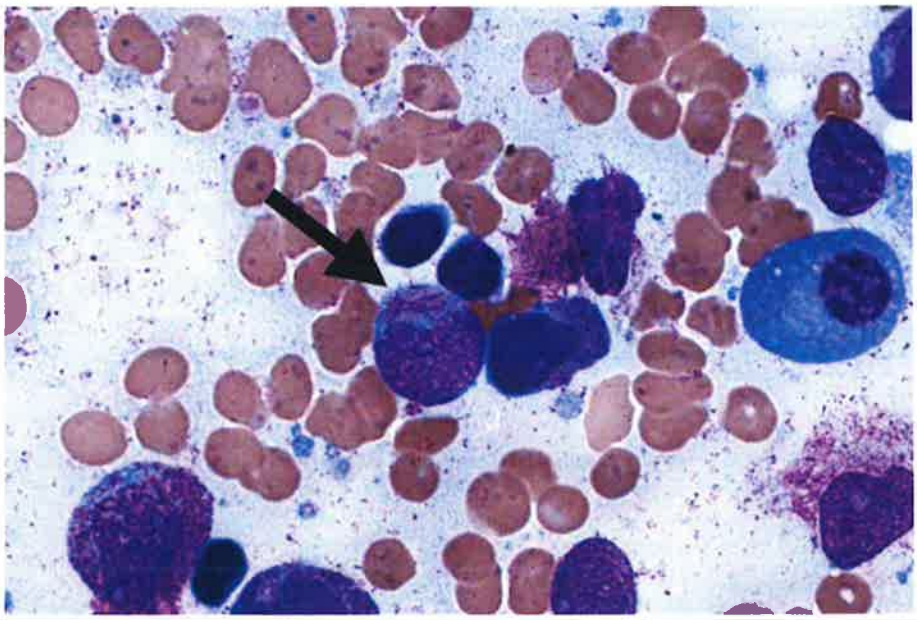
Cell Identification

BMD-08

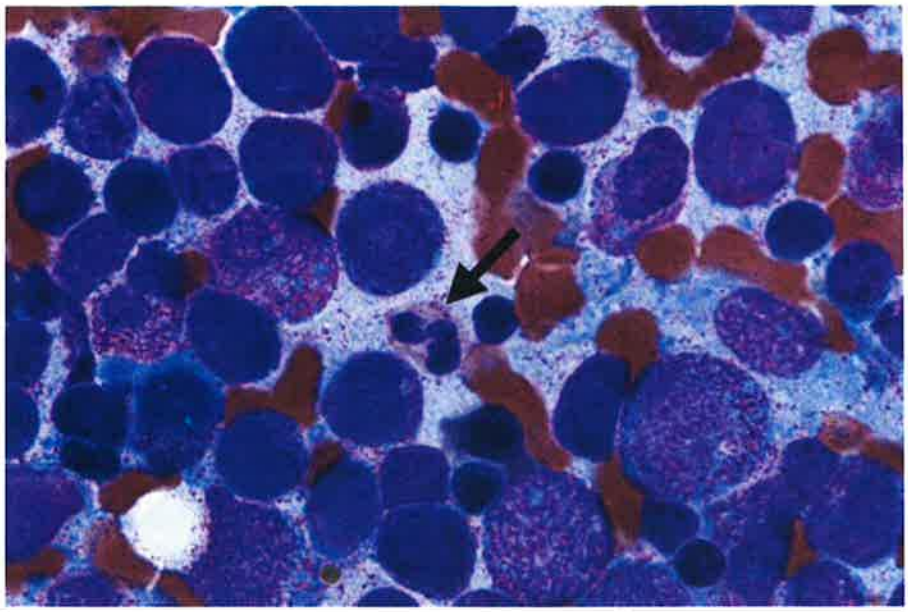


Identification	Participants No.	Participants %	Evaluation
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	224	69.1	Educational
Myeloblast with Auer rod	96	29.6	Educational
Neutrophil, promyelocyte	2	0.6	Educational
Blast cell (including lymphocyte)	2	0.6	Educational

The arrowed cell is an abnormal promyelocyte blast equivalent, correctly identified as "neutrophil, promyelocyte, abnormal, with/without Auer rod(s)" by 69.1% of participants. They are characteristic of acute promyelocytic leukemia (APL). These cells differ from normal promyelocytes. As opposed to normal promyelocytes, the nucleus is often folded, bilobed, or reniform and may show overlapping nuclear lobes. A distinct Golgi zone is typically absent, and the granules may be either coarser or finer than those seen in normal promyelocytes. A microgranular APL variant (sometimes referred to as hypgranular) occurs in which granules are so fine that they cannot be readily seen by standard light microscopy. These abnormal promyelocytic cells frequently contain numerous overlapping Auer rods (these types of cells are termed "faggot cells").

