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Acute leukemias of ambiguous lineage are defined by the World Health Organization (WHO) classification as leukemias that either lack a specific lineage association or show features of concurrent multiple lineage associations [1, 2]. Ambiguous-lineage leukemias are more rare and more diagnostically challenging than conventional acute myeloid leukemias and lymphoblastic leukemias [1–11]. Given that the cytologic features of these leukemias are generally nonspecific, the diagnosis rests upon extensive immunophenotypic analysis. Multiparametric flow cytometric immunophenotyping is the preferred method for immunophenotyping, but in some cases, immunohistochemistry may be performed and may facilitate the recognition of two distinct blast populations in tissue sections. In addition to standard immuno-

phenotypic studies, further evaluation should include cytogenetic studies to identify cases with t(9;22) (q34.1;q11.2); *BCR-ABL1* or t(v;11q23.3); *KMT2A* rearranged. Table 13.1 highlights the diagnostic subcategories of acute leukemias of ambiguous lineage, Table 13.2 lists the diagnostic criteria for lineage determination, and Table 13.3 compares lineage-specific and lineage-associated markers in acute leukemia. This chapter provides pathologic examples of various ambiguous-lineage leukemias and illustrates the key diagnostic challenges, considerations, and caveats in establishing these rare diagnoses (Figs. 13.1, 13.2, 13.3, 13.4, 13.5, 13.6, 13.7, 13.8, 13.9, 13.10, 13.11, 13.12, 13.13, 13.14, 13.15, 13.16, and 13.17).

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Table 13.1 Diagnostic subtypes of acute leukemias of ambiguous lineage

Subtype	Exemplary figures
Acute undifferentiated leukemia	Figs. 13.1, 13.2, 13.3 and 13.4
Mixed phenotype acute leukemia, T/myeloid, NOS	Figs. 13.5, 13.6 and 13.7
Mixed phenotype acute leukemia, B/myeloid, NOS	Figs. 13.8, 13.9 and 13.10
Mixed phenotype acute leukemia with t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i>	Figs. 13.11, 13.12, 13.3 and 13.4
Mixed phenotype acute leukemia with t(v;11q23.3); <i>KMT2A</i> rearranged	Not illustrated
Mixed phenotype acute leukemia, NOS: rare types	Not illustrated

NOS—not otherwise specified

Table 13.2 Criteria for lineage assignment for a diagnosis of mixed phenotype acute leukemia

<i>Myeloid lineage</i>	
Myeloperoxidase (flow cytometry, immunohistochemistry, or cytochemistry)	
Monocytic differentiation (at least two of the following: nonspecific esterase cytochemistry, CD11c, CD14, CD64, lysozyme)	
<i>T lineage</i>	
Strong ^a cytoplasmic CD3 (with antibodies to CD3 ε chain)	
Surface CD3	
<i>B lineage</i>	
Strong ^a CD19 with at least one of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10	
Weak CD19 with at least two of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10	

Adapted from Arber et al. [1]

^aStrong is defined as equal to or brighter than the normal B cells or T cells

Table 13.3 Comparison of lineage-specific and lineage-associated markers in acute leukemia

	<i>Lineage-specific markers</i>	<i>Comments</i>
Myeloid lineage	Myeloperoxidase	Myeloid only
T lineage	cCD3 or sCD3 (with antibodies to CD3 ε chain)	T lineage only
B lineage	CD19	Can be seen in AML with t(8;21)
	<i>Lineage-associated markers</i>	<i>Comments</i>
Myeloid lineage	CD13, CD33, CD117	Can be seen in B-ALL and T-ALL
T lineage	CD2, CD5, CD7	Can be seen in AML and B-ALL
B lineage	CD10, cCD22, CD79a	Can be seen in AML and T-ALL

AML, acute myeloid leukemia; B-LL, B-lymphoblastic leukemia; T-LL, T-lymphoblastic leukemia

