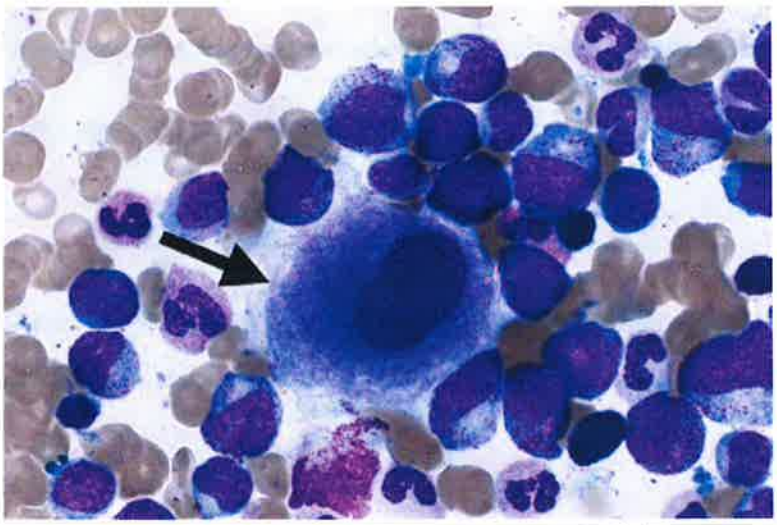
Cell Identification

BMD-02

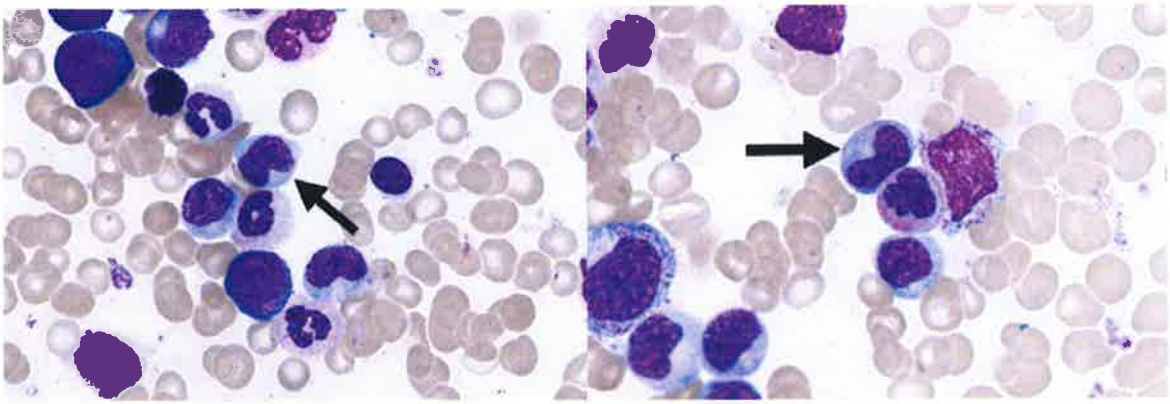


Identification	Participants No.	%	Evaluation
Megakaryocyte or precursor, abnormal	165	58.7	Educational
Megakaryocyte or precursor, normal	113	40.2	Educational
Gaucher cell, Pseudo-Gaucher cell	2	0.7	Educational
Macrophage (histiocyte)	1	0.4	Educational

The arrowed object(s) is a normal megakaryocyte, as correctly identified by 40.2% of participants. Megakaryocytes are the largest bone marrow hematopoietic cells. They are derived from bone marrow stem cells and are responsible for platelet production. During development, the cell does not divide, but instead the nucleus undergoes nuclear replication without cell division (endoreduplication) giving rise to a hyperdiploid nucleus with several lobes and each lobe roughly containing a normal complement of chromosomes. Typically, the mature megakaryocyte measures at least 25 to 50 µm in diameter. The numerous nuclear lobes are of various sizes, can be overlapping and individually indistinguishable, or connected by large bands or fine chromatin threads. The chromatin is coarse and clumped to pyknotic. The abundant cytoplasm stains pink or wine-red and contains fine azurophilic granules that may be clustered. The image clearly identifies a megakaryocyte, but with a hypolobated (single) nucleus. Thus, responses of both megakaryocyte normal and abnormal (the latter identified by 58.7% of participants) are acceptable.

