**Answers to Question for Selected Hematologic Malignancies Lab**

**Case #1 Peripheral blood-stem cell disorder**

History: The patient is a 55-year-old female who presented to her local physician with increasing fatigue, night sweats, weight loss, and abdominal "fullness." On physical examination, the patient was noted to have a firm, nontender spleen, 10 cm below the left costal margin. The liver was slightly enlarged to 2 cm below the right costal margin. No lymphadenopathy was identified. The CBC showed: WBC = 105,000 cells/mm³, Hb = 10.5 gm/dl, and platelets = 85,000/mm³.

**What are the major abnormalities on the smear ?**

Markedly elevated WBC, much higher than sepsis case examined previously. Neutophilia with many precursors (see bone marrow aspirate for comparison). Percentage of precursors higher than in most reactive causes of leukocytosis. Also more immature forms (myelocytes, progranulocytes and some blasts) in this smear compared to usual "reactive left shift". Basophils easy to spot (usually very few in normal or reactive leukocytosis). The cells are not morphologically abnormal, just too many.

**What is the differential diagnosis based on the CBC and blood smear?**

Chronic myelogenous leukemia (CML), chronic phase, versus leukemoid reaction (excessive reactive leukocytosis). Leukemoid reaction more realistic differential if WBC < 50K but still a possibility.

**Would flow cytometry help with the diagnosis?**

No. We know from the morphology that the proliferative process involves relatively normal appearing granulocytes and granulocytic precursors. There are no antigenic findings that can separate chronic phase CML from a reactive leukocytosis.

**Would molecular or cytogenetic tests help with the diagnosis?**

Absolutely since the acquired structural genetic defect that causes CML has been identified. The Philadelphia chromosome t(9;22) can be detected in the neoplastic cells using classical cytogenetics or FISH. The translocation creates a new gene, the bcr:abl transgene, that can be detected using molecular (PCR) techniques. Demonstration of either the Philadelphia chromosome or the bcr:abl transgene are required for diagnosis.

**What is the natural course of this disease ? Are there changes in the blood or bone marrow smears that one can use to monitor progression ?**

The bcr:abl transgene encodes a new protein with a constitutively active protein tyrosine-kinase domain that affects many downstream signalling cascades. Massive clonal expansion and immortalization of the affected hematopoietic stem cell occurs (common
to lymphoid and non-lymphoid or myeloid lineages). In most individuals, the mutant stem cells continue differentiating primarily down the granulocyte lineage. The bone marrow fills up with normal looking granulocytic precursors that spill out into the bloodstream and accumulate in the spleen. Over time, additional mutations accumulate (largely unknown) that result in loss of differentiation, and an increase in the percentage of minimally differentiated cells (blasts) and, ultimately, the development of lymphoid or myeloid "blast crisis" (conversion to acute leukemia).

The loss of differentiation results in a gradual increase in the percentage of so-called "blast forms" (progenitors that may require flow cytometry or cytochemical stains for lineage determination). When the percentage of blast forms in the blood or bone marrow is > 10% but < 20% then the patient is in an "accelerated" phase of CML. When the percentage of blast forms in the blood or bone marrow is 20% or greater then the patient is in "blast crisis". Flow cytometry may be required to determine whether a patient has a lymphoid or myeloid blast crisis since the cytologic appearance of the blasts frequently does not provide any clues.

Case #2 Peripheral blood- stem cell disorders

History #1: The patient is a 58-year-old factory worker who noted increasing weakness, fatigue, malaise, and weight loss over the previous six weeks. He sought medical attention when he noted several unexplained lower extremity bruises following work. Scattered petechiae and bruises were noted. The CBC showed: WBC = 155,000 cells/mm³, Hb = 9.0 gm/dl, platelets = 11,000/mm³.

History #2: The patient is a four-year-old female who presented to her local pediatrician with a recent onset of pharyngitis and otitis media, unresponsive to antibiotics. The patient was febrile to 101.3 degrees F and was noted by her mother to have sudden onset of fatigue, malaise, and nondescript bone and joint pains. Physical examination revealed a spleen tip and small petechiae on her lower extremities. The CBC showed: WBC = 55,000 cells/mm³, Hb = 7.6 gm/dl, platelets = 5,000/mm³.

What are the major abnormalities on the smear?

In all three cases, many (>20%) of the WBC are mononuclear cells that look different than both normal monocytes and activated lymphocytes. Most have nuclei with lighter staining, finely divided, less clumped chromatin and occasional nucleoli (some multiple). These fit the general category of "blast" forms. This is an imprecise cytologic category that includes hematopoietic cells from various lineages (types) with an "active" appearing nucleus and relatively modest cytoplasmic differentiation. Normal bone marrows have 1-2% "blast forms" that include the earliest stages of myeloid (non-lymphoid/non-erythroid) and lymphoid (pre-B and pre-T) differentiation. When the peripheral blood or bone marrow differential shows 20% or more "blast" forms then a diagnosis of acute leukemia is made. In most cases, there are clinical and laboratory signs of bone marrow failure at the time of diagnosis (anemia, neutropenia, thrombocytopenia and their clinical sequelae).
In the acute leukemias, like in CML, the acquired genetic defects result in clonal expansion and accumulation of leukemic stem cells and their progeny. However, the genetic defects in these disease immortalize cells and inhibit differentiation from the start. Therefore, the leukemic cells that accumulate have the cytologic appearance of blast forms (myeloid, pre-B or pre-T).