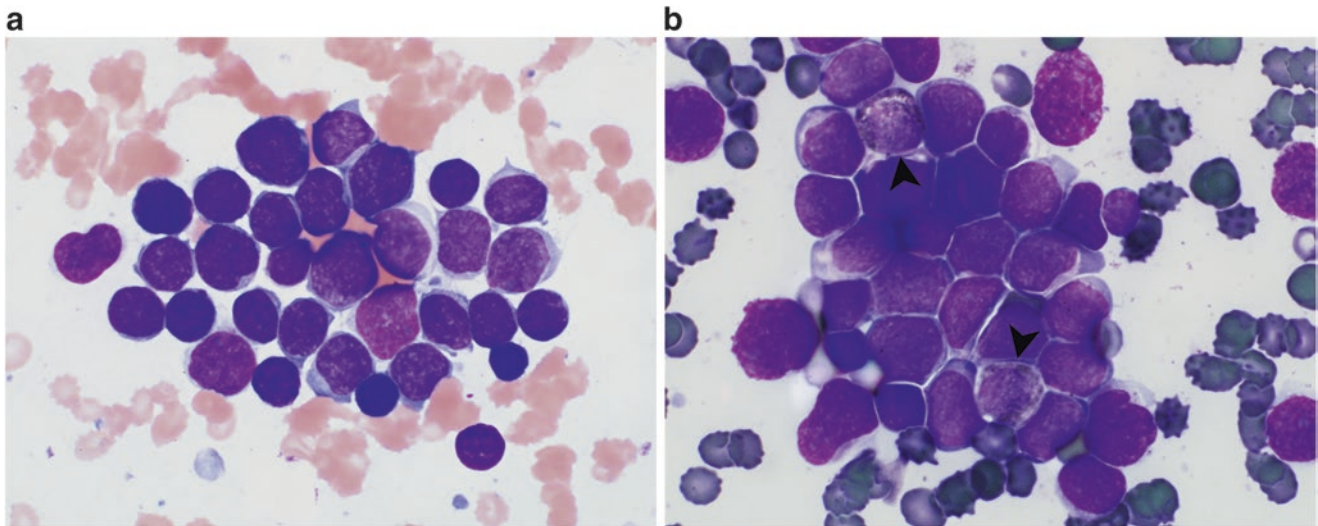


Fig. 13.1 Morphologic and cytochemical features in acute undifferentiated leukemia. **(a)** The morphologic features of the blasts in acute undifferentiated leukemia are generally nonspecific and, importantly, lack Auer rods. The blasts are small to intermediate in size, with round to slightly irregular nuclear contours, dispersed chromatin, and scant amounts of usually agranular cytoplasm. This morphologic appearance does not allow distinction of acute undifferentiated leukemia from acute myeloid leukemia with minimal differentiation and acute lymphoblas-

tic leukemia, emphasizing the significant importance of extensive immunophenotyping for lineage determination. **(b)** Cytochemical testing for myeloperoxidase (MPO) is also a helpful tool to identify myeloid lineage. In this example of an acute undifferentiated leukemia, the blasts lack cytochemical MPO positivity, with admixed residual myelocytes (arrowheads) serving as internal positive controls. In contrast, a small fraction of MPO-positive blasts (<3%) may be seen in some cases of acute myeloid leukemia with minimal differentiation

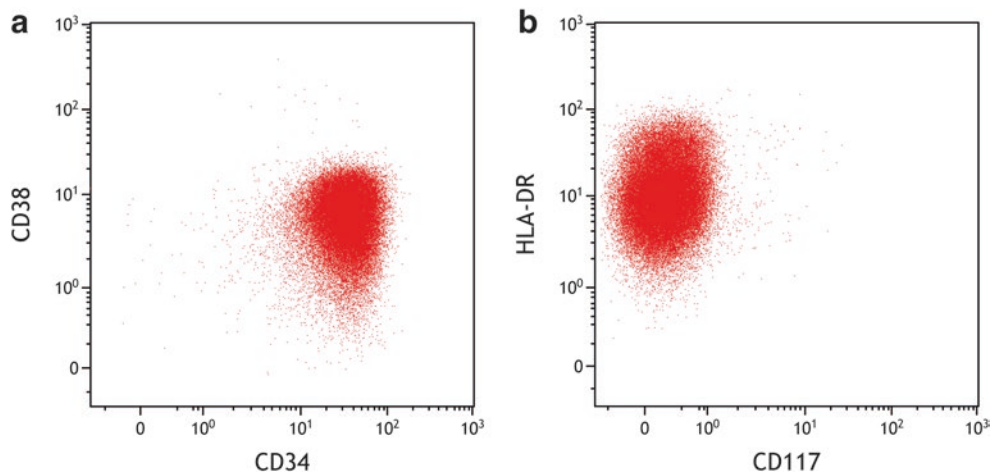


Fig. 13.2 Flow cytometric findings in acute undifferentiated leukemia. The blasts in acute undifferentiated leukemia, by definition, do not express any markers that are considered lineage specific (see Table 13.2). However, they are identified as blasts based on expression of early hematopoietic precursor markers, such as CD34 **(a)**. Commonly they also express CD38 **(a)** and HLA-DR **(b)**. The blast population is depicted in red

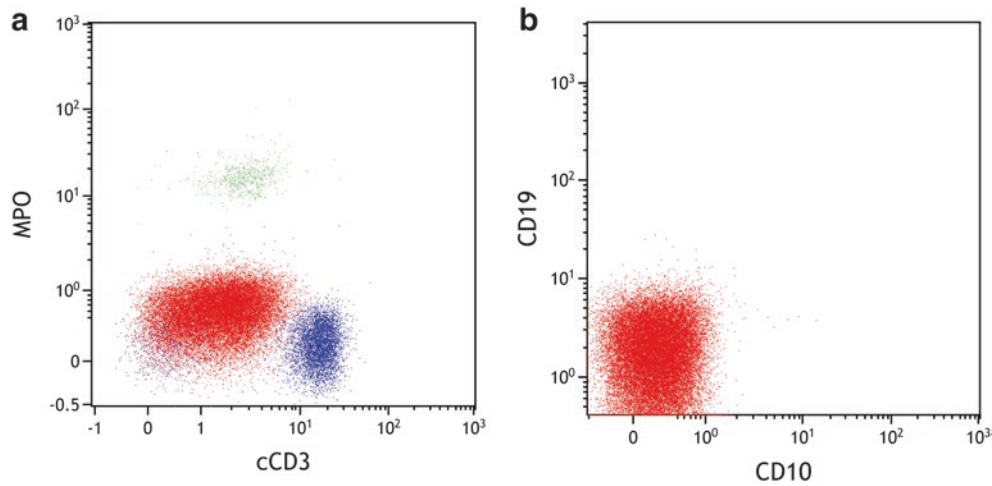


Fig. 13.3 Flow cytometric findings in acute undifferentiated leukemia. Comprehensive flow cytometric immunophenotyping is essential when diagnosing a case of acute undifferentiated leukemia. Specifically, commitment to a specific lineage(s) (myeloid, B, or T) must be excluded. To accurately determine whether lineage-specific markers are expressed or

not, proper internal controls are crucial. (a) The blasts (red) are negative for MPO and cytoplasmic CD3 (cCD3); the internal granulocytic population (green) and mature T cells (blue) serve as internal positive controls. (b) Similarly, the blasts (red) lack expression of the B-lineage markers CD19 and CD10