© College of American Pathologists

BMD-03



Identification	Participants No.	%	Evaluation
Neutrophil, metamyelocyte	162	57.7	Educational
Monocyte	91	32.4	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	11	3.9	Educational
Neutrophil, giant band or giant metamyelocyte	6	2.1	Educational
Neutrophil, segmented/band	6	2.1	Educational
Monocyte, immature (promonocyte, monoblast)	3	1.1	Educational
Histiocyte, sea blue	1	0.4	Educational
Lymphocyte	1	0.4	Educational

The arrowed object(s) are monocytes, as correctly identified by 32.4% of participants. In a normal bone marrow, mature monocytes are relatively infrequent. Monocytes are larger than mature neutrophils, approximately 12 - 20 µm in size with convoluted or folded nucleus and abundant blue-grey cytoplasm. Cytoplasmic vacuolization is common, and coarse eosinophilic granules can occasionally be appreciated.

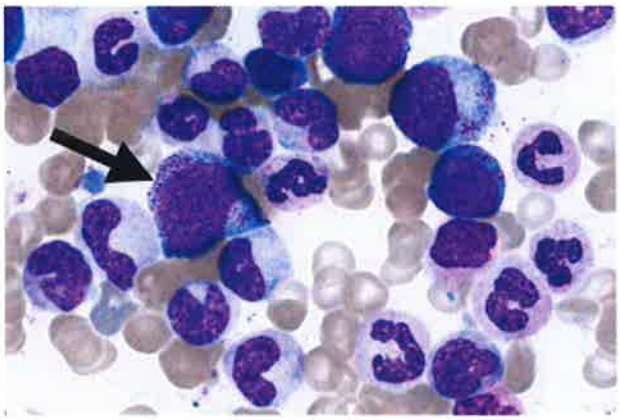
57.7% of participants incorrectly identified the arrowed objects as neutrophil, metamyelocyte. The nuclear indentation of monocytes can be similar to those of metamyelocytes, but the cytoplasm of the metamyelocytes uniquely contain rare primary granules and many fine lilac or pale orange/pink specific granules.

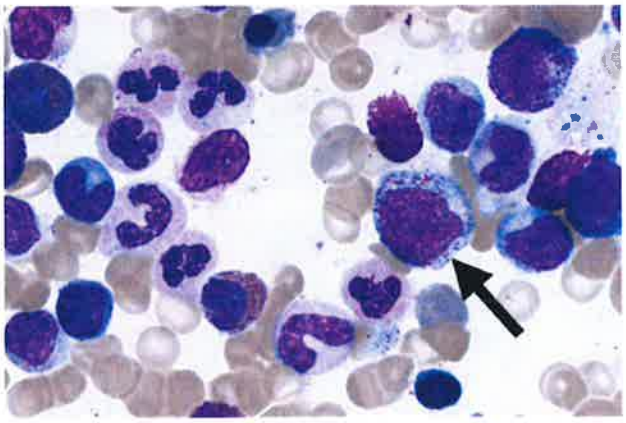
3.9% of participants incorrectly identified the arrowed objects as neutrophil with dysplastic nucleus and/or hypogranular cytoplasm. Since neutrophils in the background show normal granulation, it is unlikely that only a small subset would be hypogranular. 2.1% of participants incorrectly identified the cells as neutrophil, giant band or giant metamyelocytes. While the nuclear convolutions of monocytes can be similar to those of neutrophils with dysplastic nuclei or giant bands, cell size would argue against classification as "giant", typically 1.5 x the diameter of normal metamyelocytes and bands.

Neutrophil, segmented or band, was incorrectly assigned by 2.1% of participants. Indeed, the contours of monocytes may be difficult to distinguish from a maturing granulocyte in the bone marrow, but neutrophil band forms are typically larger and specific granules predominate in the cytoplasm of these maturing granulocytes. Neutrophils, segmented and band forms, show a predominance of specific granules that are not seen in the highlighted monocytes, but readily identified in neutrophils throughout the smear.

