37-year-old Caucasian male presented with complaints of headaches, nosebleeds, and dizziness. Hemorrhage in brain/low PLTs.

PE+ Petechiae in oral mucosa, trunk, extremities. Rest of the PE was normal/NAD.

LABS.
- WBC 32,000, platelets 17,000, H&H - 9.4/26.8. His BUN/creatinine - 19 and 1.2. LFTs-N.
- PT/INR - 19.8(9.9-13.2)/1.6, PTT is 24(21-32).
- Fibrinogen - 162(200-568), D-Dimer > 5000(0-250).
- CT head w/o contrast - 13 mm right para midline frontal lobe intraparenchymal hemorrhage.
- Peripheral Smear - majority of the circulating leukocytes are consistent with neoplastic promyelocytes.
- Flow cytometry (PB) - CD13, CD33, HLA-DR -.
- BM asp./bx- 90% involved with promyelocytes/occ. Blasts. FISH for t(15:17)PML-RARA + in 94.6% of nuclei.
A distinct subtype of AML identified by the FAB classification as **AML-M3**.

Acute promyelocytic leukemia with t(15;17)(q22;q12) in the newer WHO classification system (1999).

### French-American-British classification

<table>
<thead>
<tr>
<th>FAB subtype</th>
<th>Name</th>
<th>% of adult AML patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>Undifferentiated acute myeloblastic leukemia</td>
<td>5%</td>
</tr>
<tr>
<td>M1</td>
<td>Acute myeloblastic leukemia with minimal maturation</td>
<td>15%</td>
</tr>
<tr>
<td>M2</td>
<td>Acute myeloblastic leukemia with maturation</td>
<td>25%</td>
</tr>
<tr>
<td>M3</td>
<td>Acute promyelocytic leukemia</td>
<td>10%</td>
</tr>
<tr>
<td>M4</td>
<td>Acute myelomonocytic leukemia</td>
<td>20%</td>
</tr>
<tr>
<td>M4c</td>
<td>Acute myelomonocytic leukemia with anemia</td>
<td>5%</td>
</tr>
<tr>
<td>M5</td>
<td>Acute monocytic leukemia</td>
<td>10%</td>
</tr>
<tr>
<td>M6</td>
<td>Acute erythroid leukemia</td>
<td>5%</td>
</tr>
<tr>
<td>M7</td>
<td>Acute megakaryoblastic leukemia</td>
<td>5%</td>
</tr>
</tbody>
</table>

### WHO Classification of AML-1999

- Distinct morphologic, immunophenotypic & clinical presentation with coagulopathy which has set it apart from the other FAB morphologic subgroups.
Three periods: before, during and after treatment with all-trans retinoic acid (ATRA).

- First period (1957–1988), the disease was defined.
- Second (1988–1993), targeting the oncogenic event, dramatically improved the prognosis.

1957 by the Swedish author Leif Hillestad

1959-Bernard-Detailed features of APL & the severe hemorrhagic syndrome.
1959-Bernard- DIC or hyperfibrinolysis.
1976, the well characterized morphology led the French–American–British (FAB) Nomenclature Committee to assign them the specific classification of M3 cells (Bennett J.M. et al, 1976).

1980 & 1982 - Hypogranular and basophilic microgranular variants were described.
The number of newly diagnosed cases per year in the United States is estimated to be 1200.

Higher incidence in China and Mexico.

Compared with most patients with AML, patients with APL tend to be somewhat younger (median age, 30 to 40 years). Incidence seems higher in obese people.

At the completion of the initial work up, acute leukemia's are broadly classified:
- Acute promyelocytic leukemia (APL)
- Acute myeloid leukemia (AML), or
- Acute lymphoblastic leukemia (ALL).