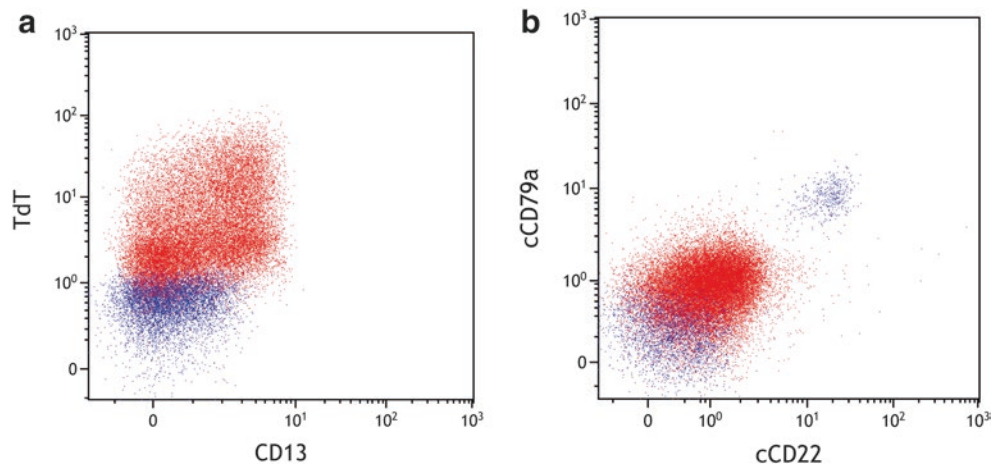


Fig. 13.4 Flow cytometric findings in acute undifferentiated leukemia. The blasts in acute undifferentiated leukemia may show positivity for terminal deoxynucleotidyl transferase (TdT) (blasts depicted in red) (a). TdT is not a lineage-specific marker and can be expressed in a variety of acute leukemia subtypes. In addition, acute undifferentiated leukemia may express some myeloid-associated markers (CD13, CD33,

CD117), but typically no more than one of these markers is present. In this case, the blasts (*red*) express CD33 (not shown), but do not express CD117 (see Fig. 13.2b) or CD13 (a). The blasts lack both monocyte-associated markers (not shown) and the B-lineage-associated markers (cCD79a and cCD22) (blasts shown in red; mature B cells in blue [upper right]) (b)

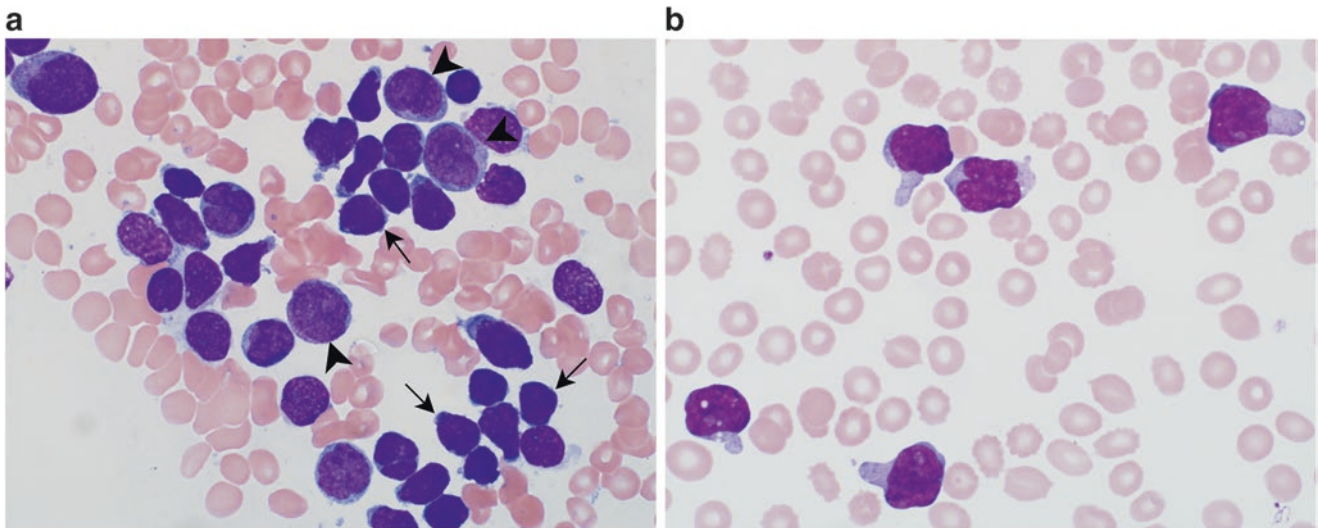


Fig. 13.5 Morphologic features of mixed phenotype acute leukemia, T/myeloid, not otherwise specified (MPAL, T/myeloid, NOS). The morphologic findings in MPAL, T/myeloid, NOS may be quite variable. (a) In this first example from a Wright-Giemsa-stained bone marrow aspirate, there is a dimorphic appearance, with one blast population smaller in size, resembling lymphoblasts (arrows), and a second blast population larger in size, resembling myeloblasts (arrowheads). (b) In

contrast, in this second example, the morphologic features of the blasts are more uniform, characterized by small to intermediate size, nuclear contour indentations, high nuclear-to-cytoplasmic ratio, and asymmetric cytoplasmic extensions (hand-mirror morphology). This appearance morphologically resembles some cases of T-lymphoblastic leukemia (Wright-Giemsa, peripheral blood)

