Educational Case: Acute Promyelocytic Leukemia With *PML-RARA*

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.

Primary Objective

Objective HWC3.1: Morphology of Acute Leukemia and Lymphoma. Describe the morphologic features that characterize typical cases of acute leukemia and lymphoma.

Competency 2: Organ System Pathology; Topic HWC: Hematopathology—White Cell Disorders; Learning Goal 3: Classification of Leukemia and Lymphomas.

Patient Presentation

A 19-year-old woman presented to the emergency department complaining of fatigue and easy bruising over the past few weeks as well as fevers without chills or recent weight loss. She had no significant past medical history. She denied any personal or family history of cancer and was taking no medications. Physical examination revealed diffuse bruising of the arms, trunk, and legs without organomegaly or palpable lymphadenopathy.

Diagnostic Findings

A complete blood count (CBC) including the automated differential is provided in Table 1.

Questions/Discussion Points

What Is the Differential Diagnosis Based on the Clinical Findings and Complete Blood Count Data and What Should be the Next Step in Evaluating This Patient?

Review of the CBC and automated differential provided by the hematology analyzer reveals pancytopenia. The differential diagnosis of pancytopenia is broad and includes nutritional deficiencies, medication effect, other toxins, acute or chronic infections, autoimmune diseases, infiltrative bone marrow processes, hypersplenism, and primary bone marrow disorders, including aplastic anemia, myelodysplastic syndrome, leukemia, and paroxysmal nocturnal hemoglobinuria. Given the patient's age and physical exam findings, a peripheral smear review with manual differential is an important initial step in narrowing the differential diagnosis. The manual differential

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Table 1. Complete Blood Count and Automated Differential Count.

Test	Patient	Reference Range
WBC	1.7×10^9/L	$4.5\text{-}11 \times 10^9$/L
Hemoglobin	7.5 g/dL	12-16 g/dL
MCV	83.3 fL	80-98 fL
RDW	11.70%	11.5%-18%
Platelets	26×10^9/L	150-400 10^9/L

Automated Differential Count	Patient Percentage	Patient Absolute ($\times 10^9$/L)	Reference Range ($\times 10^9$/L)
Granulocytes	65	1.1	1.8-7.8
Lymphocytes	35	0.6	1-2.8
Monocytes	0	0	0-1.5
Eosinophils	0	0	0-0.5
Basophils	0	0	0-0.2

Abbreviations: MCV, mean corpuscular volume; RDW, red blood cell distribution width.

Table 2. Manual Differential Count.

Manual Differential Count	Patient Percentage	Patient Absolute ($\times 10^9$/L)	Reference Range ($\times 10^9$/L)
Granulocytes	23	0.3	1.8-7.8
Lymphocytes	15	0.2	1-2.8
Monocytes	0	0	0-1.5
Eosinophils	0	0	0-0.5
Basophils	0	0	0-0.2
Blast	60	1	0
nRBC	2/100 WBC		0

Abbreviations: nRBC, nucleated red blood cell; WBC, white blood cell.

count is performed by having a trained laboratory person visually review the stained blood smear, count 100 cells, and assess for cellular morphology, count confirmation, and artifacts, such as platelet clumps.

How Does the Peripheral Smear Findings in This Case Help Narrow the Differential?

The manual differential is provided in Table 2 and images from the peripheral smear are shown in Figure 1. The manual differential confirms pancytopenia and includes circulating nucleated red blood cells and immature cells/blasts consistent with a leukoerythroblastic smear. Smear review reveals the leukocytes present to be abnormal in appearance with "figure eight"-shaped or folded nuclei (Figure 1A), numerous cytoplasmic granules, and few forms with Auer rods (Figure 1B). Auer rods represent fused primary granules. The presence of Auer rods is diagnostic of a neoplastic blast with myeloid differentiation and is important to distinguish from lymphoblasts due to different treatment regimens in acute myeloid leukemia (AML) or other myeloid neoplasms and acute lymphoblastic leukemia.