**What is the differential diagnosis based on the CBC and blood smear?**

The presence of > 20% blast forms in the blood or bone marrow smear is sufficient for a diagnosis of acute leukemia. However, this diagnosis is not sufficient for therapy since acute leukemias differ in their responses to chemotherapeutic agents. In some cases, disease specific treatments directed at the underlying acquired genetic defect are available (e.g. t(15;17), promyelocytic leukemia and ATRA containing regimens). In non-M3 leukemias, the treatment regimens for myeloid leukemias (myeloid/myelogenous, myelo-monocytic, monocytic/monoblastic, less common forms) on the one hand and the various types of lymphoid acute leukemias (pre-B and pre-T) on the other are substantial different. Therefore, classification schemes (mention FAB and WHO; mention major FAB classes by name and fact that WHO requires cytogenetics) are based on (1) blast lineage (determined either cytologically or immunologically) and (2) the presence of defining (recurring) genetic abnormalities (most frequently disease specific translocations, but other types of large structural abnormalities and point mutations occur as well).

Students should focus on distinguishing blasts form normal and reactive leukocytes. Less concerned about them being able to find a rare Auer rod or recall the characteristic nuclear appearance of an APL. They should be able to recognize the virtual slides in this case as examples of acute leukemia and know how additional tests are used to establish a precise diagnosis. They should be able to distinguish an acute leukemia form a normal smear, a reactive leukocytosis (although I won't ask them to distinguish mononucleosis from the leukemias; frequently difficult for novices), CML and CLL. They should know that immunologic studies (flow primarily), cytochemical stains, cytogenetics and molecular studies are frequently required for dx. They should see at least one Auer rod (so that they can recognize it in a JPEG).

Smear #1 - AML (few granules, few Auer rods); Smear #2 ALL

**Would flow cytometry help with the diagnosis?**

Absolutely. Can virtually always establish the lineage of an acute leukemia using a panel of antibodies that react with stem cell, granulocytic, monocytic, T and B cell antigens. Always part of the initial diagnostic work-up. Cytochemical stains (MPO, NSE) are always required for subclassification of AML.

**Would molecular or cytogenetic tests help with the diagnosis?**
Absolutely. Necessary for confirmation of APL and identification of occasional acute leukemias associated with Ph1 translocation (both may benefit from therapies targeted at neoprotein encoded by pathologic transgene). Required for WHO classification. Likely that impact of cytogenetic and molecular genetic characteristics will grow as we learn more about the genetic basis of these cancers.

Is a bone marrow aspirate and biopsy necessary to establish the diagnosis?

If the differential count shows a blast percentage that equals or exceeds 20% on the peripheral blood smear then technically the diagnosis has been made. However, bone marrow aspiration and biopsy may be needed to collect enough material for additional diagnostic studies and to define the level of bone marrow infiltration. If a patient has circulating blasts but less than 20%, particularly if there is additional evidence of bone marrow dysfunction (cytopenias, dysplastic cells), then a bone marrow aspirate and biopsy are required to rule out an acute leukemia and establish the diagnosis.

Case #3 Peripheral blood–lymphocytic disorder

History: The patient is a healthy 65-year-old male who was noted to have slightly enlarged axillary and cervical lymph nodes on an annual physical examination. The patient offered no complaints and was given a "clean bill of health" one year previously. No organomegaly was noted. The CBC showed: WBC = 45,000 cells/mm3, Hb = 13.5 gm/dL, platelets = 185,000/mm3.

Compare the blood smear in this patient to the normal and abnormal smears from other cases.

What are the major abnormalities on the smear?

Too many small, resting lymphocytes with soccer-ball nuclei (absolute lymphocytosis). Otherwise unremarkable.

What is the differential diagnosis based on the CBC and blood smear?

Reactive lymphocytosis versus chronic lymphocytic leukemia or peripheralized lymphoma. Need test that can unequivocally separate monoclonal B-cell or T-cell expansion (most likely neoplastic) from a polyclonal expansion of T and B cells. In addition, sometimes difficult to separate different types of low-grade B- and T-cell neoplasms based on smear findings (e.g. chronic lymphocytic leukemia/small lymphocytic lymphoma, marginal zone lymphoma, grade 1 follicular lymphoma, mantle cell lymphoma, cutaneous T-cell lymphoma). Remind students that lymphoid neoplasms that arise in any lymphoid organ can involve the peripheral blood at some point in their course.

How does the flow cytometry study help with the diagnosis?
Can identify a monoclonal B-cell population (generally, but not always, means neoplasm) and identify antigens that are specific for some specific types of lymphoid leukemias and lymphomas. The flow data in this case demonstrates that most of the lymphocytes express a single lightchain type on their surfaces; therefore, they are monoclonal. Normally there are many more T-cells than B-cells in the blood and the B-cells are a mixture of cells expressing kappa and lambda lightchains (K:L ratio = 0.5-2, may be slightly higher or lower in reactive conditions but virtually never less than 0.1 or greater than 10 unless the population is neoplastic). Monoclonality in this case confirms that this is a B-cell neoplasm but there are many different types. In this case, the cells express two antigens that nail the diagnosis: when CD5 and CD23 (both expressed on some normal cells) are co-expressed on small, monoclonal B-cells then the diagnosis is chronic lymphocytic leukemia/small lymphocytic lymphoma >95% of the time.