The arrowed cell was identified as "myeloblast with Auer rod" by 29.6% of participants. While Auer rods are present in the arrowed cell, the nucleus is indented with a low N:C ratio. Indeed, these features allow one to identify this cell as a promyelocytic blast and thus the choice of "neutrophil, promyelocyte, abnormal, with/without Auer rod(s)" is the preferred and most specific choice. However, because this is a blast equivalent in the WHO classification, the choice "myeloblast with Auer rod" is deemed acceptable by the committee but considered less specific.

BMD-09



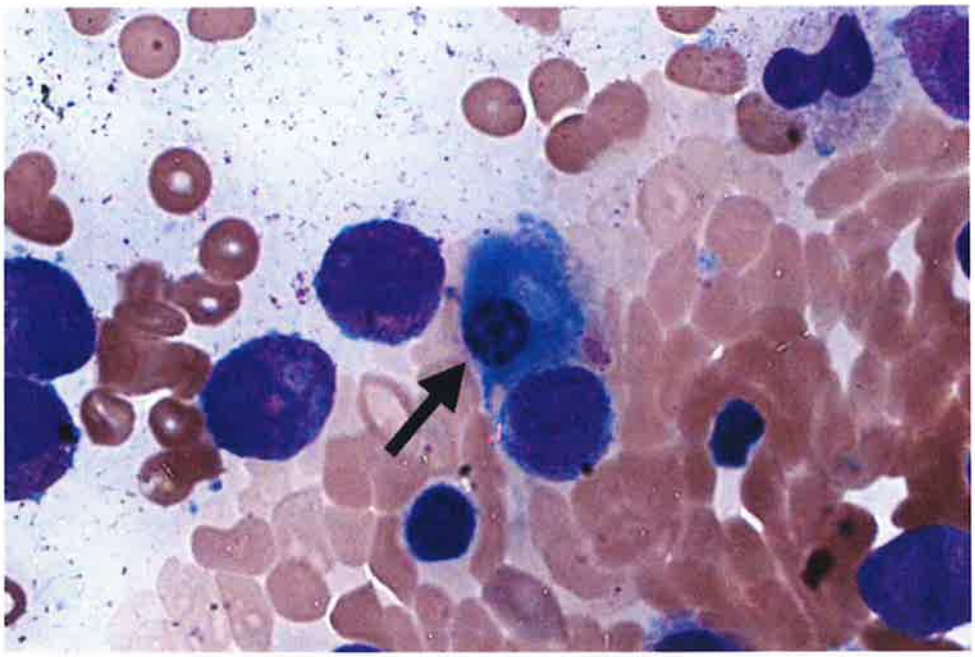
Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	313	96.6	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	7	2.2	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	4	1.2	Educational

The arrowed cell is a neutrophil, segmented or band, as correctly identified by 96.6% of participants. Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. The nucleus is segmented or lobated (with a normal range of three to five lobes). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from the band neutrophil.

The arrowed cell was incorrectly identified as "neutrophil with dysplastic nucleus and/or hypogranular cytoplasm" by 2.2% of participants. The cell in question has a segmented pattern with the mature chromatin of a neutrophil. While the cells are quite dense in this area of the smear, granules can be seen and given the variation that my occur in neutrophils from normal subjects and variation in stain quality, this is considered as normal by the committee.

The arrowed cell was incorrectly identified as a "toxic neutrophil" by 1.2% of participants. Toxic granulation is the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Döhle bodies are often also present in toxic cells. Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 µm) and shape (round, elongated, or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found in the periphery of the cytoplasm, near the cell membrane. The cell of interest does not contain these well-developed features and is therefore considered as a normal segmented neutrophil by the committee.

