Mixed-phenotype acute leukemia (MPAL) is a heterogeneous category in the World Health Organization classification that comprises acute leukemias with discrete admixed populations of myeloid and lymphoid blasts (“bilineal”) or with extensive coexpression of lymphoid and myeloid markers in a single blast population (“biphenotypic”). Flow cytometric findings suggestive of MPAL are often met with consternation by pathologists and oncologists alike, owing to unfamiliarity with the disease and uncertainty about how MPAL fits into established paradigms for treatment of acute leukemia. The purpose of this review is to explain the diagnostic criteria for MPAL, summarize its biological and clinical features, and address common diagnostic pitfalls of these unusual leukemias.


CLASSIFICATION OF ACUTE LEUKEMIA BASED ON LINEAGE

The first step in classification of acute leukemia is to assign lineage by resemblance to normal progenitor cells. This approach provides descriptive information about the blast cells that is useful for disease monitoring, provides clues to molecular pathways involved in pathogenesis, and can help to select effective chemotherapeutic regimens. The 3 main lineages of acute leukemia are myeloid (AML), B-lymphoblastic (B-ALL), and T-lymphoblastic (T-ALL). However, it is common for acute leukemias to aberrantly express protein markers more typically associated with other lineages, for example, expression of the myeloid markers CD13 and CD33 in B-ALL or T-ALL and expression of the T/NK-cell markers CD7 and CD56 in AML. The aberrant and complex patterns of marker expression in acute leukemia created a need for consensus criteria for lineage assignment.1 Furthermore, leukemias with multilineage protein expression often respond poorly to chemotherapy, suggesting that some types of multilineage expression may define a high-risk subgroup.2 Proposed reasons that mixed phenotype may portend a worse prognosis include the following: (1) mixed phenotype may indicate that the leukemic stem cells are primitive multipotent progenitors that are chemoresistant owing to slow replication, (2) mixed–phenotype blasts can adapt to therapy by switching phenotype, and (3) some mixed–phenotype acute leukemias (MPALs) express high levels of multidrug resistance proteins.2

Archetypal cases of MPAL, especially those with KMT2A (MLL) translocations, show a dramatic ability to switch lineage between myeloid and lymphoid blast proliferation,3,4 and this lineage plasticity is thought to be a key feature underlying the unusual phenotypes and aggressive behavior of MPAL. Lineage plasticity of leukemic stem cells can be demonstrated in cell culture, but currently there is no method for directly testing lineage plasticity in clinical practice. Instead, the primary clinical tool for predicting the multilineage potential of leukemic blasts is characterization of protein expression by immunophenotyping. This approach requires the elucidation of immunophenotypes that discriminate MPAL from unilineage acute leukemias.

IMMUNOPHENOTYPING of MPAL

Flow cytometry (FCM) is the primary method for blast immunophenotyping in clinical practice, and immunohistochemistry (IHC) and enzyme cytochemistry (EC) also contribute in some cases. The first consensus method for identifying MPAL was the algorithm proposed by the European Group for Immunological Characterization of Acute Leukemias (EGIL) in 1995.5 The EGIL strategy uses FCM to characterize blasts with a broad panel of markers associated with B-cell, T-cell, and myeloid lineages, and assigns a weighted score to each marker depending on how strongly it is associated with a specific lineage (Table 1). Using this algorithm, biphenotypic (or triphenotypic) leukemia is diagnosed when a score greater than 2 is calculated for more than 1 lineage. The EGIL authors defined positivity by FCM as a positive signal on at least 20% of blasts for surface markers and at least 10% for cytoplasmic markers compared to an isotype control.

