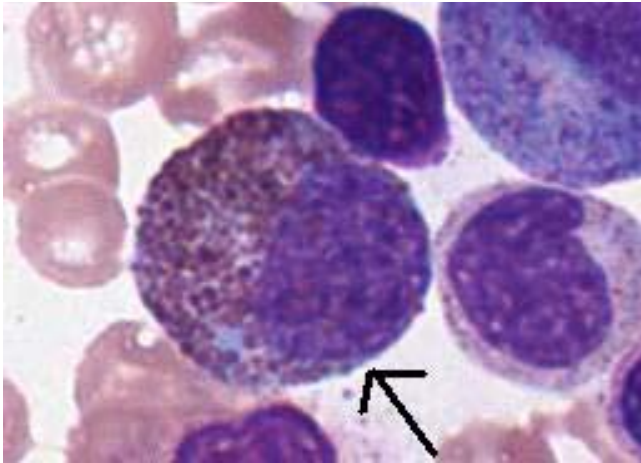


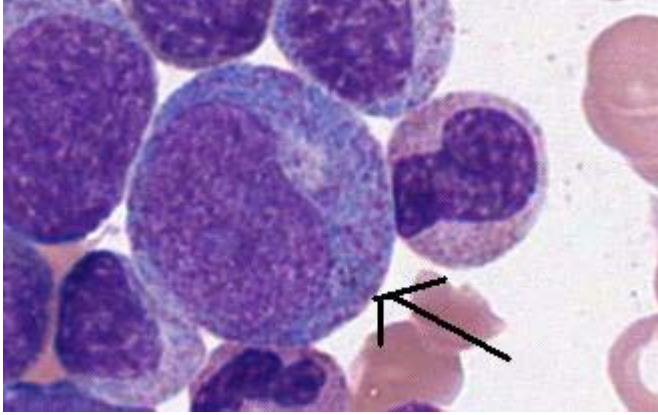
Cell Identification



EHE1-02

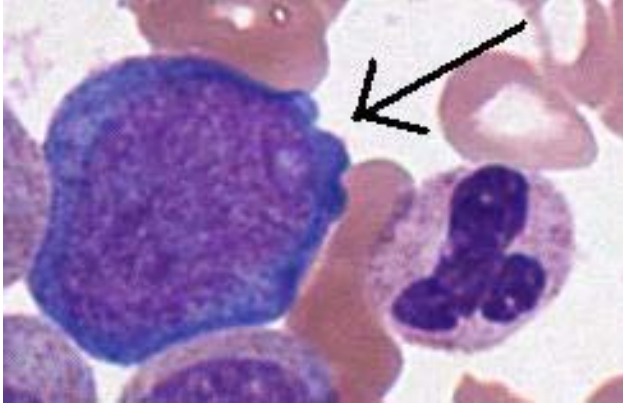
Identification	Participants		Evaluation
	No.	%	
Eosinophil, any stage	148	81.8	Educational
Neutrophil, myelocyte	14	7.7	Educational
Neutrophil, promyelocyte	12	6.6	Educational
Neutrophil, metamyelocyte	2	1.1	Educational
Basophil, any stage	1	0.6	Educational
Blast cell	1	0.6	Educational
Immature or abnormal cell	3	1.7	Educational

The arrowed cell is an eosinophil as correctly identified by 81.8% of participants. Although this cell is at the myelocyte stage of maturation, all eosinophils, including precursors and mature forms, should be classified together as eosinophils within the differential. Eosinophils usually contain an abundant amount of granules that are notably coarse and bright orange-red in colour. This granulation is easily distinguished from the finer lilac granulation of neutrophils or neutrophilic myelocytes. Eosinophils follow the same nuclear maturation sequence as neutrophils. Thus, other than the prominent coarse red-orange granulation, eosinophilic precursors appear otherwise identical to neutrophilic precursors.



Identification	Participants		Evaluation
	No.	%	
Neutrophil, promyelocyte	146	80.7	Educational
Neutrophil, myelocyte	10	5.5	Educational
Neutrophil, promyelocyte, abnormal with or w/o Auer rod(s)	8	4.4	Educational
Blast cell	5	2.8	Educational
Neutrophil, metamyelocyte	3	1.7	Educational
Myeloblast with Auer rod	2	1.1	Educational
Eosinophil, any stage	1	0.6	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.6	Educational
Immature or abnormal cell	5	2.8	Educational

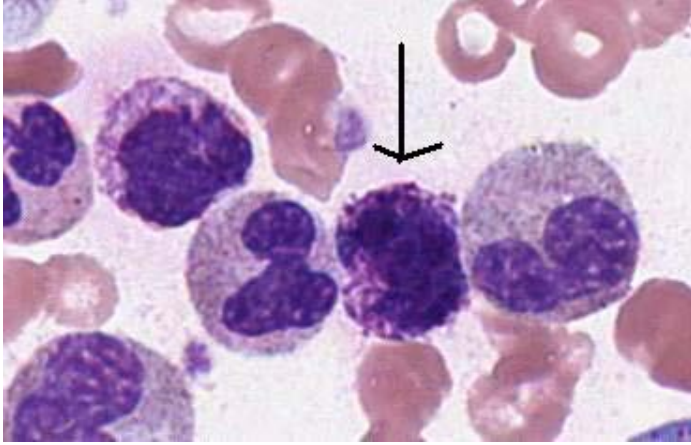
The arrowed cell is a promyelocyte as correctly identified by 80.7% of participants. These cells are the next morphologically identifiable stage in myeloid maturation following the myeloblast. Promyelocytes are slightly larger than myeloblasts and have a high nuclear to cytoplasmic ratio, open reticular chromatin, and a distinct nucleolus, which are all features that are quite similar to myeloblasts. Promyelocytes are distinguished from blast cells by identification of a clear perinuclear hof and a light to moderate amount of primary granules, which are coarse and azurophilic and often overlie the nucleus. Compare this cell with the blast cell (EHE1-04) and the myelocyte (EHE1-06).



Identification	Participants		Evaluation
	No.	%	
Blast cell	169	93.4	Educational
Neutrophil, promyelocyte	2	1.1	Educational
Basophil, any stage	1	0.6	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.6	Educational
Myeloblast with Auer rod	1	0.6	Educational
Nucleated red cell, normal or abnormal morphology	1	0.6	Educational
Immature or abnormal cell	6	3.3	Educational

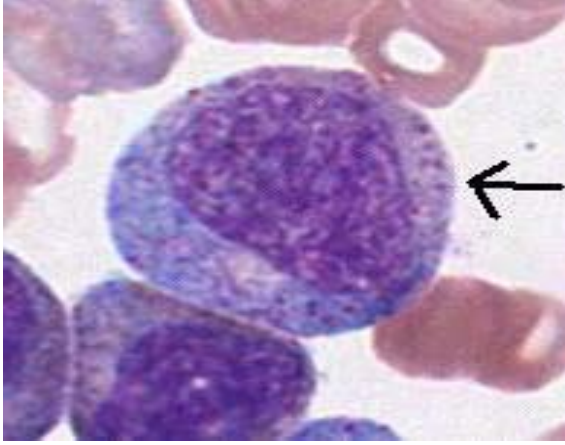
The arrowed cell is a blast cell as correctly identified by 93.4% of participants. Blasts are the most immature progenitor cells that can be recognized morphologically and are normally found only in the bone marrow, where they typically comprise less than 3% of nucleated cells. Circulating blasts are only very rarely seen in reactive conditions, such that their presence should raise concern for the possibility of marrow pathology. The presence of a small percentage of blasts, as seen in this case, is typical for CML in chronic phase.