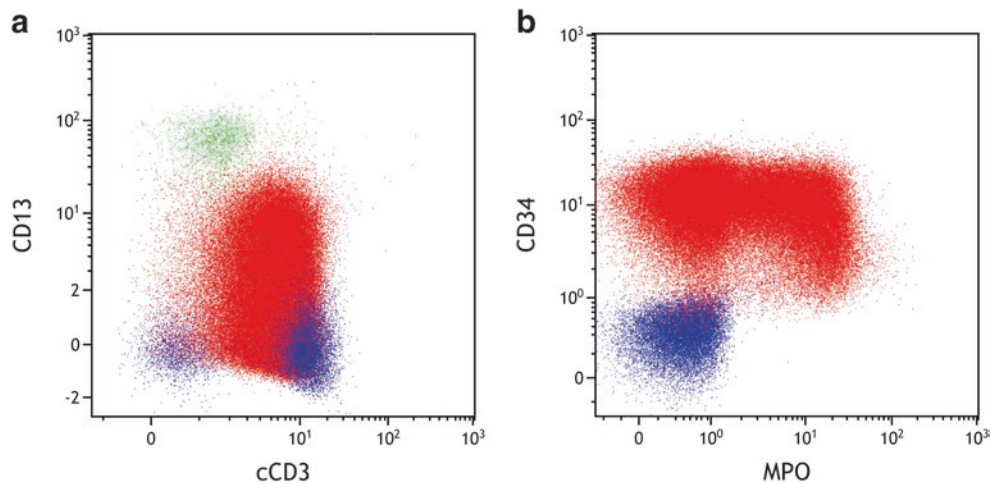


Fig. 13.6 Flow cytometric findings in MPAL, T/myeloid, NOS. As for acute undifferentiated leukemia, comprehensive flow cytometric immunophenotyping is essential for diagnosing a case of mixed phenotype acute leukemia, T/myeloid type. (a) By using antibody to the CD3 ϵ chain, the blasts (red) are positive for cCD3, confirming T-lineage derivation. This cCD3 positivity is demonstrated by a portion of the blasts

expressing at least equal intensity of cCD3 expression to that of normal control T cells (blue, lower right). Importantly, the residual granulocytes/monocytes (green) serve as negative internal controls for cCD3. (b) The blasts (red) also express MPO, which, in conjunction with the cCD3 positivity, establishes the diagnosis of MPAL, T/myeloid type. The blasts also show positivity for CD13 (dim) (a) and CD34 (b)

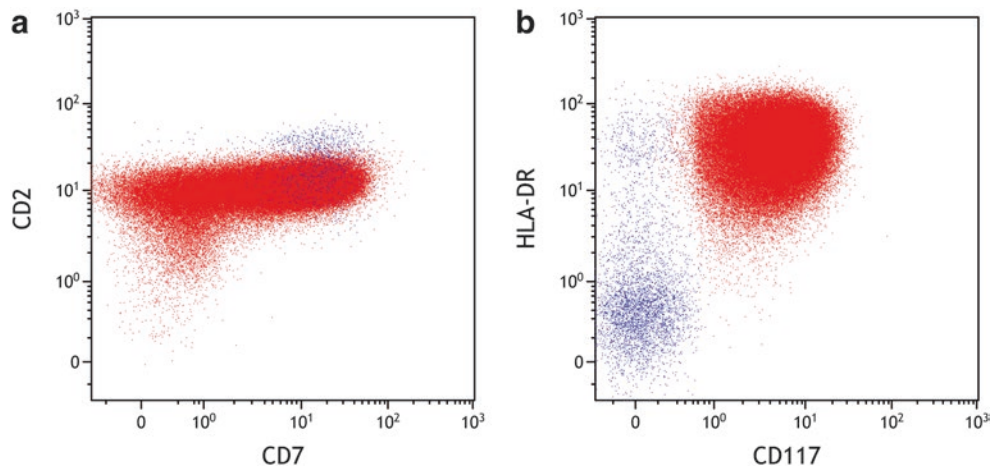


Fig. 13.7 Flow cytometric findings in MPAL, T/myeloid, NOS. In addition to the requisite cCD3 expression, the blasts in MPAL, T/myeloid, commonly express other T-lineage-associated markers such CD2, CD5, and/or CD7, but usually do not express surface CD3 (sCD3). (a) Blasts (red) are revealed that are uniformly positive for CD2 with variable expression of CD7, compared with normal T cells

(blue). In addition to MPO, the blasts in MPAL, T/myeloid frequently express one or more myeloid-associated markers, such as CD13 (see Fig. 13.6a), CD33 (not shown), or CD117 (b, blasts depicted in red). (b) Although T-lymphoblastic leukemia is normally negative for HLA-DR, T/myeloid leukemia may demonstrate HLA-DR expression