© College of American Pathologists

BMD-04





Identification	Participants No.	%	Evaluation
Neutrophil, promyelocyte	227	80.8	Educational
Neutrophil, myelocyte	32	11.4	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rods	17	6.0	Educational
Lymphocyte, large granular	2	0.7	Educational
Monocyte, immature (promonocyte, monoblast)	2	0.7	Educational
Blast cell (includes lymphoblast)	1	0.4	Educational

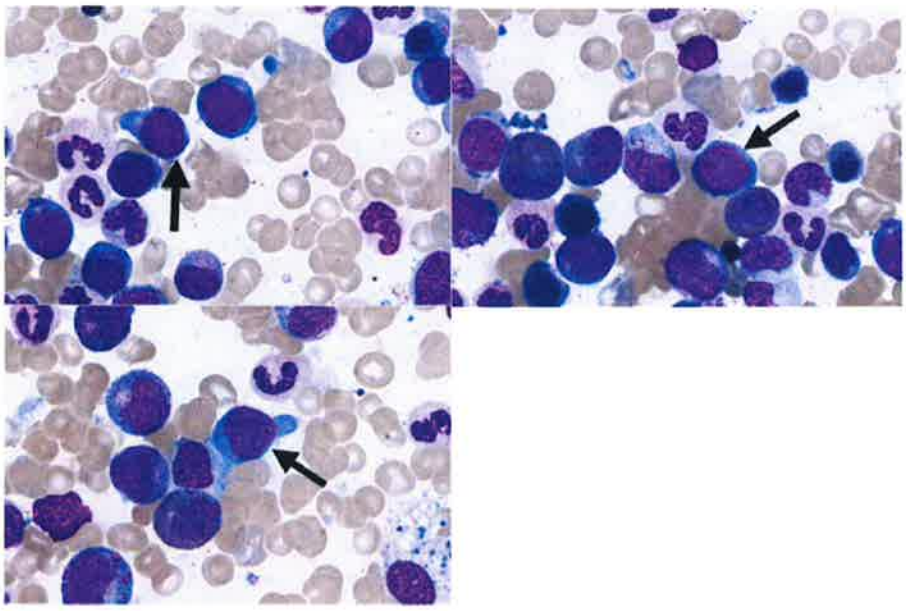
The arrowed object(s) are neutrophils, promyelocytes, as correctly identified by 80.8% of participants. Promyelocytes are round to oval cells, 12 - 24 µm in size, that are generally larger than myeloblasts. The nucleus is round or oval and contains fine, blast-like or slightly clumped chromatin with 1 - 3 visible nucleoli. The cytoplasm is basophilic and, in contrast to myeloblasts, contains distinct primary (azurophilic) granules that usually are also visible over the nucleus. Promyelocytes lack secondary (lilac) granules. A clear region or hof may be present adjacent to and slightly indenting the nucleus. Promyelocytes normally comprise 5 - 10% of nucleated cells in the bone marrow. Abundant promyelocytes are present in the provided virtual slide.

11.4% of participants incorrectly identified the arrowed objects as neutrophil, myelocyte. In comparison to promyelocytes, myelocytes are distinctly smaller, and lack nucleoli, and have an inconspicuous peri-nuclear hof.

6.0% of participants incorrectly identified the arrrowed objects as neutrophil, promyelocyte, abnormal with/without Auer rods. These typically show a folded or bilobed nucleus with an absent Golgi zone, while the promyelocytes highlighted in the virtual smear have large, round, slightly indented nuclei with a clear hof. Auer rods are rod-shaped cytoplasmic inclusions of crystalized primary granules not seen in this virtual smear.