The blasts identified here have morphologic features typical for myeloblasts, although it should be noted that confirmation of lineage requires additional studies such as flow cytometry. These blasts are intermediate to large in size, 15 to 20 micrometers in diameter. Their nuclei are usually round, oval or rhomboid, but may sometimes have mild nuclear irregularities. Blasts have a high nuclear to cytoplasmic ratio with a small amount of basophilic cytoplasm that is usually agranular. The nuclear chromatin is open and reticular, and there is usually a distinct nucleolus. These features make blast cells recognizably immature in most cases. In dysplastic or leukemic states, blast cells may have some variation in their morphology, such as unusually small size, prominent nuclear irregularities, prominent or unusual granulation, or cytoplasmic Auer rods. Compare this blast cell with the promyelocyte identified in EHE1-03.



Identification	Participants		Evaluation
	No.	%	
Basophil, any stage	155	85.6	Educational
Neutrophil, necrobiosis (degenerated neutrophil)	11	6.1	Educational
Neutrophil, segmented or band	5	2.8	Educational
Neutrophil, toxic	3	1.7	Educational
Eosinophil, any stage	1	0.6	Educational
Mast cell	1	0.6	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.6	Educational
Neutrophil, metamyelocyte	1	0.6	Educational
Neutrophil, promyelocyte, abnormal with or w/o Auer rod(s)	1	0.6	Educational
Immature or abnormal cell	2	1.1	Educational

The arrowed cell is a basophil as correctly identified by 85.6% of participants. Basophils are mature granulocytes similar in size to neutrophils, and are distinguished by the presence of coarse and densely staining dark purple or blue-black granules, which can be small to moderate in amount. Granule distribution can be uneven, and granules often overlie and obscure the nucleus. Compare the basophil granulation with that of the mature neutrophils nearby.



Identification	Participants		Evaluation
	No.	%	
Neutrophil, myelocyte	138	76.2	Educational
Neutrophil, promyelocyte	37	20.4	Educational
Neutrophil, metamyelocyte	2	1.1	Educational
Immature or abnormal cell	4	2.2	Educational

The arrowed cell is a myelocyte as correctly identified by 76.2% of participants. Myelocytes are the next stage of granulocytic maturation following promyelocytes. While these cells are still recognizably immature, there are features that indicate a degree of maturation when compared to blasts and promyelocytes. Nuclear chromatin of myelocytes is still open but shows some clumping. Nucleoli are absent. The cytoplasm is slightly more abundant than in promyelocytes and will contain both coarse azurophilic primary granules as well as finer lilac secondary granules. A perinuclear hof may or may not be present. Compare this cell with the promyelocyte in EHE1-03.

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Interpretive Questions - EHE1-07

1. Which of the following is NOT a common peripheral blood finding of chronic myelogenous leukemia (CML) in chronic phase?

Response	No.	%
Eosinophilia	9	5.1
Prominent left shift	5	2.8
Mild monocytosis	42	23.7
Neutrophilic dysplasia	119	67.2
Basophilia	2	1.1

Intended Response: Neutrophilic dysplasia

The characteristic peripheral blood film in chronic phase CML shows neutrophilia with a very prominent left shift including a disproportionate increase in myelocytes compared to metamyelocytes or bands. Basophilia is virtually always present, and most cases also have eosinophilia. There is also usually an absolute monocytosis as well, but monocytosis in CML is quite mild, with monocytes usually comprising less than 3% of the WBC differential. Neutrophil dysplasia, however, is not a characteristic finding in chronic phase CML, although some dysplasia may arise when CML progresses into accelerated or blast phase. If significant dysplasia is present upon a new presentation of a patient with myeloproliferative features, this should lead to consideration of an alternative diagnosis. The combination of neutrophilia with dysplasia as well as a more significant monocytosis is suggestive of chronic myelomonocytic leukemia, one of the myelodysplastic/myeloproliferative neoplasms.

2. The platelet count in CML is often decreased:

Response	No.	%
True	43	24.2
False	135	75.8

Intended Response: False

Chronic phase CML is often associated with thrombocytosis, but a normal platelet count is also common. Note that even when the platelet count is normal in CML, platelets tend to be unusually large in size. Thrombocytopenia, however, can occur but is an unusual finding.

3. Which of the following laboratory studies is LEAST contributory in the evaluation of a patient with suspected CML?

Response	No.	%
Cytogenetic analysis (karyotyping)	1	0.6
D-dimer	174	98.3
Bone marrow examination	-	-
Leukocyte alkaline phosphatase (LAP) score	2	1.1
Reverse transcriptase polymerase chain reaction (RT-PCR) or fluorescence in-situ hybridization (FISH) for <i>BCR-ABL1</i>	-	-

Intended Response: D-dimer

The D-dimer is a protein fragment that is released into the blood whenever fibrin (a protein involved in blood clotting) is degraded. Thus, the D-dimer assay is very non-specific, and levels may be increased in any condition associated with inflammation, injury, or malignancy. Its clinical utility is limited to a few very specific circumstances, such as to exclude the possibility of a venous thromboembolism in patients with a moderate or low pretest probability, in which case a *negative* D-dimer essentially excludes the diagnosis of thromboembolism.

Although the initial workup for patients with suspected CML is not well standardized, there is relevant information to be gained from all of the remaining answer choices. Detection of the *BCR-ABL1* fusion gene is required to establish the diagnosis of CML. Currently, this can be quickly and most efficiently done with either RT-PCR or FISH. Because there are three different possible breakpoints that need to be detected, PCR usually requires more than one assay. FISH uses probes specific for the *BCR* and *ABL1* genes and can therefore identify the *BCR-ABL1* fusion gene regardless of the breakpoint and also in the setting of complex, variant, or cryptic translocations. Bone marrow examination is commonly performed for a new diagnosis of CML. Bone marrow morphologic examination provides additional information to confirm whether or not the patient is in chronic or more advanced phase, and is the preferred specimen for cytogenetic analysis in many labs. Identification of the Philadelphia chromosome (i.e., the abnormal chromosome 22 containing the *BCR-ABL1* fusion gene due to a t(9;22) translocation) by karyotyping is straightforward and also fairly specific. However, 5-10% of CML patients lack the Philadelphia chromosome but instead have the *BCR-ABL1* fusion gene via a complex or variant translocation, or a cryptic translocation that can only be identified via molecular studies (RT-PCR or FISH). Karyotyping is also useful for the detection of additional genetic abnormalities, which can be indicators of disease progression. The leukocyte alkaline phosphatase (LAP) score is a cytochemical stain that historically was used to screen cases of suspected CML. In most cases, the LAP score is low in CML and high in reactive neutrophilia, but results are neither sufficiently sensitive nor specific to be definitive for the diagnosis or exclusion of CML. In many centers, the LAP score has been eliminated due to ready accessibility to molecular testing. The latter, however, is significantly more costly. There may still be a role for utilizing a LAP score as an initial screening test, when performed by an experienced lab and results are interpreted appropriately.