The diagnosis of AML requires a blast percentage of ≥20% blasts dedicated to myeloid differentiation including myeloblasts, atypical/neoplastic promyelocytes, monoblasts/promonocytes, and/or megakaryoblasts in the peripheral blood or bone marrow. An exception to this rule is the presence of a translocation involving one of the core binding factors *RUNX1* and *core binding factor beta* (*CBF*β), which are t(8;21)(22q;q22.1) and inv(16)(p13.1q22)/t(16;16)(p13.1;q22) or a fusion of the *promyelocytic* (*PML*) gene on 15q22 with the *retinoic acid receptor alpha* (*RARA*) gene on 17q21.[1]

Acute myeloid leukemia with t(8;21)(22q;q22.1); *RUNX1-RUNX1T1* has distinctive morphologic findings in the peripheral blood and bone marrow. Both show large myeloblasts with perinuclear hofs, abundant azurophilic cytoplasmic granules, and large salmon-colored granules.[1] *RUNX1* is also known as

core binding factor alpha (*CBF1*α) which is normally involved in regulating hematopoiesis. Translocation leads to disruption of the alpha subunit blocking the maturation of myeloid cells. A similar blockade of maturation occurs in AML with inv(16)(p13.1q22)/t(16;16)(p13.1;q22), except instead of a disruption of *CBF1*α, there is a disruption of *CBF*β. This AML also has distinctive morphologic features including blasts with myelomonocytic features and atypical eosinophils with large basophilic granules. Monoblasts are large with round nuclei with fine chromatin, abundant basophilic cytoplasm, and can have fine azurophilic granules, vacuoles, and/or Auer rods. Promonocytes are also considered blast equivalents and have irregular folded nuclei and less basophilic cytoplasm, with more obvious granules and vacuoles.[1]

Acute myeloid leukemia with a predominance of abnormal promyelocytes and a *PML-RARA* fusion represents a specific subtype of AML known as acute promyelocytic leukemia (APL), previously called AML-M3 via the French-American-British Classification. These abnormal promyelocytes often have irregular and bilobed/"figure eight"-shaped nuclei with or without cytoplasmic granules and Auer rods. There are 2 morphologic subtypes of APL including a hypergranular variant (typical) and a hypogranular (microgranular) variant (Figure 2). The hypergranular variant often presents with leukopenia and consists of abnormal promyelocytes with bilobed nuclei, numerous cytoplasmic granules, and numerous Auer rods. The hypogranular variant is more likely to present with a leukocytosis, bilobed nuclei, numerous submicroscopic granules (seen with myeloperoxidase [MPO] stain), and rare cells with multiple Auer rods.[2]

What Additional Testing Should Be Performed to Confirm the Suspected Diagnosis?