MOLECULAR CYTOGENETICS
chromosomal translocations in AML

Incidence and prognosis of cytogenetic changes in AML

<table>
<thead>
<tr>
<th>Cytogenetic change</th>
<th>Fusion gene</th>
<th>Children (%)</th>
<th>Adults (%)</th>
<th>CR (%)</th>
<th>DFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(15;17)(q22q12)</td>
<td>APL-ETO</td>
<td>2-43</td>
<td>9-12</td>
<td>8-12</td>
<td>76-80</td>
</tr>
<tr>
<td>t(10;11)(p13q23)</td>
<td>CBF-BMY11</td>
<td>6-16</td>
<td>6-16</td>
<td>6-16</td>
<td>76-80</td>
</tr>
<tr>
<td>t(11;17)(q23p13)</td>
<td>AML-ETO</td>
<td>8-15</td>
<td>6-15</td>
<td>6-15</td>
<td>40-50</td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(16;16)(q21;q22)</td>
<td></td>
<td>1-4</td>
<td>3-6</td>
<td>76-84</td>
<td></td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td></td>
<td>22-36</td>
<td>20-40</td>
<td>10-18</td>
<td></td>
</tr>
<tr>
<td>t(9;11)(q31;q23)</td>
<td>MLL-AF9</td>
<td>4-8</td>
<td>1-2</td>
<td>1-2</td>
<td></td>
</tr>
<tr>
<td>t(15;17)(q22p13)</td>
<td>AML-ETO</td>
<td>6-13</td>
<td>6-5</td>
<td>10-12</td>
<td>10-20</td>
</tr>
<tr>
<td>AML</td>
<td></td>
<td>1-2</td>
<td>7-10</td>
<td>7-10</td>
<td></td>
</tr>
<tr>
<td>Downy syndrome</td>
<td></td>
<td>3-0</td>
<td>10-12</td>
<td>10-12</td>
<td></td>
</tr>
<tr>
<td>Complex (≥3 karyotypes)</td>
<td></td>
<td>0-08</td>
<td>10-30</td>
<td>10-30</td>
<td></td>
</tr>
<tr>
<td>t(16;19)(q22)p13)</td>
<td>AML-E2A-M4</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>t(12;15)(q22;q22)</td>
<td>MLL-ENL</td>
<td>4-2</td>
<td>Rare</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>t(12;21)(q23;q22)</td>
<td>DEK-CAN</td>
<td>&lt;1</td>
<td>1-2</td>
<td>1-2</td>
<td></td>
</tr>
</tbody>
</table>

Pre-Treatment Karyotype as Prognostic Factor in AML (CALGB 8461)

AML

PMI- Promyelocytic leukemia
RARa- Retinoic acid receptor alpha
ATRA- All Trans Retinoic Acid
ATO- Arsenic tri-oxide
THE PRE-ATRA PERIOD (1957–1988)
- 1977- $t(15;17)(q22;q21)$, recognized for the first time.
- This abnormality constitutes the genetic basis for approximately 97% of all cases of acute promyelocytic leukemia.

Cytogenetic Hallmark $t(15;17)$
- Reciprocal, balanced translocation involving the PML (promyelocytic leukemia) gene on chromosome 15, band q22, and RARA (Retinoic acid receptor alpha) on chromosome 17, band q21.

The novel fusion genes of $t(15;17)$
- PML/RARa gene on chromosome 15 (100%)
- RARa/PML gene on chromosome 17 (80%)
**APL-Variant translocations-3%**

- t(11;17) - **PLZF** (promyelocytic leukemia zinc finger) gene on 11q23.
- t(11;17) - **NuMA** (nuclear matrix-associated) gene on 11q13.
- t(5;17) - **NPM** (nucleophosmin) gene on 5q35.
- dup(17) - **STAT5** gene on 17q11.

**PATHOGENESIS**

**ROLE OF PML-RARA ONCOGENE**

---

**Hematopoiesis**

- PML-RARA: Transcription factor
- The production of abnormal retinoic acid receptors
- Continuous repression of the RARα target genes
- Blocked myeloid differentiation at the promyelocyte stage
- Acute Promyelocytic Leukemia (APL)
Accumulation of cells blocked at the promyelocytic stage of granulocytic differentiation.

Pancytopenia caused by bone marrow infiltration with leukemic promyelocytes/blasts.

Anemia, fever, infection, and bleeding manifestations.

Bone pain

Hepatosplenomegaly

Chloroma- not common
**Coagulopathy**

**Hypofibrinogenemia**
- Decreased coagulation factors
- Elevated fibrin degradation products
- Increased platelet consumption

These findings are the result of both **disseminated intravascular coagulation & primary fibrinolysis**

**DIC in APL**
- Frequently present **at diagnosis** or occurs soon after the initiation of **cytotoxic chemotherapy**.
- Medical emergency, can cause **pulmonary or cerebrovascular hemorrhage** in up to **40 percent** of patients and a **10 to 20 percent incidence** of early hemorrhagic death.