4. The *BCR-ABL1* fusion gene is present in what percentage of CML cases?

Response	No.	%
20%	2	1.1
40%	-	-
60%	2	1.1
80%	46	26.3
100%	125	71.4

Intended Response: 100%

As discussed in question 3, identification of the *BCR-ABL1* fusion gene is required for the diagnosis of CML and thus is present in all cases. However, only 90-95% of patients will have the classic Philadelphia chromosome (i.e., typical t(9;22) translocation). The remaining patients have the abnormal fusion gene arise from a variant or complex translocation or through a cryptic rearrangement that cannot be identified by karyotyping. The latter patients require molecular methods, either RT-PCR or FISH analysis, to detect the *BCR-ABL1* gene.

5. Which of the following mechanisms is part of the leukemogenesis of CML?

Response	No.	%
Increased apoptosis	3	1.7
Myeloid maturation arrest	5	2.9
Unregulated myeloid proliferation	157	90.8
Decreased tyrosine kinase activity	8	4.6

Intended Response: Unregulated myeloid proliferation

The pathophysiology of CML is initiated by the abnormal tyrosine kinase enzyme that is the protein product of the *BCR-ABL1* fusion gene. As a result of the mutation, the tyrosine kinase is always active and does not respond to signals that would normally regulate its activity. This leads to the unregulated proliferation of myeloid lineages in CML. Myeloid maturation in CML is somewhat impaired but does progress, leading to proliferation and abundance of progenitors into mature forms. Apoptosis is not increased in CML. Although the mature myeloid cells in CML do undergo apoptosis, there is evidence that this process is also to some degree impaired or decreased. These features are in contrast to acute leukemias in which unregulated proliferation also occurs, but both maturation and apoptosis are also blocked.

6. Currently, what is the standard first line therapy for chronic phase CML?

Response	No.	%
Alemtuzumab	-	-
Hydroxyurea	4	2.3
Imatinib	167	95.4
Interferon	4	2.3

Intended Response: Imatinib

Prior to the development of tyrosine kinase inhibitors (TKIs), standard CML therapy included hydroxyurea and interferon, and consideration for allogeneic stem cell transplantation. The remarkable success of TKIs has led these agents to be the preferred initial therapy. The first TKI developed was imatinib, but there are two additional second-generation agents now available, dasatinib and nilotinib. Currently, recommended first line therapy is imatinib, with the second-generation agents reserved for patients who fail to respond, cannot tolerate imatinib, or develop drug resistance. Alemtuzumab is an anti-CD52 monoclonal antibody that is used for the treatment of lymphoproliferative disorders, and for immunosuppression in patients who have autoimmune disease or who have undergone organ transplantation. There is no role for alemtuzumab in the treatment of CML.

Discussion on Chronic Myelogenous Leukemia (chronic phase)

Introduction

Chronic myelogenous (or myeloid) leukemia (CML) is the most common of the myeloproliferative neoplasms and accounts for about 20-35% of all leukemias in adults. CML results from the malignant expansion of myeloid lineage hematopoietic cells within the bone marrow. The key initiating event in CML is creation of the *BCR-ABL1* fusion gene, which is present in all cases. This abnormal gene usually arises due to a chromosomal translocation, t(9;22)(q34;q11), and the abnormal chromosome 22 that results is referred to as the Philadelphia chromosome. If untreated, the disease typically has a triphasic natural history, beginning with a chronic phase, and then progressing through an accelerated phase into blast phase, which is transformation into acute leukemia.

History

CML is notable for many firsts in the history of hematology. CML is the disease for which the term *leukemia* was first coined. It is the first malignancy in which a specific genetic abnormality, the Philadelphia chromosome (named after the city where it was discovered in 1960), was identified and later found to contain the *BCR-ABL1* fusion gene. More recently, it is the very first disease for which a targeted molecular therapy was designed, the tyrosine kinase inhibitor *imatinib*. This drug has proven to be so remarkably effective in the treatment of CML that it is now the first line standard of care.

Epidemiology

The incidence of CML is 1 to 2 new cases per 100,000 people per year. Although CML can occur at any age, it most commonly presents in adults in the age range of 20 to 60 years, with a median age at diagnosis of 50 to 60. CML is uncommon in childhood, and new presentations of CML in the elderly are quite rare.

Etiology and Pathogenesis

There are no well-established exposures linked to the development of CML. The pathogenesis involves an acquired genetic error within a hematopoietic marrow stem cell, resulting in the *BCR-ABL1* fusion gene. This abnormal gene is invariably present in CML such that its detection is required for diagnosis (see *Cytogenetics and molecular testing* below).

In all cases, the *BCR-ABL1* fusion gene results in production of an abnormal BCR-ABL1 protein (enzyme) with abnormal tyrosine kinase activity. In contrast to the normal enzyme, the mutant enzyme is always active and does not respond to the cellular signals that would normally regulate its activity. As a result, myeloid lineages within the marrow (including granulocytes, monocytes, and megakaryocytes) develop unregulated proliferation and, to some degree, reduced apoptosis (cell death). Cellular differentiation/maturation is relatively normal. In contrast, acute leukemias are characterized by unregulated proliferation together with a block in maturation.

Clinical presentation

The majority of patients with CML are diagnosed while in chronic phase. Common clinical manifestations include constitutional symptoms such as fatigue, malaise, weight loss, and excessive sweating. Patients may experience abdominal fullness or discomfort due to splenomegaly. Bleeding episodes are also described, despite normal or high platelet counts, due to platelet dysfunction. 20% of patients are entirely asymptomatic and are diagnosed when blood work is done for another reason.

Peripheral blood morphology

The hallmark of chronic phase CML is marked neutrophilia with a prominent left shift, absolute basophilia, and often eosinophilia (see Figure 1). The WBC count typically exceeds $25 \times 10^9/L$ with a median WBC at presentation of $170 \times 10^9/L$. There may be a mild monocytosis, but monocytes should comprise only a small fraction of the differential (usually 3% or less). Neutrophils do not appear dysplastic. The left shift includes a much larger proportion of earlier precursors than is normally seen in a reactive left shift, including frequent myelocytes, promyelocytes, and a small population of blasts (usually less than 5%). The presence of basophilia, and sometimes eosinophilia, in CML is also helpful in the distinction from a reactive neutrophilia. Additional findings in CML include occasional nucleated red cells, rare megakaryocyte nuclear fragments, and frequent thrombocytosis, which may be marked ($>1,000 \times 10^9/L$). Thrombocytopenia can occur, but is uncommon. While granulocytes in the peripheral blood are morphologically normal, platelets can be unusually large, and sometimes not well granulated.

Due to the heightened awareness of CML and the availability of molecular testing, many patients are now diagnosed much earlier in the disease process when the full characteristic features of CML are not yet present. Such patients may present with chronic but very mild neutrophilia or isolated thrombocytosis.