New consensus criteria for MPAL were published in the 4th edition of the World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues6 (Table 2) and remain essentially unchanged in the 2016 update to the classification.7 In contrast to the EGIL approach of scoring a
detailed blast immunophenotype with numerous markers, the World Health Organization (WHO) criteria emphasize a few key lineage-defining markers with particular emphasis on CD19 for B lineage, CD3 for T lineage, and myeloperoxidase (MPO) for myeloid lineage. The WHO approach is simpler but relies heavily on the sensitivity and specificity of a few markers. Also, the WHO classification does not specify thresholds for positivity of these key markers, leaving it up to individual laboratories to decide on the definition of significant expression. In practice, the most frequent challenge in applying the WHO criteria for MPAL is interpretation of MPO expression in cases that are otherwise consistent with B-ALL or T-ALL.

### TESTING FOR MPO EXPRESSION

The WHO classification stipulates that detection of MPO by FCM, IHC, or EC is sufficient for the diagnosis of MPAL when the blasts also meet criteria for B- or T-cell lineage. However, the sensitivity of these methods for detecting MPO differs greatly. The presence of MPO mRNA in otherwise typical ALL blasts is well documented, and a strong correlation between MPO mRNA and positivity for MPO by IHC was demonstrated in a series of 57 infant ALL cases. In another series of 57 patients with MPAL, representing a broad age range (18 months–72 years), MPO mRNA was detected in 43.8% of patients, including 83% of ALL with BCR-ABL1 fusion (Ph+) and 33% of Ph−ALL. In both of these studies, all cases were negative for MPO by EC. Myeloperoxidase can also be detected by FCM in ALL, especially in Ph+ cases, even when EC is negative.

The most sensitive method for detection of MPO is reverse transcription–polymerase chain reaction, followed closely by IHC with modern signal amplification techniques. Flow cytometry is slightly less sensitive than amplified IHC, and EC is the least sensitive. In studies of AML blasts, it has been shown that cases with much stronger positivity by FCM than by EC produce relatively more of the MPO proenzyme than active enzyme. Decreased production of mature MPO might also be a factor in the low sensitivity of EC for MPO+ ALL, and the lower total quantity of MPO mRNA and protein in ALL compared to AML also contributes. For these reasons, the number of acute leukemias diagnosed as MPAL can vary significantly depending on whether FCM, IHC, or EC is used to detect MPO.

To address this problem, efforts have been made to standardize thresholds for MPO positivity. Detection of MPO by EC in 3% or more of blasts excludes most unilineage ALL cases and has been used as a threshold to define myeloid lineage since the inception of the French-American-British (FAB) classification of acute leukemia in the 1970s. A recent study used receiver-operator curve (ROC) analysis to define an FCM threshold for MPO positivity with optimal correlation with the 3% threshold by EC. This analysis identified 13% MPO+ blasts (by FCM) as the best threshold when using isotype control to define the negative control population. This result is fairly close to the 10% threshold for MPO by EC used by the EGIL algorithm. Other investigators have also shown a good correlation between a 10% threshold by FCM (using isotype control) and a 3% threshold by EC.

Importantly, Guy et al. repeated the ROC calculation by using normal lymphocytes as an internal negative control instead of using an isotype control, and found a significantly higher threshold of 28%. The main reason for the difference is that most blasts have greater autofluorescence than mature lymphocytes, and therefore a negative blast population will have a higher median fluorescence intensity than a negative lymphocyte population. Because of this difference, it is important to know what negative control was used when interpreting partial positivity for MPO by FCM.

Owing to uncertainty about the significance of weak or partial MPO expression by FCM when the question of ALL versus MPAL arises, the current practice at our institution is to perform MPO EC in cases where the FCM signal for MPO is very weakly positive or only present on a minor subset of the blasts. If MPO EC stains fewer than 3% of the blasts, then we do not consider the findings definitive for MPAL. This practice may change as more data become available, but at present we are more concerned about overdiagnosing MPAL by FCM.