BMD-10

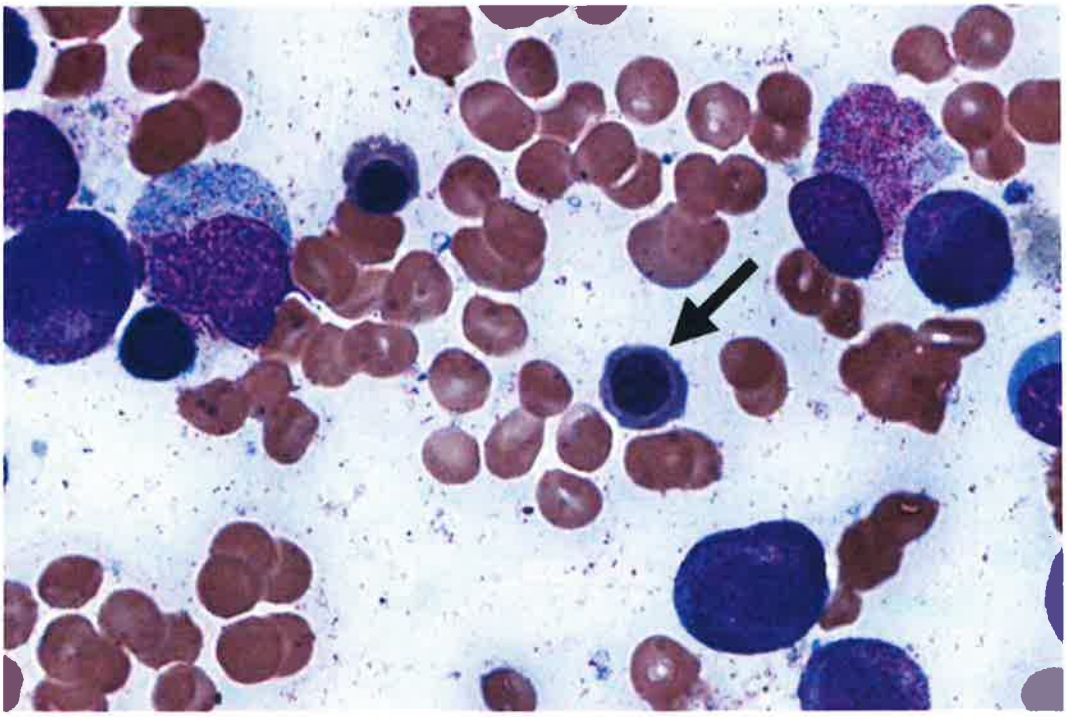


Identification	Participants No.	Participants %	Evaluation
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	315	97.2	Educational
Osteoblast	5	1.5	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic normoblast, and orthochromic normoblasts)	3	0.9	Educational
Megakaryocyte or precursor, normal	1	0.3	Educational

The arrowed cell is a mature plasma cell, as correctly identified by 97.2% of participants. Plasma cells are terminally differentiated B-cells that produce immunogloblin. They are 5% or less of cells in a normal bone marrow. Plasma cells range in size from 10 to 20 µm, and they are often oval shaped with relatively abundant cytoplasm and eccentrically located nuclei as can be seen in the arrowed cell. The N:C ratio is 1:2. Their nuclei are usually round to ovoid with prominently coarse and clumped chromatin that is often arranged in a cartwheel-like or clock-face pattern. The cytoplasm stains gray blue to deeply basophilic. A prominent hof or perinuclear zone of pale or lighter staining cytoplasm is typically seen adjacent to one side of the nucleus, corresponding to the Golgi zone.

The arrowed cell was incorrectly identified as an "osteoblast" by 1.5% of participants. Osteoblasts are bone forming cells that are located along the edge of bone trabeculae. They may resemble plasma cells in that they often have eccentric nuclei that may sometimes appear partially extruded from the cell, have abundant cytoplasm and a cytoplasmic hof. However, unlike a plasma cell the hof is separate from the nucleus as rather than closely opposed to/touching the edge of the nucleus. The chromatin of an osteoblast does not have the "clock face" appearance of the condensed chromatin seen in plasma cells, as is illustrated in BMD-10. Finally, osteoblasts are much larger (twice the size) than plasma cells.

