loss: after immunotherapy, sequence loss for *IH/TR*, lineage switch including in patients with *KMT2A* rearrangements. [3]Cytomorphology may include trephine biopsy and touch-preparations 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. *IG/TR*: immunoglobulin (*IG*) and/or T-cell receptor (*TR*) gene rearrangements.

tion 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 a fixative containing lysing reagents, optimizing cell recovery during washing 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-cell ALL, alternative primary gating strategies should be applied for leukemic cell identification.[19-21]

Molecular methods

IG/TR MRD assessment, using either qPCR or NGS, is considered the molecular gold standard to quantify MRD in ALL.[22-24] In *KMT2A*-rearranged ALL, *IG/TR* rearrangements may be absent or display clonal evolution during the course of the disease, 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)-positive 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-positive ALL,[27-29] 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 tyrosine kinase inhibitor therapy in Ph-positive ALL.[5,6]

Discordance between molecular MRD and flow cytometry or cytomorphology is also possible in other subtypes of B-cell ALL when small monocytic subpopulations derived from the leukemic clone persist after therapy (e.g., Ph-like B-cell ALL) or undergo monocytic transdifferentiation (e.g., *DUX4*-rearranged ALL). In T-cell 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 (*Online Supplementary Table S1*) and aspirate adequacy should be considered. Assessing blasts based

on BM trephine biopsy will address the concern regarding 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

Recommendations for reporting blast percentage with appropriate sample qualifiers in the integrated response assessment of acute myeloid leukemia (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 myelodysplastic syndrome (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, because of the inherent subjectivity of cytomorphology and the challenges in distinguishing myeloid blasts from regenerating myeloid precursors and other myeloid blast mimics (*Online Supplementary Table S1*). However, there are exceptions, particularly in certain subtypes of AML, such as acute megakaryocytic leukemia, erythroid (acute erythroid leukemia/pure erythroid leukemia) and acute monocytic AML. Acute megakaryocytic leukemia is often associated with significant BM fibrosis resulting in a dry tap and frequently requires trephine biopsy with immunohistochemistry studies in order to assess residual AML. Flow cytometry may underestimate residual acute erythroid leukemia/pure erythroid leukemia 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 that of mature monocytes, which limits the ability to identify leukemic monocytes accurately. In the light of these considerations, the consensus is that blast percentage should continue to be reported by cytomorphological count for these AML subtypes if BM cytomorphology shows ≥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 summarized below by blast detection methods, AML subtypes and sample quality.

Flow cytometry

The recommendation for enumerating blasts at response assessment of AML is by flow cytometry MRD assay. The guidance below also applies to standard flow blast enumeration, required for example when patients with core-binding factor AML or *NPM1*-mutated AML are monitored by an ELN