### Table 1. European Group for Immunological Characterization of Acute Leukemias (EGIL) Algorithm for Biphenotypic Blasts

<table>
<thead>
<tr>
<th>Points</th>
<th>B</th>
<th>T</th>
<th>Myeloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>cyCD79a</td>
<td>CD3 (sm or cy)</td>
<td>MPO</td>
</tr>
<tr>
<td>1</td>
<td>CD19</td>
<td>CD2</td>
<td>CD117</td>
</tr>
<tr>
<td>0.5</td>
<td>TdT</td>
<td>TdT</td>
<td>CD14</td>
</tr>
<tr>
<td>0.5</td>
<td>CD24</td>
<td>CD7</td>
<td>CD15</td>
</tr>
<tr>
<td></td>
<td>CD1a</td>
<td>CD64</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: cy, cytoplasmic; IgM, immunoglobulin M; MPO, myeloperoxidase; sm, surface membrane; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.

### Table 2. World Health Organization 2008/2016 Criteria for Mixed-Phenotype Blasts

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid</td>
<td>MPO (flow cytometry, immunohistochemistry, or enzyme cytochemistry)</td>
</tr>
<tr>
<td></td>
<td>-OR- Monocytic differentiation (at least 2 of the following: NSE cytochemistry, CD11c, CD14, CD64, lysozyme)</td>
</tr>
<tr>
<td>T lineage</td>
<td>Strong cytoplasmic CD3</td>
</tr>
<tr>
<td>B lineage</td>
<td>Strong CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10</td>
</tr>
</tbody>
</table>

Abbreviations: MPO, myeloperoxidase, NSE, nonspecific esterase.

Data derived from Bene et al. and Arber et al. Strong = at least as intense as in normal B or T cells.
classification, were not defined from experimental biology but rather from expert opinion of a safe threshold to exclude nonspecific staining based on the techniques used at the time. Current multiparameter FCM techniques allow for more precise identification of blast populations, which enables more specific detection of dim or subset marker positivity. Because the sensitivity and specificity of detection methods may vary among flow cytometry laboratories, it is important that individual laboratories develop experience interpreting true positive expression based on their selected reagents and control populations. Keeping this interlaboratory variation in mind, the preceding discussion of MPO thresholds is intended to provide a starting rule-of-thumb based on published data and personal experience.

**BILINEAL ACUTE LEUKEMIA**

The discussion up to this point has focused on diagnostic criteria for the subtype of MPAL known as biphenotypic acute leukemia. Biphenotypic blasts comprise a single population that coexpresses key markers of more than 1 lineage (Figure 1, A through F). The other major subtype of MPAL is bilineal acute leukemia. A bilineal blast population comprises 2 groups of blasts that each fulfills diagnostic criteria for different lineages of acute leukemia (Figure 2, A through F). The WHO criteria for bilineal MPAL require that the sum of the 2 blast populations is at least 20% of nucleated cells. In some cases, one of the blast populations is much smaller than the other, but no minimum count is mandated for the minor population as long as the sum is 20% or greater.

In practice, the accurate identification of minor blast populations of divergent lineage is the biggest challenge for diagnosing bilineal acute leukemia. Identification of immunophenotypic aberrancies can be essential to differentiate a small bilineal blast population from residual normal myeloid blasts or hematogones (physiological B-cell precursors). For example, in the case illustrated in Figure 2, the myeloid blasts were aberrantly positive for CD56 (not shown). It is especially important to consider the possibility of a monocytic blast population coexisting with ALL, which most often occurs in the context of KMT2A translocations, because monocytic blasts often resemble normal monocytes in FCM analysis. Furthermore, it is essential that a sufficient number of events is analyzed by FCM (≥1000 blasts and ≥20 000 total events per tube), so that a minor secondary blast population is not overlooked.