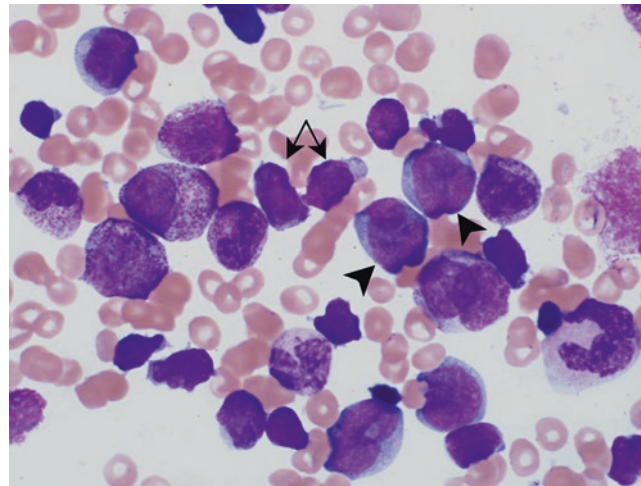


Fig. 13.8 Morphologic features of mixed phenotype acute leukemia, B/myeloid, not otherwise specified (MPAL, B/myeloid, NOS). MPAL, B/myeloid, NOS (similar to MPAL, T/myeloid, NOS) may comprise either single, uniform-appearing blasts or a dimorphic blast population,

as seen in this figure. Some blasts are small, with less open chromatin and scant cytoplasm resembling lymphoblasts (arrows); others are larger, with finer chromatin and more abundant cytoplasm, resembling myeloblasts (arrowheads)

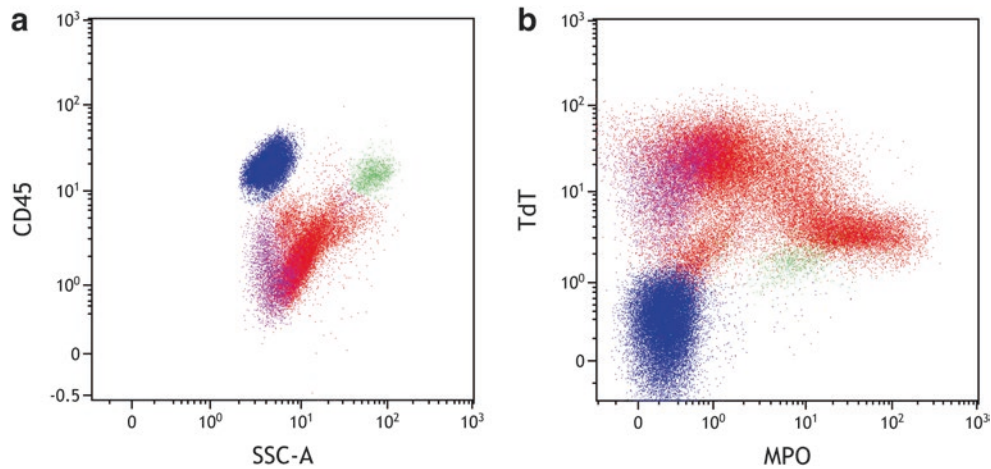


Fig. 13.9 Flow cytometric findings in MPAL, B/myeloid, NOS. The two morphologically distinctive populations demonstrated in Fig. 13.8 are also reflected in the accompanying flow cytometric immunophenotyping. (a) Two distinct blast populations (one depicted in red and the other in pink) are seen on the CD45 versus side scatter histogram, in which background granulocytes are depicted in green and mature lymphocytes in blue. (b) Both blast populations are positive for TdT, confirming immaturity, but only one population is positive for MPO (partial, red), consistent with myeloid lineage; the other blast population is negative for MPO (pink). The presence of MPO positivity meets WHO criteria for myeloid lineage

phoid cells in blue. (b) Both blast populations are positive for TdT, confirming immaturity, but only one population is positive for MPO (partial, red), consistent with myeloid lineage; the other blast population is negative for MPO (pink). The presence of MPO positivity meets WHO criteria for myeloid lineage

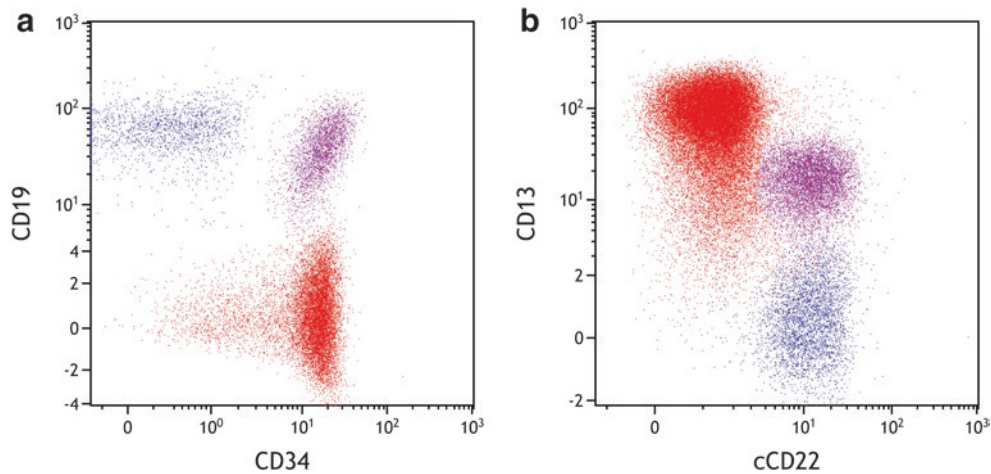


Fig. 13.10 Flow cytometry in MPAL, B/myeloid, NOS. The blasts in MPAL, B/myeloid, NOS (either one or both populations) must meet WHO criteria for B lineage and myeloid expression (Table 13.2). (a) In this example, the blasts in pink are strongly positive for CD19, suggesting but not confirming B lineage (compare with background mature B cells depicted in blue). According to the WHO classification, no single marker is sufficient to indicate B lineage, in contrast to myeloid and T

lineage. Based on the CD19 expression level, expression of additional B-cell-associated markers (CD10, cCD22, and CD79a) is required for B-lineage assignment. (b) In this case, strong CD19 expression coupled with strong cCD22 confirms the B lineage (blasts depicted in pink). In addition to MPO, the second blast population also expressed the myeloid-associated marker CD13 (red blast population)