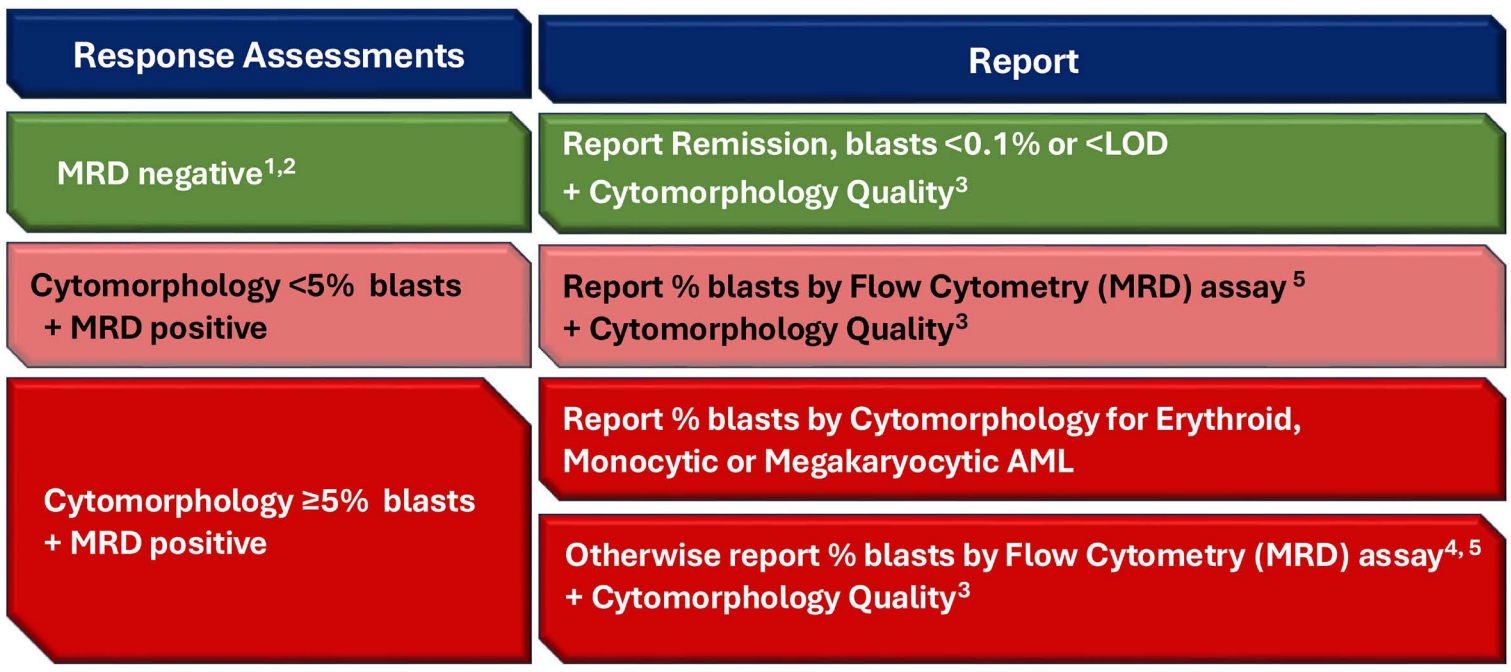

Figure 2. Response assessment in acute myeloid leukemia (including myelodysplastic neoplasm acute myeloid leukemia). [1]In an adequate measurable/minimal residual disease (MRD) sample, using European LeukemiaNet appropriate molecular or flow (that includes difference-from-normal) MRD assay. [2]If molecular MRD assay, consider potential wild-type evolution (*FLT3*, *NPM1*). [3]Cytomorphology may include trephine biopsy and touch-preparation 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 ≥5% abnormal blasts). LOD: limit of detection; AML: acute myeloid leukemia.

PCR MRD assay without parallel flow MRD testing.

The denominator for blast percentage is CD45-expressing cells (excluding CD45-negative red blood cells, 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 (Figures 3 and 4). Total myeloid blasts are most consistently quantified as CD34$^+$ and/or CD117$^+$ cells within the CD45/side-scatter (SSC) "blast region" (intermediate/low CD45 and SSC) after exclusion of technical artefacts, CD34$^+$ hematogones and CD117$^+$ non-blast cells. These last comprise immature erythroid precursors (Figure 4), immature natural killer cells (usually CD117^{weak} with higher CD45 expression, CD7$^+$, CD56$^+$), promyelocytes, subsets 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 dimly express CD34 and/or CD117. This approach provides a blast percentage 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 because of higher CD45 expression and/or SSC 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 to discriminate leukemic monocytic blasts from normal monocytic cells but with less certainity regarding immaturity and with lower specificity compared to MRD detection of CD34$^+$ and/or CD117$^+$ AML blasts.