---

**Figure 1.** Mixed-phenotype acute leukemia, B/myeloid, with biphenotypic blasts. A, Peripheral blood smear containing numerous large blasts with irregular nuclear contours and occasional cytoplasmic vacuoles. B through F, Flow cytometric evaluation of the peripheral blood shows a dominant population of CD34+, CD45-dim blasts (blue) and a smaller population of mature lymphocytes (red). The blasts are strongly positive for the B-cell markers CD19 and cytoplasmic CD79a (cCD79a), and uniformly coexpress the myeloid markers CD15 and cytoplasmic myeloperoxidase (cMPO) (Wright-Giemsa, original magnification ×1000 [A]). Abbreviations: ECD, electron-coupled dye; FITC, fluorescein isothiocyanate; PC5, phycoerythrin–cyanine 5; PE, phycoerythrin; SS, side scatter.
In the current WHO classification, biphenotypic acute leukemia and bilineal acute leukemia are both classified as MPAL, because the clinical presentation and genetic characteristics of the 2 subtypes are similar.²,⁶ However, the pathology report should note whether the blasts are biphenotypic or bilineal, because this information is helpful when testing for residual or recurrent disease. Furthermore, bilineal acute leukemias portend a somewhat poorer prognosis than biphenotypic MPAL¹⁷,¹⁸ and have a higher risk of induction failure due to lineage switch.³,⁴,¹⁷

### GENETICS OF MPAL

The WHO classification recognizes 2 genetically defined subclassifications of MPAL and also excludes some acute leukemias from the MPAL classification on the basis of genetics.⁵ The 2 genetically defined categories are MPAL with **BCR-ABL1** fusion and MPAL with **KMT2A** translocation. **KMT2A** translocation is the genetic abnormality most frequently associated with lineage switch after chemotherapy.³,⁴,¹⁹ Rare cases of lineage switch in acute leukemia with **BCR-ABL1** have also been reported.²⁰ Interestingly, some of the reported cases of lineage switch associated with **BCR-ABL1** or **KMT2A** did not show evidence of biphenotypic or bilineal blasts at the time of diagnosis, indicating that these genetic lesions confer the potential for lineage plasticity regardless of the initial blast phenotype.

**KMT2A** translocations are more common in pediatric MPAL (especially infants), and **BCR-ABL1** is more common in adults.²¹ The blast immunophenotypes are very similar to cases of B-ALL with these gene rearrangements, except that a distinct myeloid or monocytic clone is also present in bilineal cases and significant coexpression of MPO is present in biphenotypic cases. Considering that MPO mRNA is present in most B-ALLs with **BCR-ABL1** or **KMT2A** translocations, the clinical and genetic features of these cases are similar to those of B-ALL, and the classification of these cases is an important consideration for the treatment of acute leukemia.

---

**Figure 2.** Mixed-phenotype acute leukemia, T/myeloid, with bilineal blasts. A, Touch imprint of bone marrow core biopsy including a few large blasts (black arrows) with coarse cytoplasmic granules and several small blasts (yellow arrows) with scant cytoplasm. B, Bone marrow aspirate smear from the same biopsy also shows a large blast with coarse granules (black arrow) and a small blast with fine granules (yellow arrow). C, The coarse granules (black arrow) are positive for myeloperoxidase (MPO), while the small blast (yellow arrow) is negative for MPO. D, Flow cytometry of the hemodilute bone marrow aspirate shows a dominant population of mature lymphocytes (red) and an expanded population of CD45-dim blasts (orange and purple). E, Flow cytometric characterization of the blast population shows 2 distinct subpopulations, as based on differential expression of CD7 (orange) and CD117 (purple). F, The CD7⁺ blast population (orange) is strongly positive for cytoplasmic CD3 (cCD3) with c-expression of TdT. The CD117⁺ blasts (purple) are negative for cCD3 and TdT. Additional testing (not shown) demonstrated that the CD7⁺ blasts were also positive for CD5 and negative for surface CD3, CD1a, CD2, CD4, and CD8. Both populations of blasts were positive for CD11b, CD33, CD34, CD38, and CD56 (Weight-Giemsa, original magnification ×1000 [A and B]; MPO stain [3,3-diaminobenzidine], original magnification ×1000 [C]).