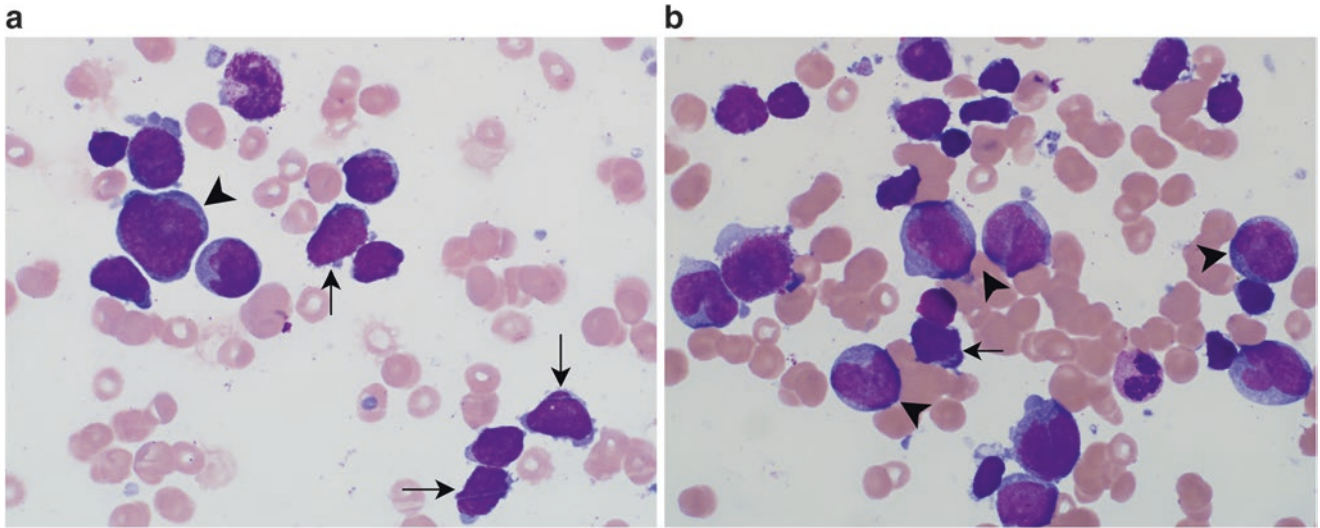


Fig. 13.11 Morphologic features of mixed phenotype acute leukemia (MPAL) with $t(9;22)(q34.1;q11.2); BCR-ABL1$. This is a rare type of acute leukemia that not only meets the criteria for MPAL, B/myeloid, but also harbors the recurring cytogenetic abnormality $t(9;22)(q34;q11.2); BCR-ABL1$. Similar to other types of MPAL, there are no diagnostic morphologic clues to the diagnosis, and many cases show dimorphic blast populations. **(a)** In this example, a dimorphic appear-

ance is evident, with some areas of the bone marrow aspirate revealing small blasts with features typical of lymphoblasts (arrows). **(b)** Other areas exhibit larger blasts with more abundant cytoplasm and monocytic appearance (arrowheads). In this circumstance, it is important to distinguish a truly separate myeloid and lymphoblast population and not misinterpret the myeloid-appearing component as a morphologic variation within a pure B-lymphoblastic leukemia

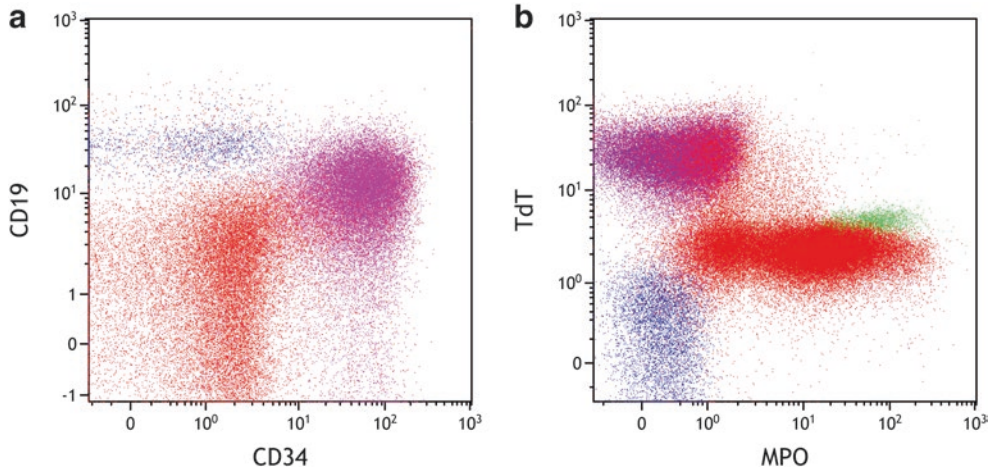


Fig. 13.12 Flow cytometric findings in MPAL with $t(9;22)(q34.1;q11.2); BCR-ABL1$. Flow cytometric immunophenotyping clearly shows two distinct blast populations (one depicted in pink and one in red). **(a)** The blasts depicted in pink are positive for CD19 (strong), CD34, and TdT and are negative for MPO (mature B cells in

blue; maturing granulocytes in green). **(b)** In contrast, the blasts depicted in red are positive for MPO and TdT (small subset) and negative for CD19 and CD34 **(a)**. The strong CD19 expression is suggestive of B-lineage blasts (depicted in pink), and the MPO expression confirms myeloid lineage of blasts (red) in this acute leukemia