---

**Mechanism of DIC**
- The following three factors may be of primary importance-
  1. **Tissue factor**, which forms a complex with factor VII to activate factors X and IX
  2. **Cancer procoagulant**, which activates factor X independent of factor VII
  3. Increased **annexin II receptor** expression on the surface of the leukemic promyelocytes

---

**APL**

**DIAGNOSIS**
**Diagnosis**

- Morphology/IHC stains
  - > 20% blasts (BM and/or PB)
  - Myeloperoxidase +
  - Nonspecific and butyrate esterase +
- Immunophenotype
  - ≥ 2 myeloid markers
  - < 2 lymphoid markers
- Cytogenetic-molecular

**MORPHOLOGY-M3 cell**

- Creased, folded, nuclei (or dumb-bell shaped).
- Heavily granulated cytoplasm. Auer rods

**Two morphologic variants**

- **Hypergranular**
  - 80% of APL
  - “faggot cells”

- **Microgranular (M3v)**
  - 20% of APL
  - ↑ WBC

**Bone marrow-heavily granulated promyelocytes**

- [Image of bone marrow with markedly granulated cells]
Promyelocytes- HLA-DR negative, CD13+, CD33+, CD34-, and CD56-.

The presence of alternative fusion genes can be suspected in the following situations:
- PLZF/ RARα — CD13+, CD56+.
- NPM/ RARα — CD13-, CD56-. 

Conventional Cytogenetics & FISH

Hybridization (FISH) technique
RT-PCR

- Mainly used to detect relapse during surveillance.
- Detects PML-RARA RNA transcripts.
- Peripheral blood or BM sample.
- High specificity & sensitivity.

Prognostic indicators - Sanz score

PETHEMA/Sanz score - 3y.RFS

- **Low risk** — WBC ≤10,000 and platelets >40,000; **RFS 98 percent**
- **Intermediate** — WBC ≤10,000 and platelets <40,000; **RFS 89 percent**
- **High risk** — WBC >10,000; **RFS 70 percent**.

Variant translocations - Prognosis

- There is **no clear evidence** that the presence of chromosomal abnormalities in addition to t(15;17)-favorable, have an adverse effect on prognosis.
Treatment

- APL is a medical emergency.
- Initiate treatment as soon as the diagnosis is suspected, and before definitive (cyto)genetic confirmation is made.

The early days

- Before 1987- Treated similar to other AML subtypes- anthracycline based (idarubicin/daunorubicin with cytarabine).
- Obsession being to avoid a fatal haemorrhage of the central nervous system during the first days of treatment.

The dialogue between clinicians and biologists

- In 1978, Leo Sachs introduced the new concept that certain agents can trigger a differentiation process in leukemic cells.
- Molecules that regulate the differentiation and proliferation of hematopoietic cells-Over 100 agents were listed.
- Breitman-1981-RA helped terminal differentiation of APL cells in cultures.

CR rates - 75% to 80% in newly diagnosed patients.
Aggravation of bleeding syndrome by CT, leading to high early death rate.
Despite such progress, the median duration of remission ranged from 11 to 25 months and only 35% of the patients could be cured by CT alone.
ATRA

- USA-13 cis-A
- Europe- Etretinate
- China/France- ATRA


ATRA- THE FIRST TARGETED THERAPY

“WEAPONS OF MASS DIFFERENTIATION”

NEJM.feb 2009,Jonathan Licht

DIFERENTIATION

- ATRA induces differentiation of the malignant promyelocytic clone, an effect which can be observed in vitro and in vivo.
- Unique mechanism-cell differentiation Rather than inducing cell death from cytotoxicity.
**ATRA-Mechanism of action**

PML-RARA behaves as a potent transcriptional repressor

ATRA: Dissociation of NCOR-promotes differentiation of Leukemic cells

- In vitro studies: differentiation of leukemic promyelocytes in the presence of retinoic acid.
- Phase 11 clinical trials in relapsed/refractory APL.
- Cooperative group studies have confirmed the remarkable efficacy of ATRA.

**ATRA/arsenic- Mechanism of action**

**ATRA-Evidence of efficacy**

**BLOOD**

The Journal of The American Society of Hematology

VOL. 76, NO 9

NOVEMBER 1, 1990

**EDITORIAL:**

Acute Promyelocytic Leukemia: Another Pseudoleukemia?

In 1975, William Pepper described the bone marrow of a 10-year-old patient as a pseudoleukemia. It was the first case of an acute promyelocytic leukemia in the presence of NCOR-promotes differentiation of Leukemic cells.

Another pseudoleukemia could be on the way out.

PETER H. WEINSTEIN
Albert Einstein College of Medicine
Bronx, NY
**North American Intergroup (protocol 10129)-92’-95’**

**Induction**
- ATRA
- Daunorubicin + Cytarabine

**Standard Consolidation Chemotherapy**
- Cytarabine + Daunorubicin

**RESULTS**

<table>
<thead>
<tr>
<th>ATRA</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>69%</td>
<td>29%</td>
</tr>
</tbody>
</table>

**ATRA/Induction**
- Complete remissions induced by ATRA alone have a median duration of only about 3.5 months.
- A combination of **ATRA plus cytotoxic chemotherapy** is necessary for long-term survival.