**Abbreviations:** ECD, electron-coupled dye; FITC, fluorescein isothiocyanate; PC7, phycoerythrin–cyanine 7; PE, phycoerythrin; SS, side scatter; TdT, terminal deoxynucleotidyl transferase.
KMT2A, the difference between B-ALL with BCR-ABL1 or KMT2A and biphenotypic MPAL with BCR-ABL1 or KMT2A is probably more a matter of degree than a fundamental biological difference.

The WHO classification of MPAL specifically excludes leukemias with t(8;21), t(15;17), inv(16), or KMT2A with myelodysplasia-related abnormalities (AML-MRC), and therapy-related AML. It is recommended to make a secondary notation of mixed phenotype in these cases, for example, AML-MRC with blasts of mixed B/myeloid lineage. The excluded categories that represent significant diagnostic pitfalls are AML with t(8;21) as AML, it is important to consider whether the leukemia has morphologic features of AML with t(8;21), for example, prominent granulocytic maturation in the bone marrow (FAB M2), when the immunophenotype shows a mixture of myeloid and B-lineage markers. Furthermore, AML with t(8;21) often shows expression of CD56 on the blasts, which is uncommon in biphenotypic B/myeloid MPAL. In difficult cases, rapid cytogenetic evaluation may be required, because B/myeloid MPAL is usually treated with ALL-directed induction regimens and AML with t(8;21) is treated with AML-directed induction.

Overlapping features between MPAL and genetically defined AML-MRC creates a diagnostic challenge. The 2 largest series of MPAL identified complex karyotype as the most common genetic abnormality; however, the WHO classification specifies that AML with complex karyotype should be classified as AML-MRC. A case series of MPAL from Stanford University (Palo Alto, California) excluded acute leukemias with complex karyotype for this reason. The use of complex karyotype and other myelodysplasia-related cytogenetic abnormalities to distinguish between MPAL and AML-MRC is problematic because these abnormalities also occur in unilineage ALL, and therefore cannot be taken as definitive evidence of myeloid lineage. In fact, a study of pediatric MPAL (an age group in which AML-MRC is rare) included several cases with complex karyotype, and most responded well to ALL-directed chemotherapy. In our opinion, karyotype should not be the sole deciding factor to distinguish MPAL from AML-MRC. If the leukemia blastsm meet criteria for MPAL and the karyotype includes myelodysplasia-related abnormalities, the patient's clinical history (eg, age and antecedent signs and symptoms), the extent of hematopoietic dysplasia, the blast morphology and immunophenotype, and the presence of somatic mutations associated with lymphoid or myeloid neoplasia should be considered to decide whether the findings are more consistent with MPAL or AML-MRC.

Characterization of the somatic mutational landscape of MPAL is limited. Eckstein et al performed whole exome sequencing on 23 cases of MPAL and found frequent NOTCH1 mutations in cases with a T-lineage component, confirming genetic overlap with unilineage T-ALL. Several RAS and TP53 mutations were detected, consistent with the aggressive behavior typical of MPAL. DNMT3A mutations were also common, especially in older patients. Yan et al performed targeted sequencing of 18 genes in 31 cases of MPAL, and found frequent IKZF1 mutations in Ph+ MPAL, similar to Ph+ B-ALL. They also identified mutations in the epigenetic regulators TET2, EZH2, and ASXL1 in a handful of cases. The 54 cases examined by these 2 groups were negative for NPM1 mutations. Kotrova et al performed whole exome sequencing on sorted T-lymphoblast and myeloid blast populations from 2 cases of bilineal acute leukemia and found no difference in mutational profile between the immunophenotypically distinct blast populations. In both cases, the mutational profiles were dominated by mutations typical of T-ALL.