BMD-11



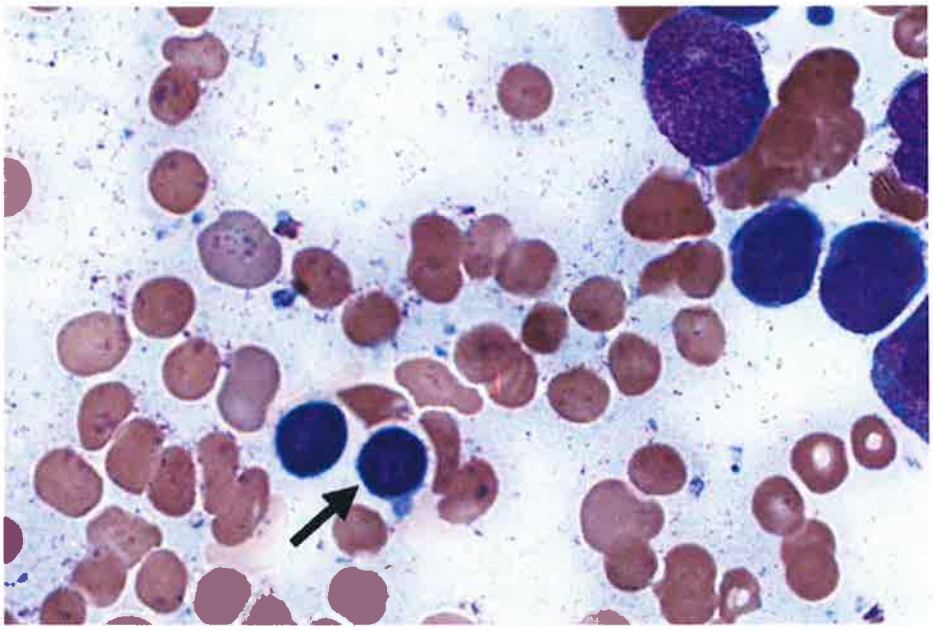
Identification	Participants No.	%	Evaluation
Erythrocyte precursor, normal (includes pronormoblast, basophilic, polychromatophilic normoblast, and orthochromic normoblasts)	310	95.7	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic normoblast, and orthochromic normoblasts)	8	2.5	Educational
Erythrocyte precursor with megaloblastic changes/maturation	4	1.2	Educational
Erythrocyte	2	0.6	Educational

The arrowed cell is an "erythrocyte precursor (includes pronormoblast, basophilic, polychromatophilic and orthochromatophilc normoblast)", as correctly identified by 95.7% of participants. Based on the size, relatively condensed chromatin and pink-gray cytoplasm reflecting a degree of hemoglobinization, the arrowed cell is best considered a polychromatophilic normoblast.

The arrowed cell was incorrectly identified as an "erythrocyte precursor, abnormal (includes pronormoblast, basophilic, polychromatophilic and orthochromatophilc normoblast)" by 2.5% of participants. Abnormal normoblasts demonstrate nuclear to cytoplasmic dysynchrony or other dysplastic features such as nuclear budding, multinuclearity, or vacuolization. The arrowed cell is a polychromatophilic normoblast and lacks these abnormal features.

The arrowed cell was incorrectly identified as an ""erythrocyte precursor with megaloblastic changes/maturation" by 1.2% of participants. Megaloblastic features such as nuclear to cytoplasmic dysynchrony are not present in the arrowed cells. The chromatin and cytoplasm are maturing well as seen by the condensed chromatin and small nucleus with hemoglobinization of the cytoplasm as evidenced by the polychromatophilic staining.

BMD-12



Identification	Participants No.	%	Evaluation
Lymphocyte	318	98.2	Educational
Blast cell (including lymphocyte)	2	0.6	Educational
Lymphocyte, large granular	2	0.6	Educational
Hematogone	1	0.3	Educational
Megakaryocyte nucleus	1	0.3	Educational

The arrowed cell is a lymphocyte, as correctly identified by 98.2% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm.

Continuing Education Information

CE (Continuing Education for non-physicians)
The CAP designates this educational activity for a maximum of 1 credit of continuing education. Participants should only claim credit commensurate with the extent of their participation in the activity.

The American Society for Clinical Pathology (ASCP) Board of Certification (BOC) Certification Maintenance Program (CMP) accepts this activity to meet the continuing education requirements.

This activity is approved for continuing education credit in the states of California and Florida.

Disclosure Statement

The following authors/planners have no financial relationships to disclose:
None

The following authors/planners have financial relationships to disclose:

Author	Commercial Interest	Your Role	What was received
Eric D. Hsi, MD, FCAP	Eli Lilly	Contract Researcher	Research Support to Institution
	Abbvie	Contract Researcher	Research Support to Institution
	Seattle Genetics	Consultant	Consultant Fee
	Miltenyi	Consultant/Advisory Board	Consultant Fee

The following In-Kind Support has been received for this activity:
None

The following Commercial Support has been received for this activity:
None

All speakers/authors of a CAP educational activity must disclose to the program audience any proprietary entity producing, marketing, reselling or distributing health care goods or services consumed by, or used on patients that may be discussed in the educational activity or with any proprietary entity producing, marketing, reselling, or distributing health care goods or services consumed by, or used on patients that is directly competitive with a product discussed in the presentation. Relevant financial relationships are considered to be any financial relationships in any amount occurring within the past 12 months that create a conflict of interest.