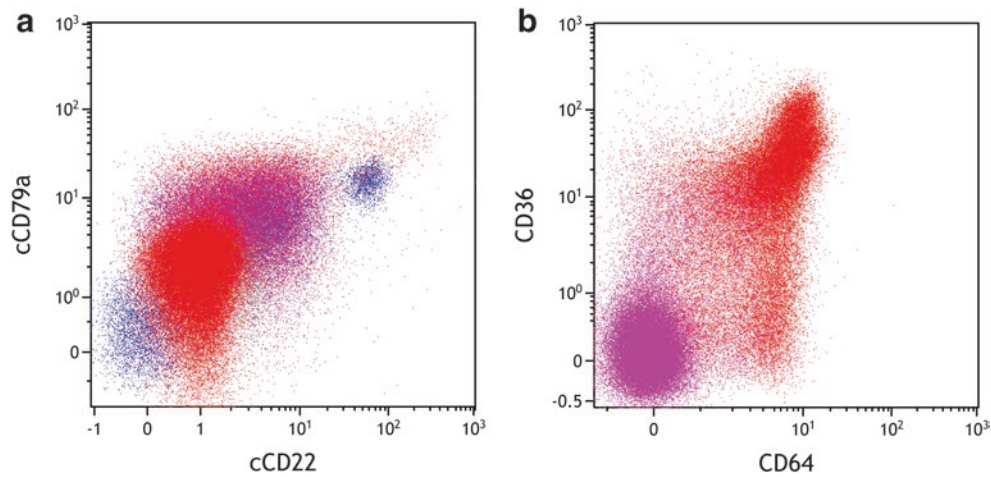


Fig. 13.13 Flow cytometric findings in MPAL with $t(9;22)(q34.1;q11.2)$; *BCR-ABL1*. (a) In conjunction with the images in Fig. 13.12, the blasts depicted in pink strongly express CD79a (in addition to strong CD19), confirming their B-lineage commitment. (b) The co-expression of bright CD36/CD64 on the separate blast population (red) confirms monocytic differentiation, as was observed in the bone marrow aspirate in Fig. 13.11b



Fig. 13.14 Fluorescence in situ hybridization (FISH) in mixed phenotype acute leukemia with $t(9;22)(q34.1;q11.2)$; *BCR-ABL1*. FISH, using a dual-color, dual-fusion probe set for *BCR* and *ABL1*, is performed on interphase cells from a patient with mixed phenotype acute leukemia. The *BCR* probe is labeled with SpectrumGreen and the *ABL1* probe, with SpectrumOrange. The two interphase cells reveal the typical abnormal pattern characteristic of a reciprocal translocation involving *BCR* and *ABL1* (i.e., one green signal (intact *BCR*), one orange

signal (intact *ABL1*), and two yellow (green/orange) fusion signals (the fusion of *BCR* and *ABL1* on both chromosomes 9 and 22). According to the WHO classification, the diagnosis of mixed phenotype acute leukemia with $t(9;22)(q34.1;q11.2)$; *BCR-ABL1* should not be rendered in patients with a history of chronic myeloid leukemia, *BCR-ABL1* positive. One other recurring genetic abnormality that may be seen in MPAL is $t(v;11q23.3)$; *KMT2A* (previously called *MLL*) rearranged (not shown)