**CLINICAL CHARACTERISTICS AND OUTCOMES OF MPAL**

Several single- and multi-institution studies describe retrospective review of acute leukemia records to identify cases of MPAL and/or biphenotypic acute leukemia by EGIL criteria, WHO criteria, or both. These studies found that MPAL accounts for approximately 2% of acute leukemias when using WHO criteria, and biphenotypic acute leukemia accounts for 3% to 5% of acute leukemias when using EGIL criteria. Studies that compared the 2 classification schemes found that fewer leukemias were classified as MPAL by WHO criteria, mostly owing to cases of ALL with expression of multiple myeloid surface markers but negative for MPO. In addition, the WHO classification excludes cases of AML with t(8;21) and AML-MRC, which were considered biphenotypic by some studies that used the EGIL criteria. Shi and Munker performed a search of the US National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database for reported leukemia cases categorized as MPAL or acute biphenotypic leukemia, and found that these represented 0.6% of acute leukemias in the database. The lower percentage in the SEER database compared to institutional retrospective reviews is likely due to failure to correctly identify MPAL in reporting to the SEER database compared to the retrospective reviews performed at academic institutions, where multiyear records of immunophenotyping data were reviewed for the purpose of consistently applying the EGIL and/or WHO criteria.

These studies showed a broad age range for MPAL with a slight male predominance, approximately 1:5:1 in most series. Outcomes for MPAL were generally worse than for comparison cohorts of patients with AML and ALL. The poor outcomes mostly correlated with enrichment for high-risk genetics in the MPAL cohorts, including BCR-ABL1 fusion, KMT2A rearrangements, and complex karyotype. In most reports, the patients were initially treated with the local standard of care for B-ALL, T-ALL, or AML, based on which lineage appeared dominant by immunophenotype and morphologic evaluation. Most reports showed better initial response to ALL-directed chemotherapy than AML-directed therapies. In some cases, poor responders were switched from AML-directed to ALL-directed therapies (or vice versa), and more than half of the patients achieved complete remission with the second regimen. A recent review of stem cell transplant experience in MPAL suggests that allogeneic stem cell transplant in first complete remission is beneficial.
DIAGNOSTIC PITFALLS OF MPAL

The most pressing concern for the diagnostic pathologist approaching a new acute leukemia is to quickly provide necessary and sufficient information for initial treatment. Currently, the most common clinical practice is to initially treat MPAL patients with ALL-directed chemotherapy. This approach is supported by the retrospective studies described in the previous section and also makes patho-physiological sense because most acute leukemias that fit the current WHO classification of MPAL more closely resemble ALL than AML in terms of genetics and immunophenotype (as reviewed above). Furthermore, the types of AML that most frequently show significant coexpression of lymphoid markers are AML with t(8;21) and AML-MRC, both of which are explicitly excluded from the MPAL category in the WHO classification. Based on current practices, the diagnostic error most likely to trigger inappropriate treatment is making a diagnosis of MPAL based on FCM when subsequent cytogenetic analysis changes the diagnosis to AML with t(8;21) or AML-MRC. Avoiding this pitfall requires careful correlation with the blood and bone marrow morphology and patient history (for AML-MRC). Most MPALs have numerous primitive blasts and/or partial monocytic differentiation, in contrast to AML with t(8;21) and AML-MRC, which usually have prominent granulocytic maturation in the bone marrow and may have prominent dysplastic features.

Interpretation of MPO expression can be a pitfall for distinguishing between ALL and MPAL, owing to the lack of precise guidelines and varying techniques for MPO detection. Fortunately, evidence that most MPALs respond to ALL-directed induction therapy relieves some of the pressure on interpreting MPO expression in blasts with predominantly lymphoid features. When evaluating an acute leukemia that seems most consistent with ALL but expresses significant MPO, it can be helpful to discuss the findings with the treating physician to ensure that he or she is aware of evidence that these leukemias usually respond best to ALL-directed therapy despite the presence of MPO.

Another common pitfall is to overlook minor populations of monocyteic blasts, lymphoid blasts, or myeloblasts that are diagnostic of bilineal acute leukemia. This error usually does not change the initial treatment; however, correctly diagnosing bilinear acute leukemia provides an early warning of high-risk disease and of the potential for sudden proliferation of a minor clone during induction therapy.