Bone marrow morphology

The bone marrow is markedly hypercellular, even in early chronic phase CML (see Figure 2). The most striking feature is marked granulocytic hyperplasia most notably involving neutrophils and their precursors, but basophils and eosinophils and their precursors are also increased. These lineages are usually left shifted but show full maturation. Erythroid precursors are often decreased. There is no significant granulocytic or erythroid dysplasia. Megakaryocytes are also increased, but are not morphologically normal. Megakaryocytes in CML are characteristically small in size and hypolobated (often monolobated). However, they usually lack other abnormal features that may be seen in myelodysplasia such as widely spaced nuclei and very small micromegakaryocytes (as small as promyelocytes).

As a result of the chronic increase in cell turnover, marrow macrophages are constantly ingesting cellular and metabolic debris, which can give them the appearance of pseudo-Gaucher cells with prominent blue “tissue paper” cytoplasm, also referred to as sea-blue histiocytes.

Cytogenetics and molecular testing

The *BCR-ABL1* fusion gene is the defining genetic mutation of CML and is present in 100% of cases. In 90-95% of cases this abnormal gene arises due to a translocation involving chromosomes 9 and 22, creating the t(9;22) translocation: the *ABL1* gene on chromosome 9 is translocated to part of the *BCR* gene on chromosome 22. The resultant abnormal chromosome 22 is referred to as the Philadelphia (Ph) chromosome. Most of the remaining cases of CML have variant genetic abnormalities that result in the *BCR-ABL1* fusion gene, but involve other chromosomes in addition to 9 and 22. These structural translocations will also be identified by conventional karyotyping. A small number of cases have cryptic *BCR-ABL1* translocations that cannot be identified by routine cytogenetic analysis. When this is suspected, molecular genetic studies, such as reverse transcriptase polymerase chain reaction (RT-PCR) analysis or FISH analysis, are indicated. The sensitivity of RT-PCR for detecting the *BCR-ABL1* fusion gene is approximately 99% and for FISH analysis it is greater than 99%.

Natural history

Without therapy, CML has a triphasic or biphasic clinical course. These phases are *chronic phase*, *accelerated phase*, and *blast phase*. Most patients are diagnosed during the indolent *chronic phase*, with splenomegaly, leukocytosis, and a hypercellular marrow. Without treatment, the disease invariably progresses into *blast phase*, which is transformation to acute leukemia. In some patients, the progression of disease from chronic phase towards blast transformation can be identified as a discrete *accelerated phase*.

Markers for disease progression in the peripheral blood include a rising white count despite treatment, increasing basophilia, new or an increase in circulating blasts, dysplastic features within neutrophils, or progressive anemia. Within the bone marrow, one can see increasing blasts, the development of dysplastic features, foci of blasts within the trephine biopsy, or progressive marrow fibrosis. Cytogenetic studies may also reveal evidence of clonal evolution, which can manifest as additional copies of the Ph chromosome, additional cytogenetic abnormalities, or further mutations of the *BCR-ABL1* fusion gene that render it resistant to imatinib treatment.

The blasts in blast phase CML are usually of myeloid lineage (acute myeloid leukemia), but in about 20-30% of cases are of lymphoid lineage, usually precursor B lymphoblasts. Rarely, the blasts are of mixed lineage. The ability of CML to transform to either a myeloid or lymphoid leukemia confirms that this disorder arises from a pluripotent bone marrow stem cell that is capable of multilineage differentiation.

Treatment

Historically, CML was treated with low dose chemotherapy such as hydroxyurea or low dose cytarabine (to control cell counts) and interferon, which could induce remission in some patients, but at the cost of poorly tolerated medication side effects (flu-like symptoms). The only proven long term cure is allogeneic stem cell transplantation, a therapy with significant associated risks.

The development of targeted tyrosine kinase inhibitors (TKIs), such as imatinib, has revolutionized the treatment of CML (see Figure 3). These agents specifically target the enzymatic pocket of the mutant *BCR-ABL1* enzyme, effectively blocking its activity and disrupting the pathway that leads to unregulated myeloid proliferation. The first of this new class of agents was imatinib, which proved to be able to induce hematologic, cytogenetic, and molecular remission in the vast majority of patients with chronic phase CML, and in most is well tolerated without significant side effects. Since the introduction of imatinib, two additional “next generation” TKIs have been developed, dasatinib and nilotinib, which increase the treatment options for the few patients who do not respond well or do not tolerate imatinib.

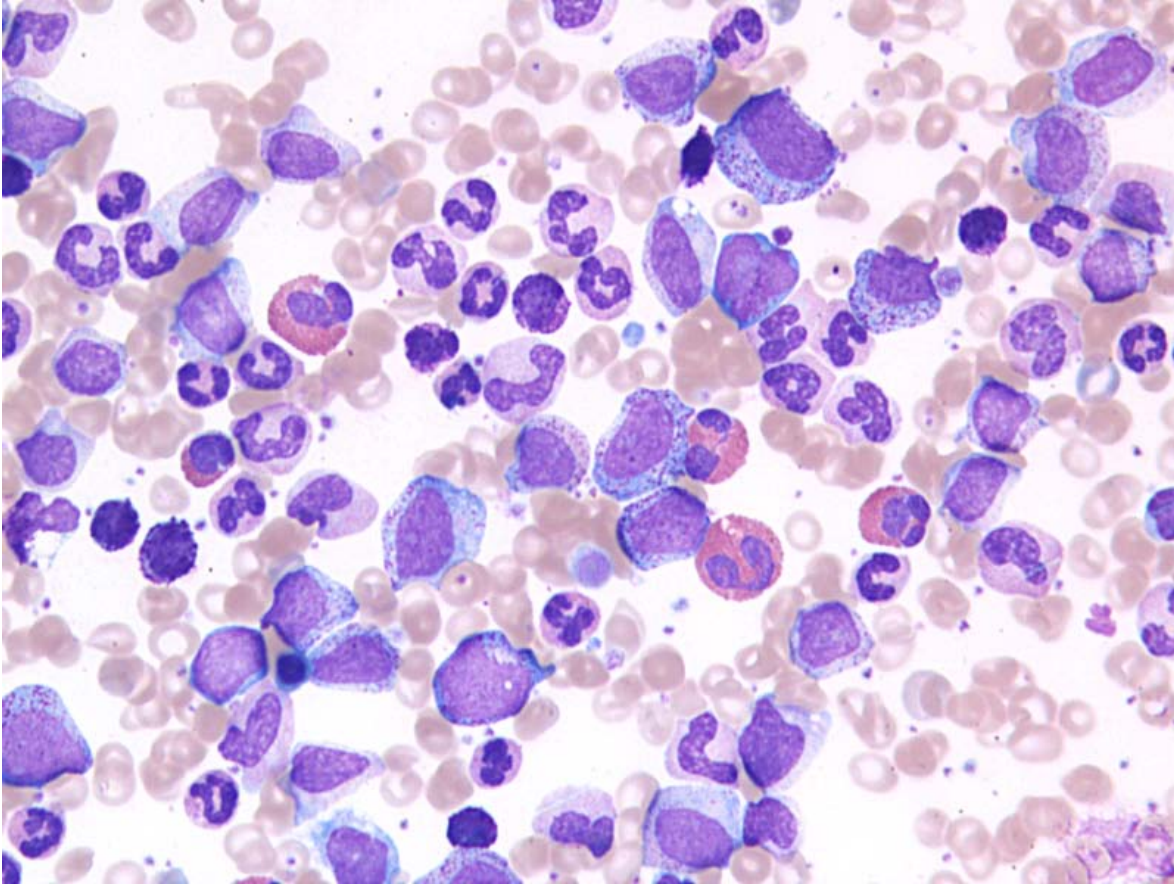
At present, TKI therapy provides very effective control for most patients with CML, but this treatment is not curative. Furthermore, there are small numbers of patients who either do not respond to TKI therapy or develop drug resistance and progress to *accelerated* or *blast phase*. For these patients, conventional chemotherapy and allogeneic stem cell transplantation provide a chance for disease control or cure.