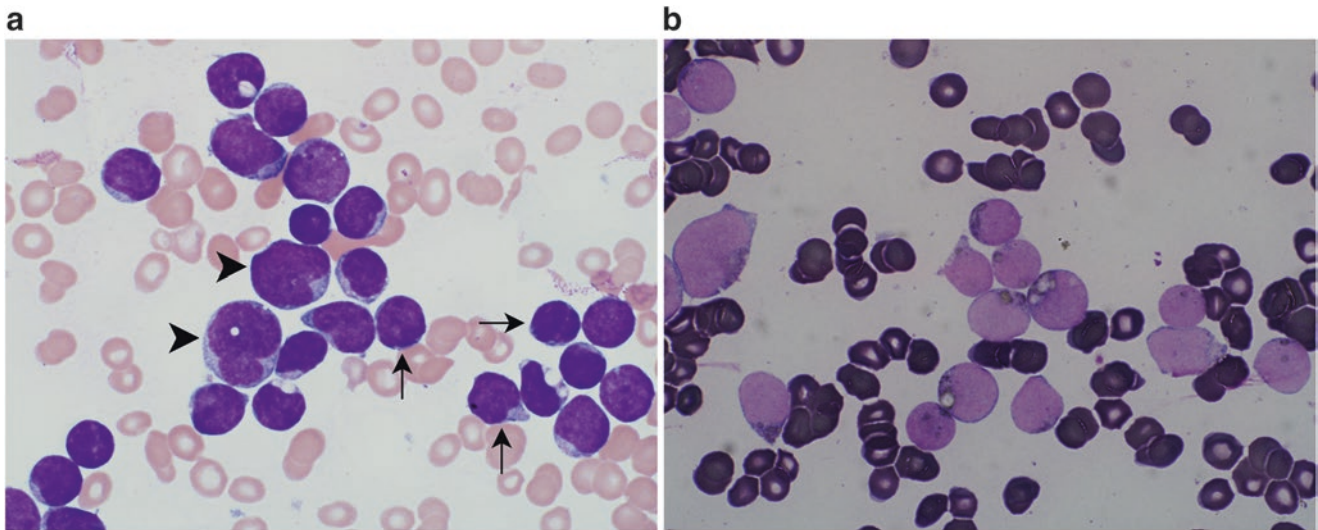


Fig. 13.15 Morphologic and cytochemical features in acute myeloid leukemia with dimorphic populations. MPALs may have dimorphic blast populations, but the presence of a dimorphic population does not necessarily indicate MPAL. (a) A dimorphic blast population is present in this example, which shows lymphoblast-like cells (arrows) and myeloblast-like cells (arrowheads). (b) All of the blasts are essentially positive for MPO, indicating uniform myeloid lineage commitment

(cytochemical MPO, bone marrow aspirate; cytoplasmic brown stain connotes positivity). Flow cytometric immunophenotyping also confirms a single blast population, which expresses MPO, CD117 (bright), and CD33 (bright) and is negative for cCD3, CD19, CD79a, or cCD22 (not shown). For cases with a dimorphic population, it is important to perform an extensive immunophenotypic workup to avoid an overdiagnosis of MPAL

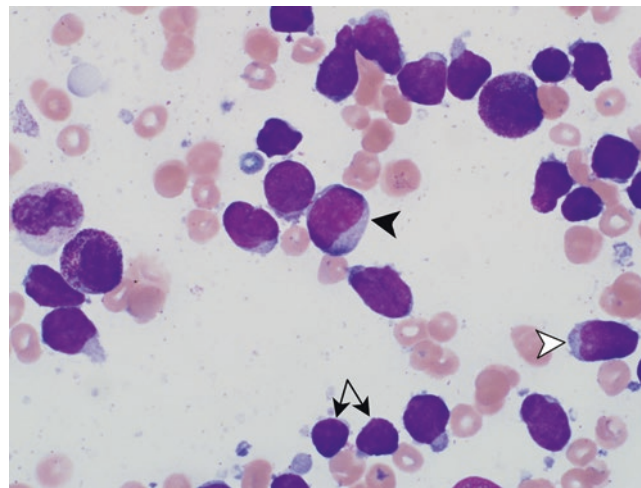


Fig. 13.16 Spectrum of morphologic features in pure lymphoblastic leukemia. The presence of a spectrum of blast morphologies or dimorphic appearance is not uncommon in both B- and T-lymphoblastic leukemia. In this example of B-lymphoblastic leukemia, the blasts range from small with scant cytoplasm, condensed chromatin, and

inconspicuous nucleoli (arrows) to intermediate ones with more cytoplasm and open chromatin (open arrowhead) and to large blasts with abundant amounts of light blue cytoplasm and dispersed chromatin (arrowhead). Immunophenotypic studies are essential to make an accurate diagnosis

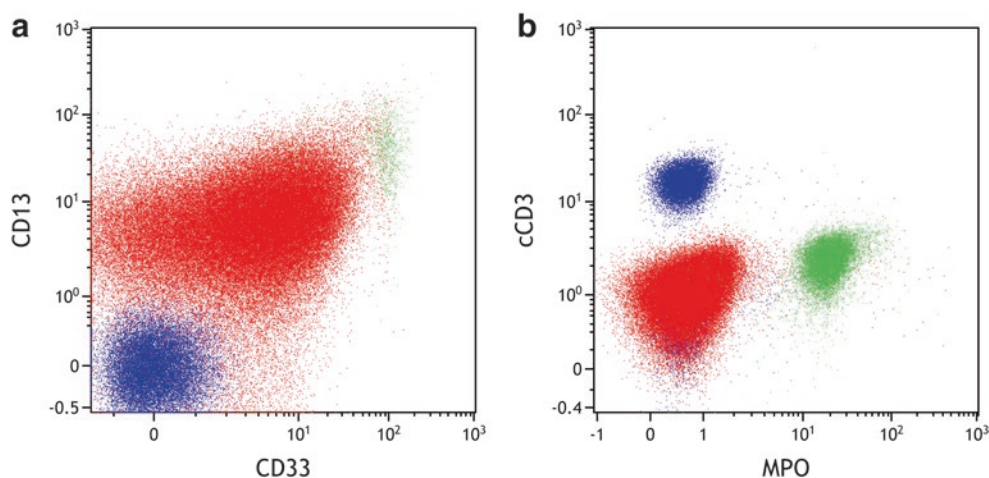


Fig. 13.17 Aberrant myeloid antigen expression in lymphoblastic leukemia. Both B- and T-lymphoblastic leukemias may express one or more myeloid-associated markers, including CD13, CD33, and/or CD117 (see Table 13.3). However, it is important to not overinterpret the expression of these markers as MPAL (either B/myeloid or T/myeloid). For MPAL, the expression of MPO or monocytic differentiation is required for myeloid lineage (see Table 13.2). (a) This B-lymphoblastic leukemia shows aberrant CD13 and CD33 expression (blasts depicted

in red; T cells in blue; granulocytes in green) (bright CD19, bright cCD22, and bright CD79a expression on the blasts, not shown). (b) Importantly, this leukemia does not express MPO (blasts, red; T cells, blue; granulocytes, green) or monocytic differentiation (not shown); therefore, the diagnosis is B-lymphoblastic leukemia with aberrant myeloid antigen expression and not mixed phenotype acute leukemia, B/myeloid type

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