The College of American Pathologists does not view the existence of these interests or uses as implying bias or decreasing the value to participants. The CAP feels that this disclosure is important for the participants to form their own judgment about each presentation.

All College educational activities hold to the highest standards of educational quality and are dedicated to promoting improvement in healthcare quality and not a specific proprietary business interest of a commercial interest. All recommendations and/or planning criteria involving clinical medicine are based on evidence that is accepted within the profession of medicine as adequate justification for this indication and contraindication in the care of patients.

Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Understand the clinical characteristics of acute promyelocytic leukemia (APL).
2. Describe the morphologic, immunophenotypic, and molecular genetic characteristics of APL.
3. Understand the therapeutic implications associated with a diagnosis of APL.

Case Study

A bone marrow aspirate smear is from a 70-year-old woman presenting with fatigue, fever, and pancytopenia. Laboratory peripheral blood data include: WBC = 3.0 x 10E9/L; RBC = 3.20 x 10E12/L; HGB = 9.6 g/dL; HCT = 27.3%; MCV = 90 fL; and PLT = 14 x 10E9/L. Flow cytometry studies showed a dominant immature myeloid cell population with the following phenotype: CD117+, CD34-, CD33+, CD36-, HLA-DR-, CD15 partial +, CD13+, CD11b-, CD16-, and myeloperoxidase positive. These cells represent approximately 38% of total events. CD34+ myeloblasts represented < 1% of events.

INTRODUCTION

This is a case of acute promyelocytic leukemia (APL) with *PML-RARA*, one of the acute myeloid leukemias (AML) with recurrent genetic abnormalities defined in the updated 2017 World Health Organization (WHO) classification. These leukemias with recurrent genetic abnormalities are distinct biologic entities defined by their specific genetic alterations (in this case, fusion of the *PML* and *RARA* genes). The new fusion gene plays a central role in leukemogenesis and imparts differentiation arrest of myeloid progenitor cells at the promyelocyte stage. APL accounts for approximately 5% - 8% of AML cases in younger patients but is less common in patients over age 60. APL has an annual incidence of 0.08 cases per 100,000 people.

CLINICAL PRESENTATION

Patients may present with fatigue due to anemia associated with bone marrow replacement by leukemic cells and a bleeding diathesis/coagulopathy due to disseminated intravascular coagulation (DIC). DIC is a known complication of APL and is a cause of early death in some APL patients. Patients may have elevated white blood cell (WBC) counts, but more frequently have low WBC counts. The hypogranular variant is associated with elevated WBC counts (see Differential Diagnosis section below).

PATHOLOGIC FEATURES

Blast morphology is very characteristic in APL. In the common hypergranular variant, blasts are large with bilobed or reniform nuclei and open chromatin. The cytoplasm has dense azurophilic granulation that can obscure nuclear features and contains Auer rods. In some cells, numerous Auer rods may be present (so-called "faggot cells") and this feature is considered specific for APL. However, they may not be found in some cases even with extensive searching. The hypogranular variant has numerous small granules that are not apparent by routine light microscopy, and these cells have gray-blue cytoplasm that can mimic monocytic or myelomonocytic blasts. Cytochemical stains for myeloperoxidase are intensely positive in both variants.

By flow cytometry, APL blasts express the myeloid markers CD13 and CD33, with most cases expressing CD117. Although not specific, APL blasts often lack CD34 and HLA-DR and this phenotype can prompt one to perform specific genetic testing. CD2 expression is associated with the microgranular variant morphology and presence of *FLT3*-internal tandem duplication (ITD). Definitive diagnosis requires demonstration of a *PML-RARA* gene fusion. Multiple methods can be used to demonstrate a *PML-RARA* gene fusion including RT-PCR and fluorescence in situ hybridization. Standard karyotyping can also be performed to demonstrate

the t(15;17)(q22;q11-12) abnormality. There are uncommon variant rearrangements involving *RARA* that have similar but sometimes subtly different morphologic features that are beyond the scope of this exercise but can be explored in the accompanying reference list. However, it should be noted that two variants in particular, involving *ZBTB16-RARA* and *STAT5B-RARA*, are important in that these cases do not respond to typical APL therapy.