Summary

Chronic myelogenous leukemia is a neoplasm involving unregulated proliferation of myeloid lineages that arises due to a *BCR-ABL1* fusion gene mutation in a hematopoietic progenitor cell. Peripheral blood features of chronic phase CML are neutrophilia with a prominent left shift out of keeping with most reactive processes, along with basophilia, eosinophilia, and often thrombocytosis.

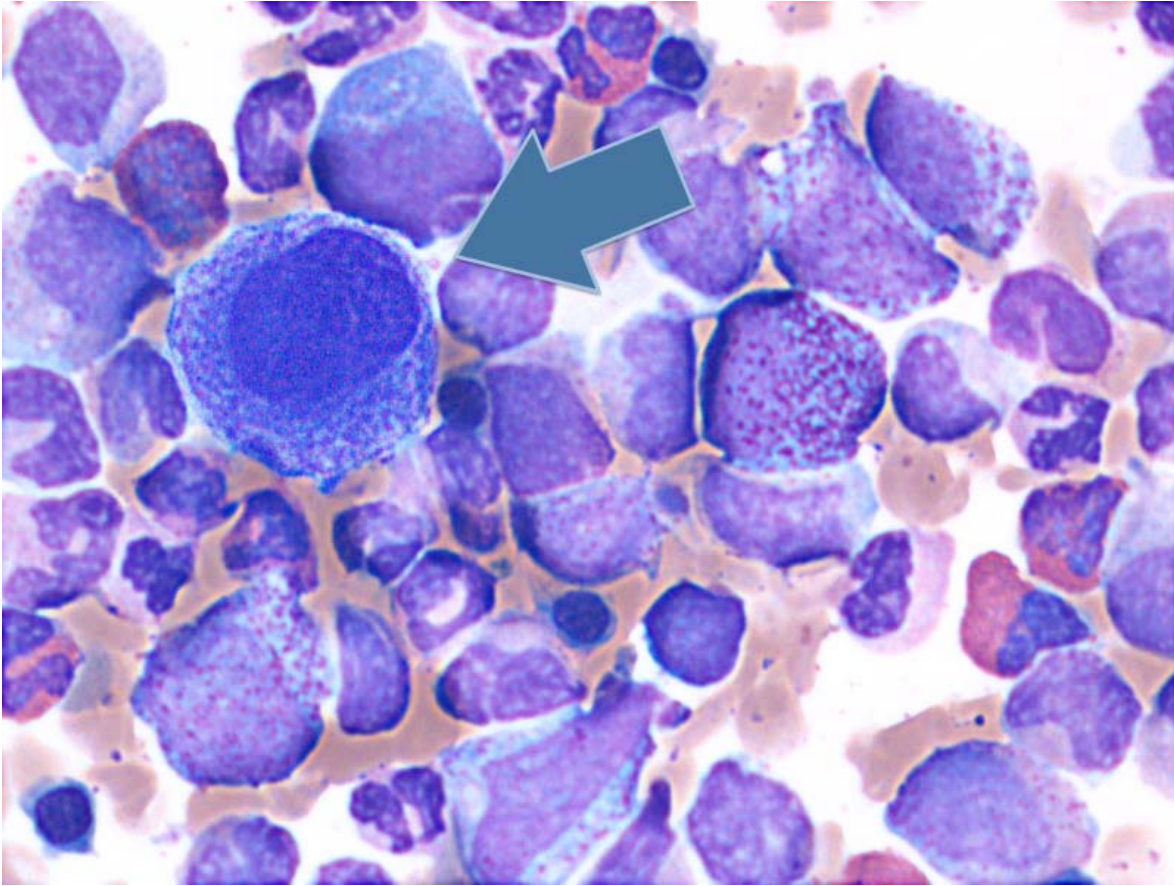
The natural history is progression from an indolent chronic phase to accelerated and blast phase transformation. Current therapy for CML is a class of targeted molecular drugs known as tyrosine kinase inhibitors, which have remarkably changed the treatment of CML and have replaced other treatments, including stem cell transplantation, as the first line treatment of choice. These agents induce remission in most patients with durable responses. For the small number of patients who do not respond, chemotherapy and stem cell transplantation remain effective treatment options.

Figure 1: Peripheral blood in chronic phase CML



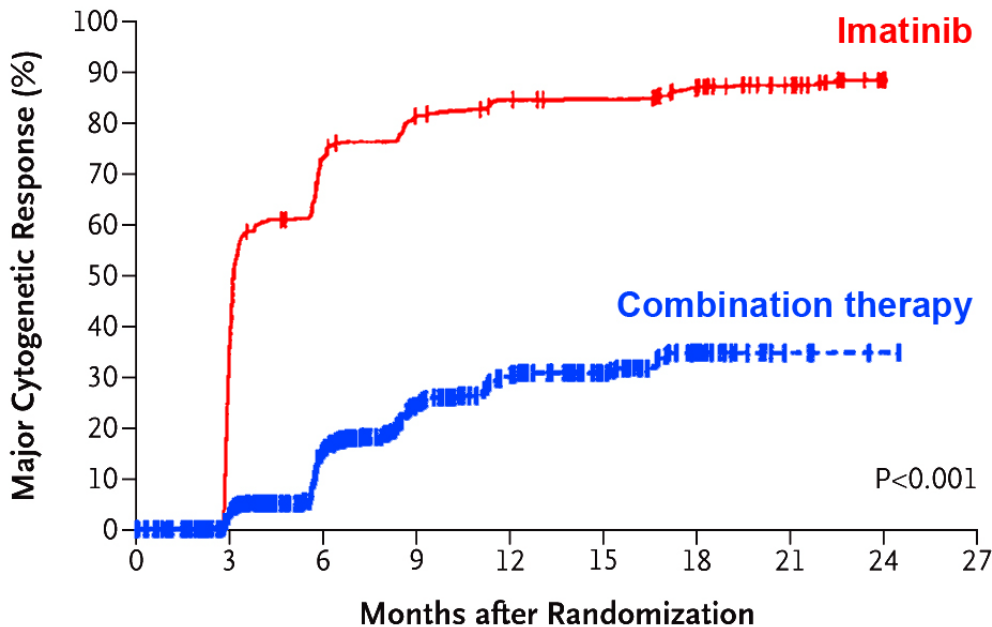
The image illustrates the marked neutrophilia with left shift, eosinophilia, and basophilia that are characteristic of CML in chronic phase. When significant leukocytosis is present, the peripheral blood smear resembles a bone marrow aspirate smear.