Morphologically, the increased blasts with "figure eight"-shaped nuclei, numerous cytoplasmic granules, and Auer rods are suspicious for APL. To confirm this diagnosis, flow cytometric evaluation of the peripheral blood as well as rapid fluorescence in situ hybridization (FISH) to evaluate for *PML-RARA* fusion should be performed.[3] Recognition and early diagnosis of APL is essential due to the high risk of disseminated intravascular coagulation (DIC), a syndrome that
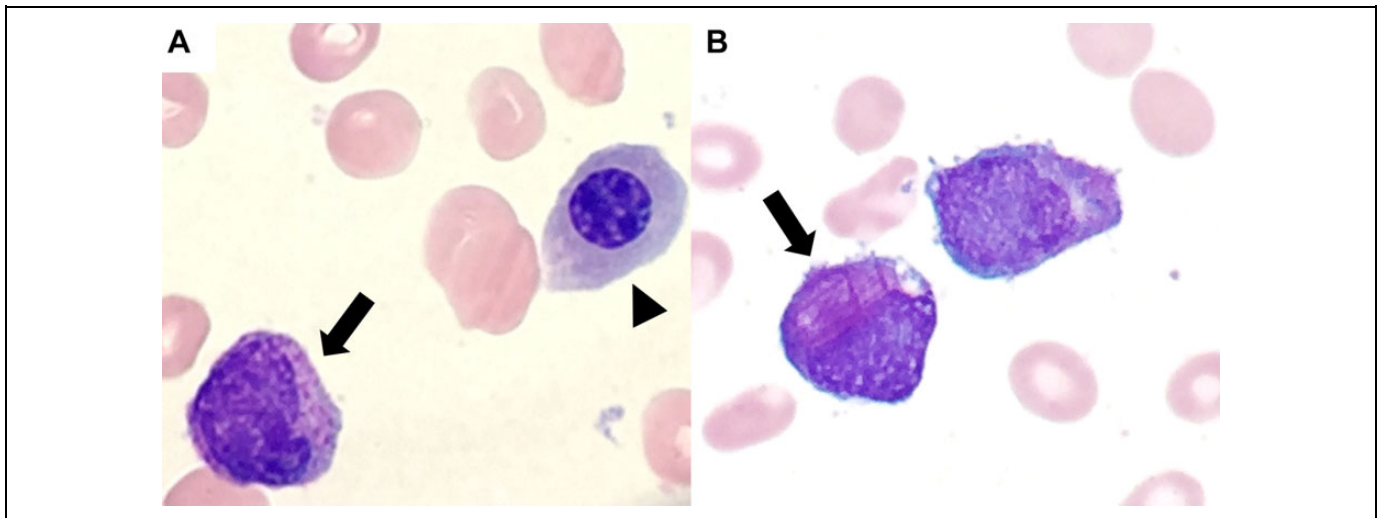
Figure 1. A, Leukoerythroblastic peripheral smear (×1000 magnification). Note the abnormal leukocyte with folded nucleus and numerous cytoplasmic granules (arrow) as well as the nucleated red blood cell (arrowhead). B, Leukocyte with numerous Auer rods (arrow) (×1000 magnification). Auer rods result from a fusion of primary granules and are diagnostic of a neoplasm with myeloid lineage.
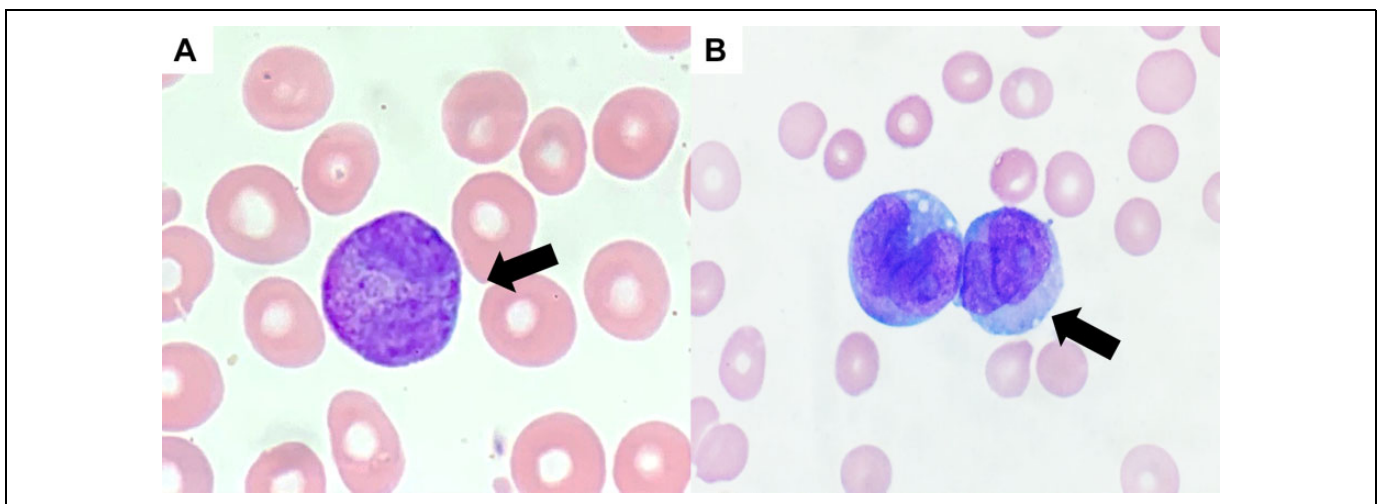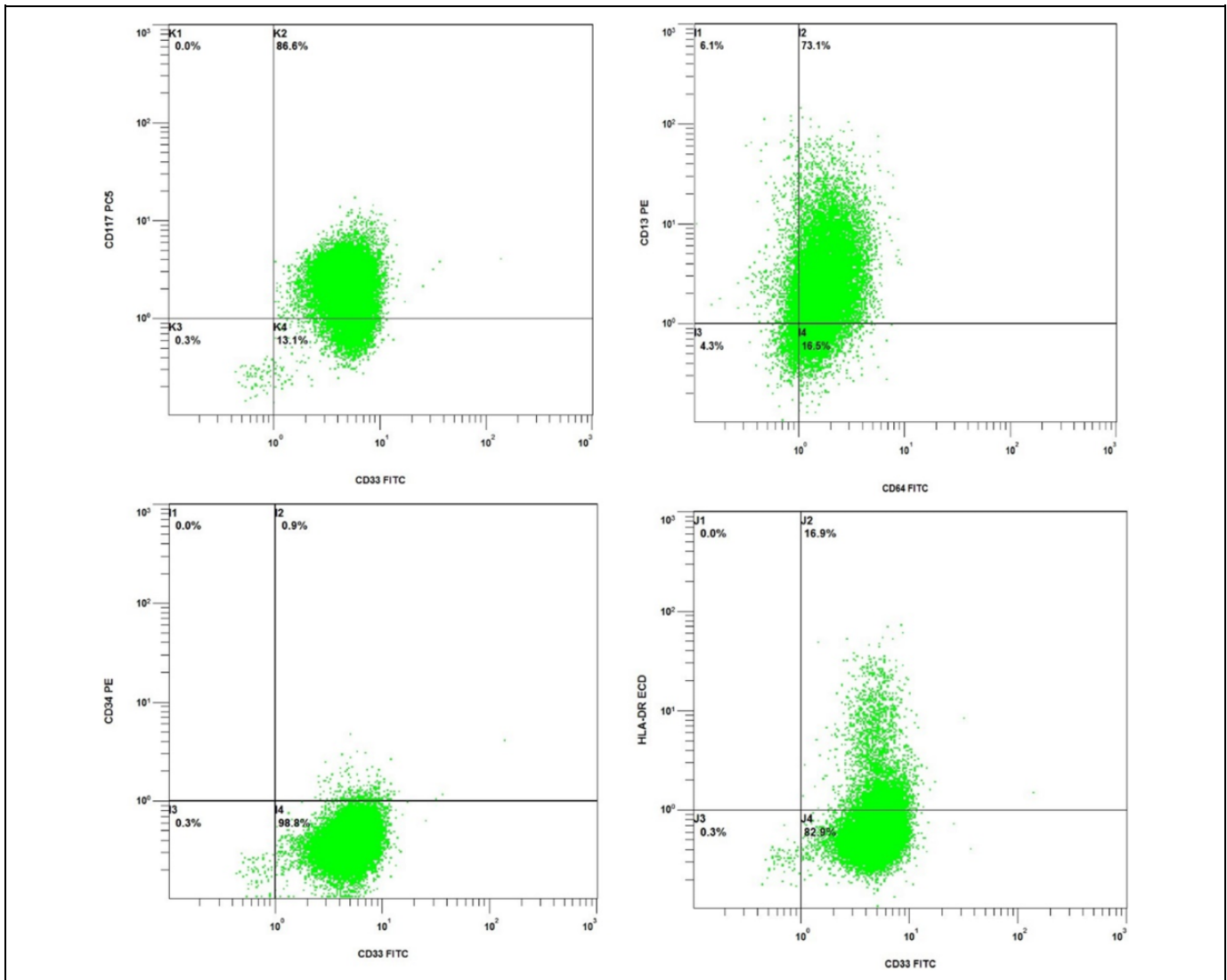