© College of American Pathologists

BMD-05



Identification	Participants No.	Participants %	Evaluation
Blast cell (includes lymphoblast)	281	82.2	Educational
Monocyte, immature (promonocyte, monoblast)	15	5.3	Educational
Myeloblast with Auer rod	15	5.3	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polchromatophilic, and orthochromic normoblasts)	5	1.8	Educational
Erythrocyte precursor with megaloblastic changes/maturation	4	1.4	Educational
Erythrocyte precursor, normal (includes pronormoblast, basophilic, polchromatophilic, and orthochromic normoblasts)	4	1.4	Educational
Immature/abnormal cell	2	0.7	Educational
Neutrophil, promyelocyte	2	0.7	Educational
Hematogone	1	0.4	Educational
Malignant lymphoid cell (other than blast)	1	0.4	Educational
Plasma cell, mature/abnormal morphologically mature/abnormal/containing inclusion (eg. Dutcher body, Russell body)	1	0.4	Educational

The arrowed object(s) are blasts, as correctly identified by 82.2% of participants. Blasts are typically large cells (10 - 20 µm) with round or slightly irregular nucleus and characteristic dispersed, finely reticulated chromatin. One or more conspicuous nucleoli can often be seen. The cytoplasm is typically scant and basophilic but not as basophilic as that of an erythrocyte precursor (pronormoblast/proerythroblast). If present, Auer rods, rod-shaped crystalline inclusions of polymerized myeloperoxidase, are definitional of the myeloid lineage (myeloblasts). If Auer rods are absent, blast lineage may be assigned by flow cytometric immunophenotyping, often in conjunction with cytochemical staining for myeloperoxidase, sudan black, and/or nonspecific esterase. Myeloblasts normally comprise 0 - 2% of nucleated cells in the bone marrow. Increased numbers of myeloblasts can be seen in myeloid neoplasms and $\geq$ 20% myeloblasts in the blood or bone marrow is diagnostic of acute myeloid leukemia.

© College of American Pathologists

BMD-05, (cont.)

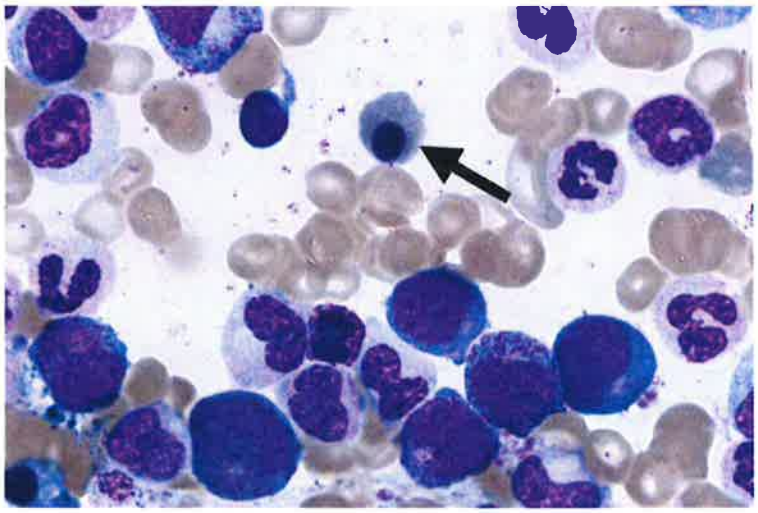
BMD-05 Discussion, Cont'd:

5.3% of participants identified the arrowed objects as monocyte, immature (promonocyte, monoblast). Monoblasts are large (15 - 25 mm in diameter) and have moderate amounts of gray-blue, sometimes sparsely granular cytoplasm. One of the arrowed blasts has a monoblastic features and this was considered an acceptable response.

5.3% of participants incorrectly identified the arrowed objects as myeloblast with Auer rod. Unlike the blasts seen in this virtual smear, blasts with Auer rods show one or multiple sharp, eosinophilic, needle-like crystalline cytoplasmic inclusion(s). Myeloblasts can retain occasional or rare granules, but rod-shaped inclusions are not present in the arrowed cells.