Figure 2: Bone marrow aspirate in chronic phase CML



The image demonstrates the classic bone marrow morphology of CML, with marked granulocytic hyperplasia, eosinophils, basophilic precursors, and a dwarf megakaryocyte (indicated by the arrow).

Figure 3: Cytogenetic response to imatinib in the IRIS trial



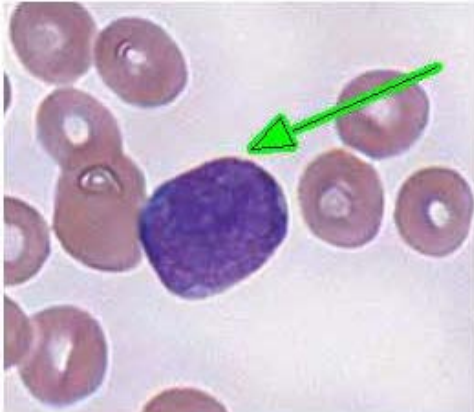
The IRIS trial demonstrated markedly superior cytogenetic responses in patients with CML treated with imatinib in comparison to interferon-alpha with low dose cytarabine (combination therapy). Patients on imatinib also experienced better progression-free survival. [From: O'Brien GS *et al.* N Engl J Med. 2003; 348(11):994-1004.]

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Recommended Reading

1. Vardiman JW, Melo JV, Baccarani M, Thiele J. *Chronic myelogenous leukaemia, BCR-ABL1 positive*. In: Swerdlow SH, ed. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th Edition. IARC Press, Lyon, 2008.
2. Melo JV, Hughes TP, Apperley JF. *Chronic myeloid leukemia*. Hematology Am Soc Hematol Educ Program 2003: 132-152.
3. Savage DG, Szydlo RM, Goldman JM. *Clinical features at diagnosis in 430 patients with chronic myeloid leukaemia seen at a referral centre over a 16-year period*. Br J Haematol 1997; 96: 111-116.
4. Goldman JM. *How I treat chronic myeloid leukemia in the imatinib era*. Blood 2007; 110: 2828-2837.
5. Cervantes F, Mauro M. *Practical Management of Patients with Chronic Myeloid Leukemia*. Cancer 2011; 117: 4343-4354.

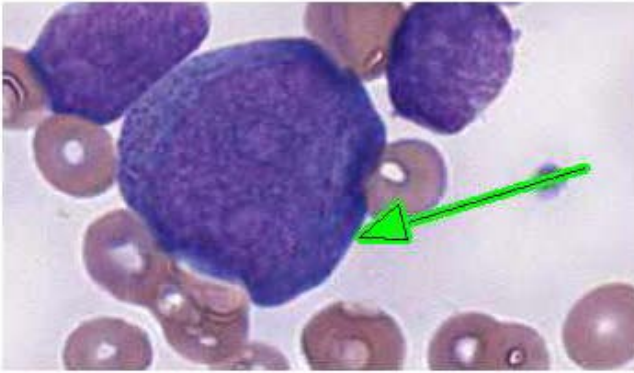
Cell Identification



EHE1-09

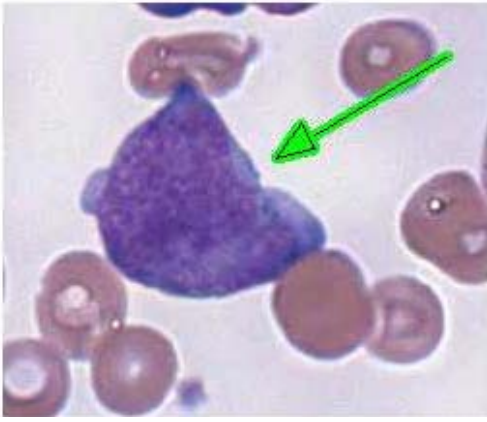
Identification	Participants		Evaluation
	No.	%	
Blast cell	149	82.3	Educational
Lymphocyte, reactive	10	5.5	Educational
Lymphocyte	9	5.0	Educational
Myeloblast with Auer rod	3	1.7	Educational
Lymphoma cell (malignant)	2	1.1	Educational
Monocyte, immature (promonocyte, monoblast)	2	1.1	Educational
Immature or abnormal cell	6	3.3	Educational

The arrowed cell is a small blast, as correctly identified by 82.3% of participants. Small blasts predominate in this case and range in size from approximately 10-20 μm , about 1.5 to 3 times that of a normal RBC and contrast with larger blasts that are less frequently seen in this case (see EHE1-11). These small blasts have variable but generally high N/C ratios with scant agranular blue cytoplasm and variable nuclear profiles ranging from round to oval or irregular, sometimes with prominent nuclear clefts. Chromatin is variable, ranging from fine to coarse and reticulated, smudged, or irregularly clumped. Nucleoli are generally small and indistinct or absent. The morphology of the small blasts is most consistent with lymphoid blasts, such as are seen in de novo acute lymphoblastic leukemia or, as in this case, lymphoid blast crisis arising from CML; however, assignment of a definitive lineage (such as myeloid or lymphoid) requires additional testing, such as immunophenotyping.



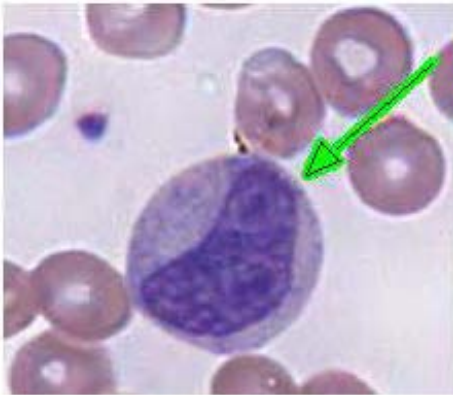
Identification	Participants		Evaluation
	No.	%	
Neutrophil, promyelocyte	137	75.7	Educational
Blast cell	15	8.3	Educational
Neutrophil, promyelocyte, abnormal with or w/o Auer rod(s)	13	7.2	Educational
Neutrophil, myelocyte	3	1.7	Educational
Myeloblast with Auer rod	2	1.1	Educational
Basophil, any stage	1	0.6	Educational
Lymphocyte, reactive	1	0.6	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.6	Educational
Plasma cell, morphologically mature	1	0.6	Educational
Immature or abnormal cell	7	3.9	Educational

The arrowed cell is a promyelocyte, as correctly identified by 75.7% of participants. Promyelocytes are a part of the spectrum of immature myeloid cells seen in the blood in CML. Promyelocytes are generally larger than blasts (12-24 μm) with slightly more cytoplasm and lower N/C ratios. The cytoplasm contains few to many azurophilic granules that extend over the nucleus. The chromatin is slightly coarser than in blasts, and nucleoli may be present.