Myeloid cells displaying markers of immaturity, e.g., 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 that of 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

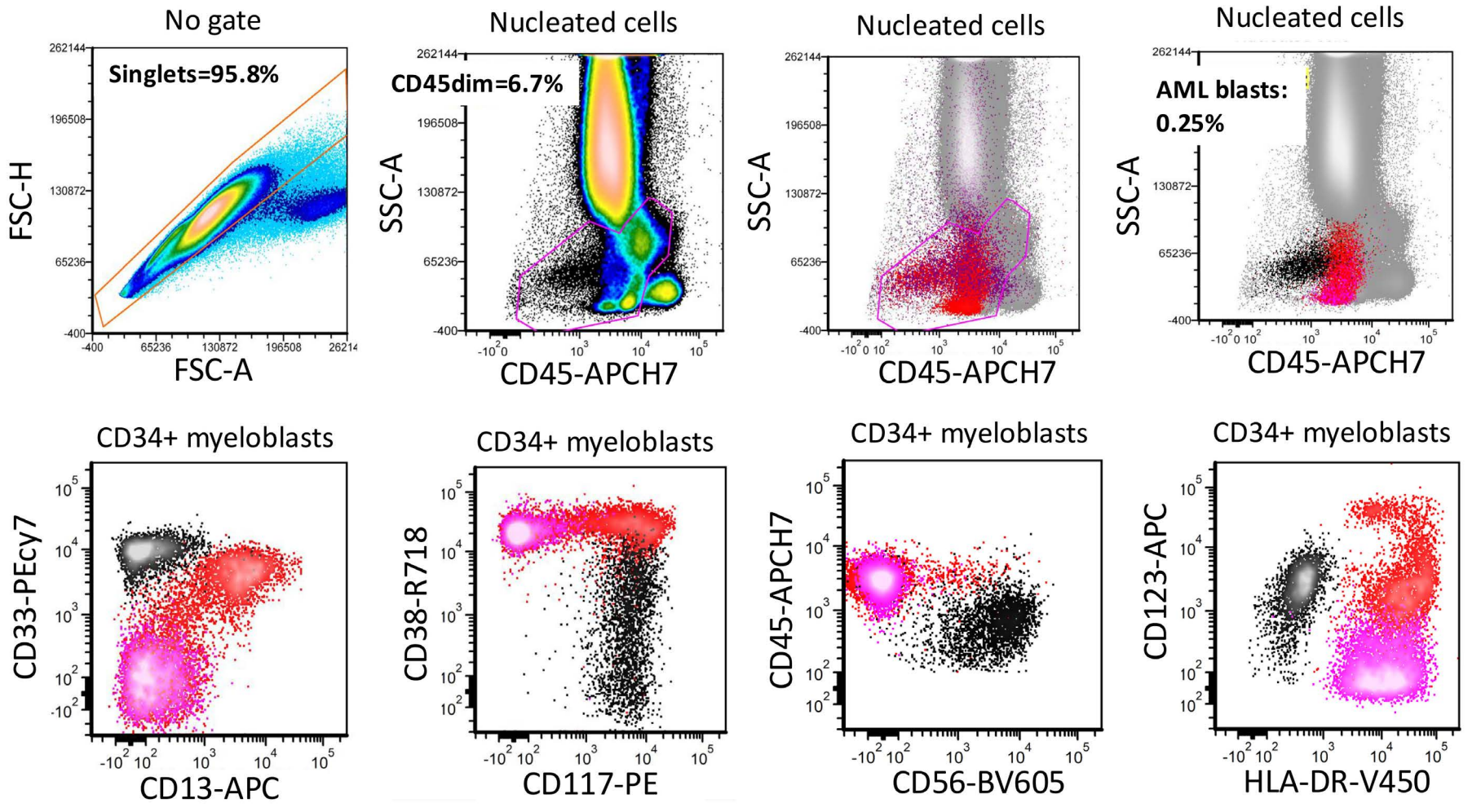

Figure 3. Flow cytometric blast gating at response assessment with an acute myeloid leukemia measurable/minimal residual disease panel. Analysis starts with a singlet gate, followed by removal of CD45$^-$ debris, unlysed red blood cells and platelets. The CD45/SSC "blast" gate should include all CD34$^+$ cells (highlighted in red) and most CD117$^+$ cells (in dark purple) and may be extended to include monocytic cells as appropriate. In this response assessment of bone marrow, further analysis shows that among the CD34$^+$ cells, there are many stage I hematogones (pink), normal myeloid precursors (red) and plasmacytoid dendritic cell precursors (CD123$^{bright+}$HLA-DR$^+$). Acute myeloid leukemia blasts are highlighted in black, identified by aberrant expressions of bright CD33, loss of CD13 and HLA-DR, positivity for CD56 with markedly decreased CD45 and CD38, at 0.25% of CD45-expressing cells. FSC-H: forward scatter height; FSC-A: forward scatter area; SSC-A: side scatter area; AML: acute myeloid leukemia.