**ATRA**
- Optimization of the ATRA-based regimens combining ATRA and CT has raised the CR rate up to 90% to 95%, and a 6-year DFS up to 86%.
- Cure rates – from 35% with CT alone to 86% for ATRA + CT.
- Significant decrease in Induction mortality seen with CT alone.
- **ATRA + Anthracycline** is standard induction Rx.
"This agent represents one of the most spectacular advances in the treatment of human cancer, providing the first paradigm of molecularly targeted treatment". European leukemianet, 2/2009.

Third post-ATRA period (1991–current), a new discovery: the beneficial effect of arsenic

ATO-History

- 1878 - Boston City Hospital - Fowler's solution on the reduction of white blood cell counts in "leucocythemia".
- 1930's - CML treatment
- China/1991 - ATO in relapsed/refractory APL
- 9/2000 - FDA approval for relapsed/refractory APL.

Arsenic Trioxide (ATO)

- Probably the most biologically active single drug in APL, has provided a valuable addition to the armamentarium.
- A number of studies have shown that single-agent ATO is a very effective therapy for patients with newly diagnosed APL (Iran, India).
If coagulopathy is present, administer frequent platelet transfusions to maintain platelet count greater than 30,000 to 50,000/mL and cryoprecipitate to maintain fibrinogen level greater than 100 to 150 mg/dL. There is no role for routine use of heparin or antifibrinolytic agents.

APL – Treatment overview

**DIC-supportive care**

- If coagulopathy is present, administer frequent platelet transfusions to maintain platelet count greater than 30,000 to 50,000/mL and cryoprecipitate to maintain fibrinogen level greater than 100 to 150 mg/dL.
- There is no role for routine use of heparin or antifibrinolytic agents.

**CONCLUSIONS**

- APL
  - **Historical**
    - Course: Fatal
    - Prognosis: Poor
    - Frontline Rx: Anthracycline
  - **Modern**
    - Course: Curable
    - Prognosis: Excellent
    - Frontline Rx: ATRA + anthracycline
Differentiation Syndrome - ATRA/ATO

- Fever, dyspnea, pleural-pericardial effusions, weight gain, renal failure.
- CXR-infiltrate, Hypoxia
- 2-21d of Rx(ATRA or ATO).
- Mortality-1-7%
- Dexamethasone. Rarely-stop ATRA/ATO

Combining ATRA + ATO.

- ATRA + ATO can be reserved for patients who cannot tolerate anthracyclines (esp. if over 60y).
Prior to 1992, induction therapy for APL was similar to other AML subtypes (anthracycline + ara-C).

Early 70's, leukemic cells from patients with APL are peculiarly sensitive to anthracyclines.

The dose of anthracycline in most of these trials was roughly 1.8 times that used for AML induction.

The introduction of all-trans retinoic acid (ATRA) prompted several study groups to exploit this peculiar sensitivity to anthracyclines by combining it with ATRA.

**Italian GIMEMA trial-93'**

**Spanish PETHEMA LPA 94 trial-94'**

Induction regimen were pared down to ATRA + Idarubicin. CR-95%. 

<table>
<thead>
<tr>
<th>Study</th>
<th>Anthracycline</th>
<th>No. of patients</th>
<th>Complete remission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernard et al. 1973</td>
<td>Daunorubicin</td>
<td>34</td>
<td>55</td>
</tr>
<tr>
<td>Collins 1978</td>
<td>Daunorubicin</td>
<td>7</td>
<td>71</td>
</tr>
<tr>
<td>Maruyama et al. 1982</td>
<td>Daunorubicin</td>
<td>83</td>
<td>84</td>
</tr>
<tr>
<td>Park et al. 1987</td>
<td>Daunorubicin</td>
<td>30</td>
<td>67</td>
</tr>
<tr>
<td>Sant et al. 1988</td>
<td>Daunorubicin</td>
<td>34</td>
<td>69</td>
</tr>
<tr>
<td>Anawalt et al. 1990</td>
<td>Idarubicin</td>
<td>27</td>
<td>81</td>
</tr>
<tr>
<td>Annaratone et al. 1990</td>
<td>Idarubicin</td>
<td>131</td>
<td>78</td>
</tr>
<tr>
<td>Falcini et al. 1991</td>
<td>Daunorubicin</td>
<td>20</td>
<td>88</td>
</tr>
</tbody>
</table>
APL - Treatment overview

- ATRA (all Trans Retinoic acid)
- ATO (Arsenic Tri oxide)
- Anthracyclines
- Cytarabine
- Supportive care for Coaguopathy

Induction/APL - ATRA + Anthracycline.