Identification	Participants		Evaluation
	No.	%	
Blast cell	169	93.4	Educational
Myeloblast with Auer rod	2	1.1	Educational
Neutrophil, promyelocyte	2	1.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.6	Educational
Immature or abnormal cell	7	3.9	Educational

The arrowed cell is a large blast, as correctly identified by 93.4% of participants. Large blasts are much less frequently seen in this particular case and, unlike the small lymphoid blasts demonstrated in EHE1-09, are part of the spectrum of immature myeloid cells seen in cases of CML. Typically, larger myeloid blasts would be much more frequent in cases of CML in myeloid blast crisis. Myeloid blasts range from 3 to 5 times the size of RBCs and often have round to oval nuclear shapes. Nuclear profiles may be more irregular with grooves or folded contours. Unlike typical lymphoblasts, large myeloblasts have finer nuclear chromatin. Nucleoli are generally distinct, may be multiple, are often large, and sometimes have well defined “punched-out” borders. Cytoplasmic azurophilic granules may be seen in variable, but usually small numbers.



Identification	Participants		Evaluation
	No.	%	
Neutrophil, metamyelocyte	161	89.0	Educational
Neutrophil, giant band	5	2.8	Educational
Neutrophil, myelocyte	5	2.8	Educational
Monocyte	3	1.7	Educational
Monocyte, immature (promonocyte, monoblast)	2	1.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.6	Educational
Neutrophil, promyelocyte	1	0.6	Educational
Immature or abnormal cell	3	1.7	Educational

The arrowed cell is a neutrophilic metamyelocyte, as correctly identified by 89.0% of participants.

Neutrophilic metamyelocytes are immature myeloid cells that are no longer capable of self-renewal. The defining distinction from a myelocyte is that metamyelocytes have visibly begun the process of nuclear segmentation and have a discernable nuclear indentation. Metamyelocytes also have somewhat more clumped nuclear chromatin than myelocytes but less than that seen in terminally differentiated band and segmented neutrophils. The cell has relatively abundant cytoplasm with specific granules, similar to those seen in mature neutrophils.



Identification	Participants		Evaluation
	No.	%	
Basophil, any stage	178	98.3	Educational
Nuetrophil, necrobiosis (degenerated neutrophil)	1	0.6	Educational
Immature or abnormal cell	2	1.1	Educational

The arrowed cell is a basophil, as correctly identified by 98.3% of participants. Basophils contain a moderate number of coarse densely stained granules that vary in color from blue-black to purple-red in Wright stained preparations and frequently overlay and obscure the nucleus. The granules are larger and more variable in size and shape than the granules in neutrophils. Basophils are often increased in relative and absolute number in CML; this observation is often helpful in distinguishing neutrophilic leukemoid reactions from CML.

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Interpretive Questions - EHE1-14

1. Common features of CML at diagnosis do not include:

Response	No.	%
Fatigue and weight loss	6	3.4
Thrombocytopenia	141	80.1
An increase in the absolute number of circulating basophils	18	10.2
Circulating immature granulocytes	3	1.7
Splenomegaly	8	4.5

Intended Response: Thrombocytopenia

Typically patients with CML present with weight loss, splenomegaly (increased spleen size) and fatigue. Typical CBC and peripheral blood morphological analysis shows a leukocytosis with myeloid left shift with an increase in myelocytes versus metamyelocytes, an increase in basophils and eosinophils, normal to low hemoglobin levels and normal to increase platelet count.

2. T precursor lymphoblastic disease is not a recognized subtype of blastic phase CML:

Response	No.	%
True	54	31.0
False	120	69.0

Intended Response: False

CML blast phase may consist of a myeloid blast immunophenotype or a lymphoblast immunophenotype. The immunophenotype, which is evaluated typically by flow cytometric analysis, will determine the blast lineage. Although B lymphoblasts are the much more commonly seen subtype in a lymphoid blast transformation of CML, either B or T lymphoblasts may comprise the blast population.

3. Common secondary chromosomal abnormalities associated with progression of CML include:

Response	No.	%
22q-	28	16.2
Isochromosome 17	46	26.6
Monosomy 7	-	-
Trisomy 8	97	56.1
Loss or gain of Y	2	1.2

Intended Response: monosomy 7

Common secondary abnormalities associated with CML progression include 22q-, isochromosome 17, trisomy 8 and loss or gain of chromosome Y. Additional mutations associated with progression include mutations of the p53, C-RAS or N-RAS genes. Monosomy 7 is typically associated with myelodysplastic syndromes and therapy related myeloid neoplasms.

**** This question was intended to be:** "Which of the following is *not* a common secondary chromosomal abnormality associated with the progression of CML?" with the answer being Monosomy 7.

However, the question was incorrectly stated. Answers 22q-, isochromosome 17, trisomy 8, and loss or gain of the Y chromosome are all correct answers to the question as it was stated on the Result Form.

4. A genetic abnormality reported present in about 50% of patient in lymphoid but not myeloid blast phase of CML is:

Response	No.	%
Homozygous deletion of exon 2 of <i>INK4A/ARF</i>	88	51.2
der(9)	11	6.4
Extra copies Ph chromosome	36	20.9
del p16 tumor suppressor gene	37	21.5

Intended Response: homozygous deletion of exon2 of *INK4A/ARF* but del p16 tumor suppressor gene is also acceptable

The *INK4A* gene is also known as the p16 tumor suppressor gene and is associated with *de novo* cases of *T* lymphoblastic leukemia and is seen in >50% of lymphoid blast crises of CML, including both B and T-cell lymphoid crisis. Der (9) is found in a small percentage of CML patients, typically at presentation. Extra copies of Ph chromosome are found in patients with CML progression and are not restricted to patients with lymphoblastic blast crisis.

Discussion:

B Lymphoblast Crisis (B Lymphocytic Leukemia) of Chronic Myelogenous Leukemia

This peripheral blood smear is from a 52-year old female with a history of chronic myelogenous leukemia, BCR-ABL1 positive. Laboratory data include: WBC = $62.1 \times 10^9/L$; RBC = $1.37 \times 10^{12}/L$; HGB = 3.8 g/dL; HCT = 11.0 %; MCV = 80.3 fL; MCH = 27.7 pg; MCHC = 34.5 g/dL; PLT = $83 \times 10^9/L$; MPV = 8.3 fL; RDW =16.9%. Flow cytometry was performed on the peripheral blood primarily to evaluate blasts. Blasts are increased, accounting for 74% of leukocytes, and show a B-lymphoblast phenotype, expressing CD19, CD10, TdT, CD79a, HLA-DR, CD34, partial CD20 and partial CD22. They do not express surface light chains, T-cell antigens, CD25, CD117, CD13, CD33, CD15, MPO, CD14, CD64, CD61, CD56, or CD38.

Chronic Myelogenous Leukemia, *BCR-ABL 1+* (CML) is a myeloproliferative neoplasm which presents in the peripheral blood as a neutrophilia and typically has associated thrombocytosis. CML is differentiated from other myeloproliferative neoplasms on the basis of morphological features and, importantly, the presence of the *BCR-ABL 1* genetic fusion produced by the translocation between chromosomes 9 and 22 (also known as Philadelphia chromosome). The initial diagnostic evaluation in most patients begins with evaluation of the peripheral blood smear morphologic findings and CBC parameters.