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 variant allele 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 after all-*trans* retinoic acid 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 acute promyelocytic leukemia, 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 (*Online Supplementary Table S1*) and aspirate adequacy should be considered. For promonocytes that are considered as blast equivalents, 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 acute promyelocytic leukemia BM samples after all-*trans* retinoic acid and arsenic trioxide induction.

Assessing BM trephine biopsies will address concerns regarding BM aplasia, necrosis or fibrosis. BM fibrosis is common in acute megakaryocytic leukemia, AML progressed from myeloproliferative neoplasms or myelodysplastic syndromes 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. Acute megakaryocytic leukemia 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 pure erythroid leukemia.[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

Acute leukemias of mixed or ambiguous lineage (ALAL) include acute undifferentiated leukemias and mixed phenotype acute leukemias (MPAL).[36] While acute undifferentiated leukemias 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 in which 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 in which core blast markers such as CD34 may show heterogenous 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 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 acute undifferentiated leukemia 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 appropriate MRD panels.

As genetic rearrangements are frequent in MPAL, MRD

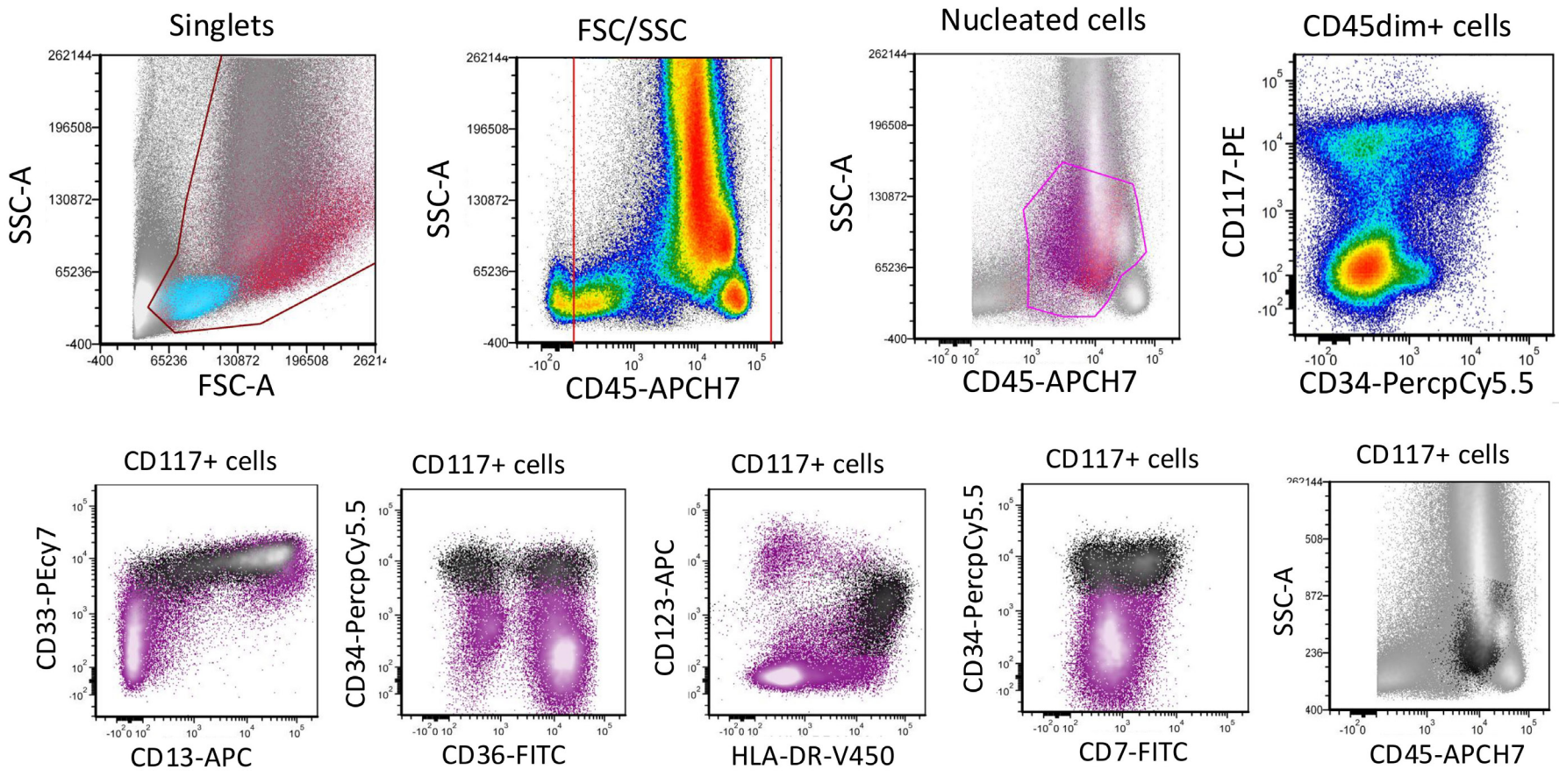

Figure 4. Flow cytometric blast gating in a response assessment of bone marrow with significant background dysplasia in a patient with acute myeloid leukemia. In bone marrow with marked erythroid hyperplasia, removing red blood cells is more effective using the side scatter (SSC)/forward scatter (FSC) plot, since nucleated erythrocytes may fall into the CD45 very low region. The CD45/SSC blast" gate is ill-defined due to dysgranulopoiesis/granulocyte hypogranulation, and only a subset of cells expressing immature markers ($CD117^{+}CD34^{+}$). The $CD117^{+}$ cells (9.8%) do not represent blasts; in fact, they include erythroid precursors ($CD36^{+}CD123^{-}CD13^{-}HLA\text{-}DR^{dim/neg}$); basophils ($CD123^{bright+}HLA\text{-}DR^{-}$), and promyelocytes ($CD33^{+}CD13^{+}CD34^{-}$). The AML blasts are highlighted in black, $CD34^{+}CD117^{+}CD7^{+}CD123^{uniformly+}$, 1.3% of the CD45-expressing cells.