Current standard of care

- French APL 2000 trial - A benefit for High risk subtype of APL (lowered the 3y Relapse).
- Current recommendations - High risk patients - Cytarabine offers some benefit & should be considered (if there is no trial available).
**Post Remission Therapy**

**CONSOLIDATION**

**MAINTENANCE**

**Consolidation**
- **Goal** - Converting morphologic and cytogenetic remission into **durable molecular remission**.
- Consolidation with anthracycline is needed for all risk groups.
- For intermediate and high risk - add ATRA (15 days).
- High risk - Add Cytarabine (French trial or ATO as in the US intergroup trial).

**Trials - Consolidation**
- PETHEMA-LPA 99
- FRENCH-APL 2000
- Second US INTERGROUP trial.

**Maintenance**
- If PCR is negative - Maintenance ATRA for 1-2 y, which may be combined with 6 mercaptopurine & methotrexate.
- **French APL 93 (ATRA+6MP+Mtx)** - benefit.
- **US intergroup (ATRA vs. Obsv.)** - benefit.
- **AIDA** trial - No Benefit
**APL - Treatment overview**

- ATRA (all Trans Retinoic acid)
- ATO (Arsenic Tri oxide)
- Anthracyclines
- Cytarabine
- Supportive care for Coaguopathy

**DIC - Supportive care**

- If coagulopathy is present, administer frequent platelet transfusions to maintain platelet count greater than 30,000 to 50,000/mL and cryoprecipitate to maintain fibrinogen level greater than 100 to 150 mg/dL.
- There is no role for routine use of heparin or antifibrinolytic agents.

---

**APL - Trials/Induction**

- **Shanghai group-1988.** CR rates of 85% as a single agent.
- **North American Intergroup study-70%** single agent CR (same as for a combination of ara-C/ Daunorubicin).
- **French APL 93’ trial-ATRA f/b chemo vs. ATRA + Chemo.** 92% CR in both arms but relapse rate at 2y- 6% in combined arm vs. 16% for sequential arm.
Relapse

- Arsenic trioxide alone or in combination with ATRA (if ATRA was not used during consolidation).
- After achieving remission-consider Autologous HSCT.
- European APL-7y OS-75% for autologous SCT vs. 52% for allogenic SCT.
- If patients are not candidates for SCT or clinical trial- post remission ATO x 6 cycles.

Relapse

- Gemtuzumab ozogamicin (GO)-
  - Relapse within 6mo of ATO therapy.
  - Second line salvage for patients in relapse after initial rescue with ATRA+ATO.
- Use it with caution in potential candidates for SCT-Increased incidence of hepatic venoocclusive dz.

Supportive therapy

- Coagulopathy
- APL differentiation syndrome with ATRA or ATO.
- Hyperleuocytosis
- Monitoring during ATO.
### APL-Distinctive features

- 10%–15% of adult acute myeloid leukemia (1200 new patients per year in the United States)
- Leukocytosis in 85%
- Coagulopathy (disseminated intravascular coagulopathy, fibrinolysis, prothrombosis)
- t(15;17)
- Promyelocytic leukemia-retinoic acid receptor α (PML-RARA) fusion transcript
- Differentiation with all-trans retinoic acid
- Apoptosis with arsenic trioxide
History

- 1959-Bernard-Detailed features of APL & the severe hemorrhagic syndrome.
- 1959-Bernard-DIC or hyperfibrinolysis.
- 1973- Bernard et al demonstrated that APL leukemic cells were relatively sensitive to chemotherapy (daunorubicin).

APL

- 1957- Hillestad, Sweden. “a very rapid fatal course of only a few weeks’ duration,” with a white blood cell (WBC) picture dominated by promyelocytes and a severe bleeding tendency. “seems to be the most malignant form of acute leukemia”.

HISTORY OF EVENTS

Three periods: before, during and after treatment with all-trans retinoic acid (ATRA).

- First period (1957–1988), the disease was defined
- Second (1988–1993), targeting the oncogenic event, dramatically improved the prognosis.

ATRA

ATRA maintenance x 1y

- In CR

Observation

- In CR

APL-CASE

- **PMH-** Melanoma-12y ago-s/p wide excision
  - Gout.
- **SH/FH-** He does work around a lot of chemicals, including paints. + smoker.
  No Hx of cancers in family.
- **ROS-** No fever, wt. loss, night sweats.
- **PE-** + Petechiae in oral mucosa, trunk, extremities. Rest of the PE was normal/NAD.