Figure 2. Acute promyelocytic leukemia (APL). A, Hypergranular APL (×1000 magnification). Note the abnormal promyelocytes with figure eight-shaped nucleus (arrow) and numerous cytoplasmic granules. In hypergranular APL, the granules can become so numerous that they obscure the nuclear features of the cell. B, Hypogranular APL (×1000 magnification). Note the absence of cytoplasmic granules on Wright Giemsa stain (arrow). The granules in these cases are submicroscopic and can be seen with a myeloperoxidase (MPO) stain.

results in inappropriate systemic activation of coagulation and fibrinolytic pathways with subsequent consumption of coagulation factors and platelets leading to bleeding, thrombosis, or both. There is a high risk of DIC in patients with APL due to increased production of procoagulants including tissue factor, cancer procoagulant, and microparticles as well as numerous cytokines which lead to endothelial damage and hypercoagulability. At the same time, APL cells have increased cell surface expression of annexin II and proteases leading to plasmin generation and hyperfibrionolysis.[4]

Flow cytometry and FISH can be performed rapidly on peripheral blood or bone marrow aspirate. Flow cytometry involves labeling cells with fluorescently tagged antibodies that are specific for hematolymphoid differentiation. These cells are then passed, single file, through a laser beam which detects the fluorescent label and separates the cells by antigen profile. In this case, the neoplastic cells were positive for CD117, CD33, CD13, and CD64 and mostly negative for CD34 and human leukocyte antigen-DR (HLA-DR) (Figure 3). The expression of CD33, CD13, and CD64 favor myeloid lineage, and this can be confirmed by MPO expression. Myeloperoxidase is an enzyme found in myeloid cells that aids in immune and inflammatory functions. The expression of CD117 is a marker of immaturity, and the hypergranular variant of APL is typically negative for other markers of immaturity including CD34 and HLA-DR. This is in contrast to the microgranular variant which typically expresses CD34 in conjunction with CD2. The flow cytometric findings are characteristic of AML and most

Figure 3. Flow cytometric phenotype of acute promyelocytic leukemia (APL). The neoplastic cells (green) are positive for CD117, CD33, CD13, and CD64 and mostly negative for CD34 and human leukocyte antigen-DR (HLA-DR).

consistent with hypergranular APL. The increased suspicion of APL in this case can be confirmed by FISH which uses DNA probes to recognize specific sequences located in a particular chromosomal region. These DNA clones are labeled with fluorescent dyes which bind to the desired chromosomal region and create a signal that is visualized under a fluorescent microscope.[5] Each gene is assigned a specific color probe, and within the cells being evaluated, there should be 2 signals for each gene (red and green, for example). When a translocation occurs, the 2 separate color probes fuse and a third color signal (yellow) is present due to the overlapping of the other 2 colors (Figure 4).

How Does the Fusion of These 2 Genes Result in Leukemia?

Promyelocytic gene is a nuclear regulatory factor gene which acts as a tumor suppressor gene. *Retinoic acid receptor alpha* is a transcriptional activator that suppresses cell growth and promotes cell differentiation in the presence of retinoids.[6] Translocation resulting in fusion of these 2 genes converts *RARA* to a transcriptional repressor which no longer responds to physiologic levels of retinoids. The *PML-RARA* fusion protein disrupts the signaling of both genes and results in a blockade of myeloid differentiation. This leads to an increase in abnormal promyelocytes that replace normal marrow elements.[6] This blockade of differentiation is necessary for development of APL but is not sufficient to cause leukemia.[3] Additional oncogene mutations cooperate with the *PML-RARA* fusion protein to cause leukemia including cytogenetic abnormalities such as gain of chromosome 8 as well as mutations in *FLT3*.[1]

A subset of cases will demonstrate variant translocation partners with the *RARA* gene. The most common of these variant partners are shown in Table 3.
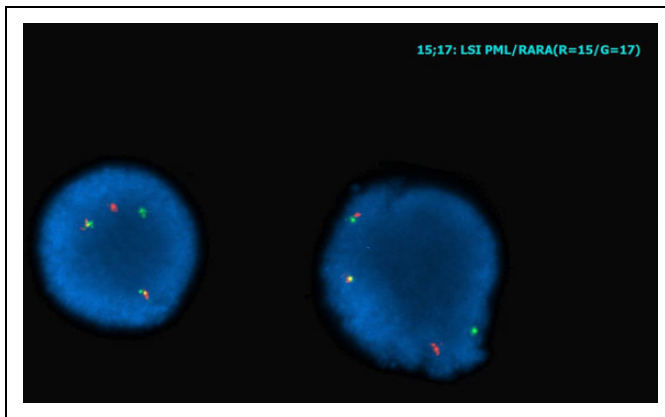
Figure 4. Fluorescence in situ hybridization (FISH) for *PML-RARA* fusion. When a translocation occurs, the *PML* gene on chromosome 15 (red) and the *RARA* gene on chromosome 17 (green) fuse together to produce a third color signal (yellow). PML gene indicates *promyelocytic* (*PML*) gene; RARA, retinoic acid receptor alpha.