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 standardized 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 identify residual leukemia directly, while morphologically identified blasts are a mixture of both non-neoplastic and leukemic progenitors. Consequently, the linkage between morphological 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 in cases of recognized 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 (*Online Supplementary Table S1*). This underlines the importance of ensuring appropriate clinical information (leukemia subtype, treatment, timepoint, peripheral blood counts and use of granulocyte colony-stimulating factor) is included at request. The application of artificial intelligence with digitalized morphology slides and flow cytometric data may further enhance the role of morphology and improve standardized 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 clincial 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 the turnaround times of these assays.

Disclosures

FB has provided consultancy services and/or participated in advisory boards for Jazz Pharmaceuticals, Laboratoires Delbert and Novartis and has been a speaker for Astellas, Bristol Myers Squibb, Janssen-Cilag and Servier. MB has been a speaker for and/or received travel support from Amgen, BD, Janssen and Pfizer, and has participated in advisory boards for Amgen, AstraZeneca, Hello Healthcare and Incyte. WK reports part ownership of MLL Munich Leukemia Laboratory. BLW has participated in advisory boards for Amgen and Kite Pharma. SDF has been a member of an advisory board for MPAACT, been a speaker for Novartis and Jazz Pharmaceuticals, and has received research funding (to her institute) from Jazz Pharmaceuticals, Bristol Myers Squibb/Celgene, AstraZeneca and Cytek. SAW, LA, MM, AP, LS, DW and DAW have no conflicts of interest to disclose.

Contributions

SAW and SDF were panel co-chairpersons and wrote the manuscript. LA, FB, MB, WK, MM, AP, LS, DW, DAW and BLW were panel members and wrote the manuscript. All panel members contributed equally.

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