CML is divided into three phases: chronic phase, accelerated phase and blast phase. Prior to the utilization of specific therapies that target the *BCR-ABL 1* protein target, CML would typically progress from chronic phase to accelerated phase to the final phase, which is the blast phase. In a smaller percentage of patients however, CML would initially present in the blast phase. Each phase of CML has characteristic morphologic, prognostic and genetic features. However, these criteria have evolved in recent years due to use of protein-targeted therapy.

Our current case is an example of a patient with a history of CML in chronic phase progressing to blast phase. Blast phase of CML is characterized by a variety of pathologic features that differentiate it from chronic phase (see TABLE 1). Morphologically, blast phase is generally characterized similar to acute myeloid leukemia classification where either the blast percentage is $\geq 20\%$ in the peripheral blood and/or bone marrow. Additionally, the diagnosis may be made when there is a discrete extramedullary blast proliferation or large area of blasts in the bone marrow biopsy. Additional parameters denoting blast phase include progression seen on the cytogenetic/molecular level and manifested by acquisition of new or additional cytogenetic abnormalities or increasing levels of BCR-ABL1 transcripts. The peripheral blood analysis of our case shows a blast population of greater than 20%, consistent with blast phase.

TABLE 1.

FEATURES OF BLAST PHASE, CHRONIC MYELOGENOUS LEUKEMIA, *BCR-ABL 1+*

- $\geq 20\%$ blasts in bone marrow or peripheral blood
- Extramedullary proliferation of blasts
- Bone marrow biopsy shows a focal significant accumulation of blasts
 - Approximately 70-80% of cases will consist of myeloblasts
 - Approximately 20-30% of cases will consist of lymphoblasts, mostly B-cell
- Granulocytes may develop dysplastic features (pseudo-Pelger Huet nuclei, cytoplasmic hypogranularity)
- May demonstrate persistence of basophilia in association with eosinophilia
- Evidence of cytogenetic evolution

Interestingly, the blasts comprising the blast phase of CML are not restricted to a myeloid phenotype/morphology, reflective of the concept that CML is a disorder of bone marrow stem cells capable of multiple pathways of differentiation. Approximately 20-30% of CML blast phase cases have a lymphoblast phenotype, typically B lymphoblasts. Fewer cases show megakaryocytic and/or erythroid blast phenotypes. Although morphologically the blasts may resemble lymphoblasts, definitive phenotyping requires immunophenotypic analysis such as flow cytometry and/or immunohistochemistry. The blast population in our current case contains a phenotype consistent with B lymphoblasts (CD19, CD10, TdT, CD79a, HLA-DR, CD34, partial CD20 and partial CD22 positive) and lacking myeloid and T lymphoblast antigens.

A small percentage of patients may initially present in CML blast phase, lacking a prior history of CML chronic phase. It is thus occasionally difficult to morphologically differentiate between CML blast phase and a *de novo* (non-CML associated) acute lymphoblastic leukemia (ALL). Additionally, *de novo* B acute lymphoblastic leukemia (B-ALL) may be Philadelphia chromosome positive, particularly in adult cases. However, there are a few morphologic and genetic clues that may indicate a possible CML blast phase diagnosis.

Morphologically, it is important to evaluate the other leukocytes present in the peripheral blood smear. Are there increased numbers of basophils? (FIGURE 1) Basophilia is not a common finding in *de novo* B-ALL. Basophilia in association with eosinophilia and myeloid left shift is a common finding in CML. Basophils may be degranulated and challenging to recognize. Also, it is important to know if the patient has a prior history of CML, as seen in the current case, which would indicate a B-ALL blast phase.

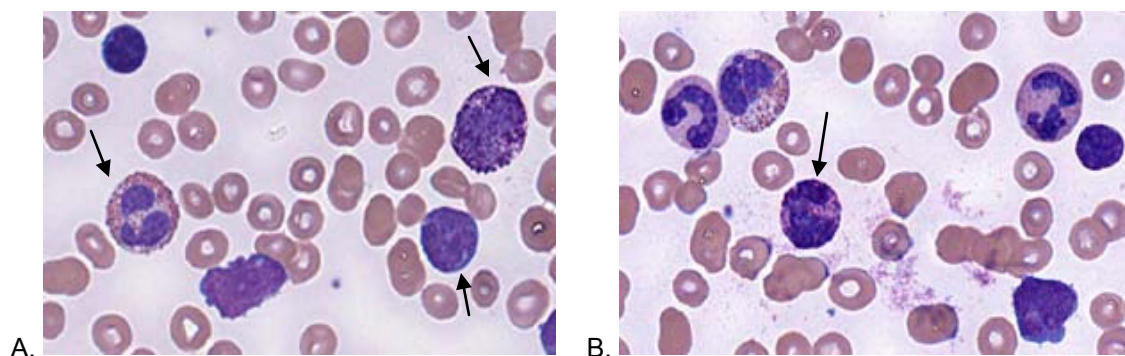


FIGURE 1. CML IN BLAST PHASE. A. This image from the current case shows two cells with lymphoblast morphology at the bottom of the picture with an eosinophil (left center), basophil (right upper center), and small lymphocyte (left upper). **B.** This image from the current case shows decreased granulation in the arrowed basophil (center).

Cytogenetic/molecular analysis may provide additional clues. The combination of the *BCR* and *ABL1* gene may occur at different locations of the *BCR* gene. The different breakpoint locations will cause a change in the protein size. The typical predominant *BCR-ABL1* protein produced in CML is known as p210; however, in most *de novo* B-ALL the typical *BCR-ABL1* protein produced is smaller and known as p190. *BCR-ABL1* translocations are thought to directly cause genomic instability, leading to progressive accumulation of genetic defects. Such defects disrupt critical cellular pathways and are likely responsible for impaired cell maturation, increased proliferation, and blockage of apoptosis that lead to clinical progression of the disease to accelerated and blast phases. Common chromosomal abnormalities linked

to disease progression in CML include: +22q-, isochromosome 17, extra copies of chromosomes 8, 9, 19, and 21 and either deleted or extra copy of chromosome Y. Molecular genetic abnormalities reported in CML that have been associated with progression include inactivating mutations in p53 in 30% of patients entering blast phase with less commonly reported loss of the retinoblastoma gene, inactivating mutations in *C-MYC* or *N-RAS* and deletion of the p16 tumor suppressor gene. Lymphoid blast crisis is most commonly associated with deletion of exon2 of *INK4A/ARF* (*INK4A* is also known as the p16 tumor suppressor gene), which is seen in over 50% of cases.

Dramatic changes have occurred in recent years with the advent of directed therapy for CML. The first line drug imatinib specifically targets the functional part of the BCR-ABL1 protein. This targeted therapy has increased 5-year progression-free and overall survival. However, it is not a cure, and additional cytogenetic aberrations may occur, allowing for development of resistance to the targeted therapy and increased risk for transformation to blast crisis. Thus, it is important to monitor CML patients for signs of morphological and cytogenetic progression so that therapies may be adjusted accordingly. Once blast transformation occurs, patients tend to be relatively resistant to leukemia therapies and have a poor prognosis.

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