© College of American Pathologists

BMD-06



Identification	Participants No.	%	Evaluation
Erythrocyte precursor, normal (includes pronormoblast,basophilic, polchromatophilic, and orthochromic normoblasts)	250	89.0	Educational
Erythrocyte precursor, abnormal /dysplastic nuclear features (includes pronormoblast, basophilic, polchromatophilic, and orthochromic normoblasts)	14	5.0	Educational
Erythrocyte precursor with megaloblastic changes	10	3.6	Educational
Erythrocyte	3	1.1	Educational
Blast cell (includes lymphoblast)	1	0.4	Educational
Macrophage (histiocyte)	1	0.4	Educational
Stain precipitate	1	0.4	Educational
Stromal cell	1	0.4	Educational

The arrowed object(s) is a normal erythroid precursor (polychromatic normoblast), as correctly identified by 89.0% of participants. Polychromatophilic normoblasts are round or ovoid cells, but are slightly smaller (10 to 15 µm in diameter) than earlier erythroid precursors (pronormoblasts). The nucleus is round and may show prominent chromatin condensation and clumping, but nucleoli are not present. A perinuclear halo is visible and the N:C ratio is approximately 4:1. The cytoplasm is abundant and stains blue- to pink-gray, depending upon the relative proportions of residual RNA or degree of hemoglobinization.

5.0% of participants incorrectly identified the arrowed objects as erythroid precursor, abnormal/dysplastic nuclear features, and 3.6% incorrectly identified them as erythroid precursor with megaloblastic changes/maturation. While the morphology of abnormal erythroid precursors can be variable, the nuclear characteristics are typically striking. Compared to the round nucleus of normal erythrocyte precursors, dysplastic erythrocytes have misshapen nuclei, demonstrate multi-nucleation, nuclear budding, or abnormal nuclear bridging. Megaloblastic changes represent dyssynchronous maturation of the nucleus and cytoplasm, characterized by delayed nuclear maturation (large nuclei with immature chromatin) relative to the degree of cytoplasmic maturation. The arrowed object shows a densely clumped, round nucleus with abundant pink gray cytoplasm of polychromatophilic normoblast. 1.1% of participants incorrectly identified the arrowed object as an erythrocyte, which is a terminally differentiated and anucleate mature red cell.

© College of American Pathologists

Case Presentation:

This bone marrow aspirate smear is from a 69-year-old woman presenting with bone pain and fatigue. Laboratory peripheral blood data includes: WBC = 26.1 x 10E9/L; RBC = 3.24 x 10E12/L; HGB = 9.7 g/dL; HCT = 29.2%; MCV = 110 fL: and PLT = 422 x 10E9/L.

(BONE MARROW, WRIGHT-GIEMSA)

Case Discussion: Chronic Myelomonocytic Leukemia:

The bone marrow aspirate is abnormal, with a marked granulocytic hyperplasia. Additionally, the granulocyte lineage is left-shifted with increased numbers of promyelocytes, characterized by distinct primary granules and a perinuclear clearing adjacent to the nucleus. Erythropoiesis is generally orderly. The megakaryocytes are normal number, but some are dysplastic with a small, hypolobated nucleus (BMD-02). Blasts represent ~12% (median) of cells by manual differential count (see BMD-01 differential results). Monocytes (BMD-03) and monocytic precursors, typically infrequent in bone marrow aspirates, are conspicuously present. Flow cytometric phenotyping of the monocytic cells showed expected pattern of CD14 and CD64 expression, decreased expression of CD13 and CD14, and aberrant CD56 overexpression. The karyotype was normal and fluorescence in situ hybridization (FISH) was negative for the *BCR-ABL1* fusion. In isolation, the morphologic features of the bone marrow aspirate are not specific, but together with peripheral blood and flow cytometric findings, are consistent with a diagnosis of chronic myelomonocytic leukemia (CMML).

Chronic myeloid neoplasms can be generally classified as either myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPN). While both are typically associated with bone marrow hypercellularity, MDS is characterized by ineffective hematopoiesis and peripheral blood cytopenias, while MPN is associated with excessive proliferation and peripheral blood cytoses. Rarely, myeloid neoplasms with overlapping features of MDS and MPN can occur, with frequent transformation to acute myeloid leukemia (AML). While a number of neoplasms fall within this "overlap" category, including Juvenile myelomonocytic leukemia (JMML), and atypical chronic myeloid leukemia (aCML), CMML is the most frequent in adults, with a median patient age around 70 years.