We have noticed occasional misapplication of the WHO criteria for assigning mixed lineage to a single blast population (Table 2) to decide whether an acute leukemia can be assigned to a specific lineage or is undifferentiated/unclassifiable. These criteria are only intended to be used for the diagnosis of biphenotypic MPAL. For example, AML with minimal differentiation (FAB M0) is negative for MPO by EC by definition but it is still classified as myeloid lineage. Also, the lack of strong CD3 or CD19 expression does not exclude the diagnosis of ALL, so long as the blasts lack definitive features of myeloid or monocytic differentiation. In contrast, acute undifferentiated leukemia lacks any expression of lineage markers, typically only expressing CD34, CD38, and/or HLA-DR.

OUTSTANDING QUESTIONS

More than 40 years after the FAB classification of acute leukemias was proposed, the role of MPO in defining myeloid lineage or mixed lineage is still a topic of discussion and investigation. As described above, it is well documented that MPO mRNA and protein can be detected in blasts with lymphoid phenotype that are negative for MPO by EC. A recent study examined a cohort of 293 patients with B-ALL excluding leukemias with BCR-ABL1 fusion or KMT2A rearrangement, and it identified 29 cases that were positive for MPO by FCM and negative for other myeloid markers. Only 1 case was positive for MPO by EC. Compared to the rest of the B-ALL cohort, MPO+ B-ALL was associated with a higher rate of relapse and shorter event-free survival. These findings suggest that detection of MPO by FCM is a prognostically significant biomarker, even when MPO enzymatic activity is undetectable. However, it is unclear whether isolated expression of MPO in B-ALL is evidence of the lineage plasticity characteristic of MPAL.

The significance of MPO expression in T-ALL is also uncertain. Studies of FLT3 mutations in T-ALL found that these mutations correlate with an early T-precursor (ETP)–like immunophenotype, and most cases expressed MPO. FLT3 mutations have also been detected in several cases of bona fide ETP-ALL. Based on 2016 WHO criteria, the presence or absence of MPO is a discriminating factor between the diagnosis of ETP-ALL and MPAL, T/myeloid. It would be interesting to know if cases of MPAL, T/myeloid, with FLT3 mutation have a gene expression profile that matches the previously defined profile for ETP-ALL, because it seems likely that these leukemias are closely related.

Exclusion of AML-MRC from the MPAL category in the WHO classification has helped to restrict the MPAL designation to a group of acute leukemias that are more closely related to ALL than to AML. However, the significance of mixed phenotype within the context of AML-MRC is uncertain. Studies of the prognostic impact of immunophenotype in AML suggest that aberrant expression of lymphoid markers has minimal prognostic significance when cytogenetic risk groups are included in the analysis.

To the best of our knowledge, the impact of MPAL immunophenotype on outcomes in AML-MRC has not been specifically addressed. A recent submission to the American Society of Hematology’s Image Bank described a bilinear acute leukemia comprising B lymphoblasts and myeloblasts. Owing to a complex karyotype including monosomy 7, the leukemia was classified as AML-MRC by WHO criteria, and significant dysgranulopoiesis was also present. Details of therapy and outcome were not provided; however, it seems unlikely that an acute leukemia with a prominent B-lymphoblast population would behave as a typical AML-MRC. Further investigation is needed to evaluate the overlap between AML-MRC and MPAL to ensure optimal classification and treatment decisions.

CONCLUSIONS

Mixed-phenotype acute leukemia is a diagnostic and therapeutic challenge owing to its heterogeneity, overlapping features with other types of ALL and AML, and lineage plasticity. Multiparameter FCM can detect immunophenotypic profiles typical of MPAL, and international consensus guidelines have contributed greatly to the standardization of diagnosis and clinical investigation of these unusual leukemias. There are several unresolved questions about the diagnostic criteria for MPAL that will require further...
studies of the correlation between immunophenotype, genotype, lineage plasticity, and therapeutic response.

References