Differential Diagnosis

The differential diagnosis of APL, from a morphologic standpoint, includes other AMLs, most notably acute monocytic or myelomonocytic leukemia as the lobulated blasts may be mistaken for cells of monocytic lineage. This is particularly true of the hypogranular variant. According to the WHO classification, AML with recurrent cytogenetic abnormalities such as APL can be diagnosed in the presence of fewer than 20% circulating or bone marrow blasts. Thus, when the blast count is low, reactive/regenerative conditions and myelodysplastic or myeloproliferative processes might be considered in the differential diagnosis. Reliance on morphologic features and recognition of the characteristic nuclear features of APL blasts, a high index of suspicion, and appropriate molecular studies enables an accurate and timely diagnosis in the vast majority of cases.

Therapy and Prognosis

Initial induction therapy for APL consists of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) for low-risk APL, and chemotherapy with ATRA and ATO for high-risk patients. Early initiation of therapy and supportive care to maintain fibrinogen levels, the platelet count, and prothrombin time is important to minimize morbidity and mortality from bleeding complications/DIC.

When appropriately managed, the prognosis of APL is favorable relative to other types of AML, with complete remission rates and long-term survival rates in the 80% - 90% range. Adverse prognostic factors include hyperleukocytosis, advanced age, and *FLT3*-ITD.

References

1. Arber DA. Acute myeloid leukemia. In: Hsi ED, ed. *Hematoathology*. 3rd ed. Elsevier; 2018:459-466.
2. Sanz MA, Fenaux P, Tallman MS, et al. Management of acute promyelocytic leukemia: updated recommendations from an expert panel of the European LeukemiaNet. *Blood*. 2019;133(15):1630-1643.
3. Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues*. Revised 4th ed. IARC; 2017:134-136.

Author

Eric D. Hsi, MD, FCAP, is a professor of pathology at the Cleveland Clinic Lerner College of Medicine and chair of the Department of Laboratory Medicine at Cleveland Clinic. He serves as chair of the Hematology and Clinical Microscopy Committee for the CAP, chair of the Hematopathology and Laboratory Hematology Committee for the American Society of Hematology, and chair of the Pathology Committee for ALLIANCE. Dr. Hsi's research interests include diagnosis and classification of lymphoma and leukemias and predictive and prognostic biomarkers in these diseases.

COLLEGE of AMERICAN PATHOLOGISTS

Don't Miss Out On This Opportunity to Earn Continuing Education Credit

***** Enter the information below and distribute to your laboratory staff. *****

Program Mailing and Year:	BMD-B 2020
Activity Start Date:	September 23, 2020
Activity Expiration Date:	September 22, 2021

How to Access Your Online Education Activities

1. **Access Your Account**
   a. Go to cap.org.
   b. Under the MY CAP menu, click **Log In**.
      - If you are unsure whether you have an *individual* web account with the CAP, or do not remember your user ID and password, click on the **Reset password** or **Email temporary password.**
      - If you do not have an individual web account, click **Create An Account.** Complete and submit the account request form. You will be notified within one business day that your individual account has been activated.

   c. **If you are associated to one lab** that purchased kits for this activity, the system will register you into the activity. A kit will be associated to your registration.
   d. **If you are associated to more than one lab** that purchased kits for this activity, you will need to select the lab by clicking the **Select or Change Laboratory** button. The system will register you into the activity. A kit will be associated to your registration.
   e. **If you are not associated to a lab**, you need to add lab affiliation.
      - Under the MY CAP menu, click **Update My Profile.**
      - Click the **Business/Professional** tab, then (in the upper-right corner) click **+ Add Affiliation** to enter the appropriate information.

2. **Access Your Online Education Activities**
   a. Go to cap.org.
   b. Under the MY CAP menu, click **Log In**.
   c. Click on the **Learning** tab.
   d. Enter the Program code in the Search box (eg, BMD, CGL), then click the arrow icon ».
   e. In the list of results, click the **Register** button of your activity.
   f. After reviewing the Activity Details page, click the **Register** button.
   g. Click **Resume** to access the Activity.
   h. Click the confirmation checkbox at the bottom of the Activity Overview page, and then click the **Continue** button.
   i. If you choose to return to the activity later, it can be found on the In-Progress Learning tab. Click the activity title to return to the activity.

View courses with one of the following browsers: Internet Explorer 7.x or newer, Firefox, Google Chrome, or Safari. Pop-up blockers must be turned off to complete the activity.

Important: Refer to the System Requirements document located on cap.org.

For assistance, please call a Customer Contact Center representative at 800-323-4040 or 847-832-7000 option 1.