Question 1: Common presenting symptoms of CMML include:

A. > 20% bone marrow blasts
B. Leukopenia
C. Persistent monocytosis
D. Polycythemia

The initial diagnosis of CMML can be very challenging, particularly as the bone marrow features are not specific. Moreover, other myeloid neoplasms, including chronic myeloid leukemia (CML), primary myelofibrosis, and myeloid neoplasms associated with eosinophilia and specific gene rearrangements can appear similar and require extensive ancillary testing to distinguish them from one another. Importantly, bone marrow blast count is an important prognostic indicator in CMML, as blast counts greater than 20% are diagnostic of acute myeloid leukemia (AML). Thus, to establish a diagnosis of CMML, the World Health Organization (WHO) classification requires a persistent peripheral blood monocytosis of $\geq$ 1 x 10E9/L (with monocytes typically accounting for > 10% of the white blood cells) and specifically demands exclusion of: (1) other reactive causes of monocytosis, (2) myeloid neoplasms with defining molecular characteristics, such as CML, and (3) blast counts $\geq$ 20%.

© College of American Pathologists

As in this case, flow cytometric studies can be helpful in identifying and characterizing the peripheral blood and bone marrow monocytes. In some cases, the abnormal findings can be subtle, and include decreased expression of markers typically seen on mature monocytes. Aberrant expression of CD2 or overexpression of CD56 is frequent, but expansions of monocytes with CD56 expression alone is not definitive of malignancy.

Question 2: Which of the following immunophenotypes is consistent with aberrant mature peripheral blood monocytes in CMML?

A. CD13+/CD14+/CD64+/CD33+/CD56-/CD2-/CD34-
B. CD13+/CD14-/CD64+/CD33+/CD56-/CD2-/CD34+
C. CD13+/CD14dim+/CD64+/CD33+/CD56+/CD2+
D. Myeloperoxidase+/CD13+/CD14-/CD64-/CD7+/CD34+

Cytogenetic studies remain an important component in the work-up of myeloid neoplasms. With appropriate morphology, specific cytogenetic abnormalities can be diagnostic of CML, AML, or myeloid neoplasms associated with eosinophilia. However, in most cases of CMML, as presented above, the karyotype is normal. If present, karyotypic abnormalities include trisomy 8, and deletion of chromosome 7 or 7q. Gene mutations are frequent in CMML, and the introduction of next generation sequencing assays into clinical laboratories has been particularly helpful in the evaluation of these cases. *TET2*, *ASXL1*, *SETBP1*, and *SRSF2* are the most commonly mutated genes in CMML, followed, less frequently, by mutations in *CBL*, *RUNX1*, *KRAS*, and *NRAS*.

Question 3: Which abnormality can be seen in CMML?

A. *PDGFRA* rearrangement
B. *SF3B1* mutations
C. t(9;22); *BCR-ABL1* fusion
D. *TET2* mutations

All chronic myeloid neoplasms carry a risk of AML, as defined by a blast count of ≥ 20% in the blood or bone marrow. The blast count is a significant predictor of disease progression, and the WHO further stratifies CMML based on blasts counts:

WHO category:	CMML-0	CMML-1	CMML-2	AML
Peripheral blood blast count	< 2%	2 - 4%	5 - 19%	≥ 20%
Bone marrow blast count	< 5%	5 - 9%	10 - 19%	≥ 20%

However, irrespective of category, prognosis of CMML is generally considered poor amongst the chronic myeloid neoplasms. White cell count at presentation, rather than blast count, has also shown to be a predictor of survival in CMML, with higher leukocyte counts being associated with a worse overall survival.