Table 3. Common Translocation Partners in APL With Variant *RARA* Translocations.

Chromosome Region	Involved Gene	Expected Response to ATRA
11q23.2	ZBTB16	Resistant
11q13.4	NUMA1	Likely responsive
5q35.1	NPM1	Likely responsive
17q21.2	STAT5B	Resistant

Abbreviations: APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; RARA, retinoic acid receptor alpha.

What Is the Preferred Treatment Regimen for Acute Promyelocytic Leukemia? Are There Findings That Help Predict the Clinical Course or Treatment Response?

Rapid diagnosis and initiation of treatment of APL is critical due to the high risk of early death related to DIC. The successful treatment of APL with all-trans retinoic acid (ATRA) and arsenic trioxide is well-documented and is one of the first examples of differentiation therapy.[6] The *RARA* gene usually promotes cell differentiation in the presence of retinoids, but upon formation of the *PML-RARA* fusion protein, a conformational change prohibits the binding of these retinoids at physiologic levels. Pharmacologic doses of ATRA are able to overcome this state and bind to the *PML-RARA* fusion protein resulting in a conformational change that leads to transcription activation. The ability to differentiate is restored, and the neoplastic promyelocytes progress to neutrophils.[6] These neutrophils have limited life spans and subsequently die leaving the marrow clear to resume normal hematopoiesis. The arsenic trioxide therapy targets *PML* also leading to maturation and apoptosis. Remission is achievable with ATRA alone, but relapse can occur requiring standard induction chemotherapy to be given with or after ATRA.[1]

Two of the known variant translocations that are resistant to ATRA therapy, thus rendering patients more prone to developing DIC, include t(11;17)(q23;21); *ZBTB16-RARA* and t(17;17)(q11.2;q21); *STA5B-RARA*.[2] Previous cases, with older age, hyperleukocytosis, CD56 expression, and *FLT3* internal tandem duplication (ITD) mutation, have reported a worse prognosis in patients with APL with *PML-RARA*.[1] FLT3 is a receptor tyrosine kinase whose signals mimic normal growth factor signaling. When an activating mutation occurs (ITD), this leads to increased cellular growth and survival. Minimal residual disease monitoring can be assessed at the end of consolidation therapy utilizing quantitative real-time polymerase chain reaction.[3] Sequential levels of the *PML-RARA* transcripts are measured, and if detected after morphologic and cytogenetic complete remission strongly predict the risk of relapse. Identifying relapse early in these patients allows for delivery of preemptive therapy while the patient is still in subclinical disease, thus reducing the risk of treatment-related toxicity and death due to potential coagulopathy that comes with clinical relapse.[3]

Teaching Points

- Acute myeloid leukemias have distinct morphologic features based on their genetic aberrations.
- Acute promyelocytic leukemia is a specific subtype of AML with a characteristic t(15;17)(q22;q12); *PML-RARA* translocation.
- Acute promyelocytic leukemia is characterized by atypical promyelocytes that can be hypergranular with "figure eight" nuclei, abundant cytoplasmic granules, and bundles of Auer rods, or hypogranular with similar nuclear shape but indistinct cytoplasmic granules.
- Rapid diagnosis by flow cytometry and FISH is essential due to the common association with DIC.
- There is an excellent clinical response to therapy with ATRA and arsenic trioxide which act as differentiating agents, and when used in combination with standard induction chemotherapy in high risk patients can achieve complete remission.

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