As mentioned above, accurate diagnosis of CMML is based on a multiparametric approach which includes among other the assessment of morphologic and genetic findings. Other specific MDS/MPN overlap neoplasms include JMML and aCML. As the name implies, JMML occurs in children, with most patients presenting before age 3. Children with neurofibromatosis type 1 are at a significantly increased risk for developing JMML. The peripheral blood in JMML shows proliferative and dysplastic features, namely an expansion of granulocytes and monocytes with an associated anemia and thrombocytopenia in most cases, similar to CMML. Bone marrow findings can also resemble CMML, with significant granulocytic hyperplasia. The prognosis is variable, depending on other genetic associations, but JMML

© College of American Pathologists

is an aggressive and fatal neoplasm and typically treated with allogeneic hematopoietic stem cell transplant.

Question 4: Features that favor CMML over JMML include:

A. Age > 50 years
B. Bone marrow granulocytic hyperplasia
C. Family history of neurofibromatosis, type 1
D. Peripheral blood monocytosis

Yuri Fedoriw, MD
Hematology and Clinical Microscopy Committee

References:

1. Swerdlow SH, Campo E., Harris N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., Arber, D. A.,Hasserjian, R. P., Le Beau, M. M., Orazi, A.,Siebert, R. ed *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (ed Revised 4th). IARC; 2017.

2. Elena C, Gallì A, Such E, et al. Integrating clinical features and genetic lesions in the risk assessment of patients with chronic myelomonocytic leukemia. *Blood.* 2016;128(10):1408-1417.

3. Loghavi S, Sui D, Wei P, et al. Validation of the 2017 revision of the WHO chronic myelomonocytic leukemia categories. *Blood Adv.* 2018 Aug 14;2(15):1807-1816.

Answers to questions:

Question 1: C. Persistent monocytosis
Monocytosis is a hallmark feature of CMML, necessary to establish the diagnosis. Blast counts > 20% would qualify as AML rather than a chronic myeloid neoplasm. CMML typically presents with an associated anemia, and not polycythemia, and leukocytosis, rather than leukopenia, is common.

Question 2: C. CD13+/CD14dim+/CD64+/CD33+/CD56+/CD2+
Flow cytometry is a critical component of bone marrow and peripheral blood evaluations for myeloid neoplasms. In some instances, the peripheral blood monocytes of CMML are immunophenotypically normal (choice A), but in most cases show some variation from the expected pattern. This includes decreased CD14 expression, aberrant expression of CD2 or aberrant overexpression of CD56 (choice C). The other immunophenotypes listed show expression of CD34, a blast marker not seen in mature monocytes.

Question 3: D. *TET2* mutations
Gene mutations are common in CMML and mutations in a handful of genes can be identified in most cases by DNA sequencing. *TET2* mutations are identified in nearly 60% of CMML. *PDGFRA* rearrangements are identified in cases of myeloid neoplasms with associated eosinophilia, and *SF3B1* mutations in MDS associated with ring sideroblasts. The t(9;22); *BCR-ABL1* fusion is characteristic of CML and would exclude the diagnosis of CMML.

Question 4: A. Age > 50 years
The diagnosis of CMML can be very challenging but almost uniformly occurs in older adults. JMML is a disease of children that shares many clinical and laboratory features with CMML. Both demonstrate peripheral blood monocytosis and bone marrow granulocytic hyperplasia. Children with neurofibromatosis, type 1 are at a significantly increased risk for developing JMML.

© College of American Pathologists

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants below have completed the review of the CAP BMD-A 2020 (Product Mailing, Year) Participant Summary/Final Critique report and can self-report the recommended 0.5 (Education Hours) hours towards fulfilling education and certification of maintenance requirements.

Participant	Date	Participant	Date

Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity. Date

Retain this page for record-keeping and auditing purposes.

1. Go to www.cap.org
2. Click **LOG IN / LOG IN** and enter your User ID and Password.
   - 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 **PASSWORD HINT**.
   - 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.
3. Click **Learning** from the top menu bar
4. Click **Learning Transcript** from the menu bar
5. Click **Add My Own Activity**
6. Follow prompts to enter 'Self-Reported Training Activities'.

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

**CAP Self-Reported Training activities do not offer CE credit, but can be used towards fulfilling requirements for certification of maintenance by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*

© College of American Pathologists