Blood disorders leading to cytopenias

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Objectives

Overview of conditions/ diseases that lead to blood cytopenias

Select disorders reviewed in more detail

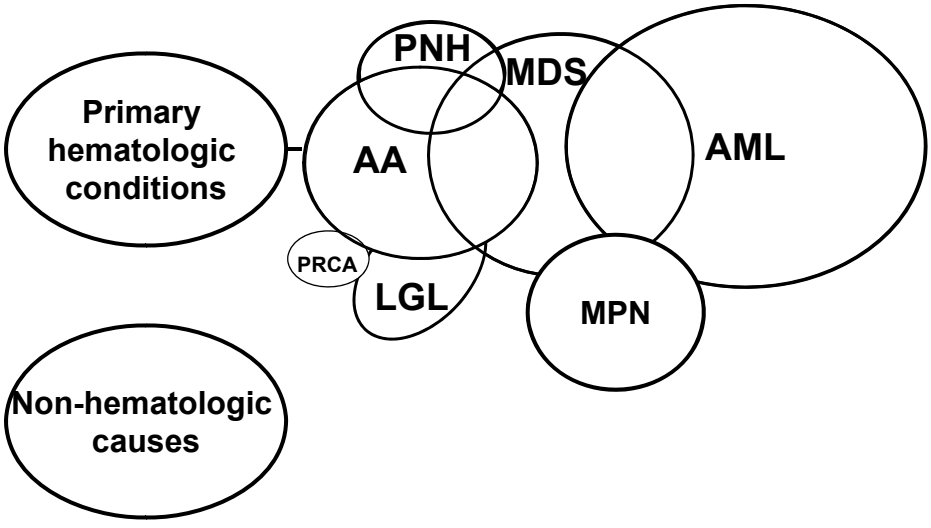
Focus on evolving understanding of molecular biology
- **Hematologic disorders as a paradigm for "next generation" medical understanding of medical disease**

Personalized health care has arrived!

Case 1

- 31 year old female with fatigue, neuropathy
- WBC 1.8 (10% segs), Hgb 8.3g/dL, Plt 277
  - Bone marrow biopsy with dyserythropoiesis and dysmegakaryopoiesis, low blasts
  - Normal female karyotype
- Referred for transplantation consultation for myelodysplastic syndrome

"Blood disorders causing cytopenias…" its not quite so straightforward…



Non-hematologic conditions in the differential diagnosis (partial list)

- Drug Effects
- Nutritional Anemias
- HIV
- Hypothyroidism
- Rheumatologic Disorders
- Copper deficiency
- Alcoholism

Case 1 continued

- Exam findings: dentures in place, severe sensory and motor neuropathy
- She mentioned that she had recently seen on ABC news a report of Zinc toxicity from Poligrip. She stopped using the product about a month prior, but had used it for 4-5 years.

Case 1 continued

- Her Zinc level was high at 2800ug/L in the urine with Zn/Cr ratio of 5456/ug/g creat (nl 100-900)
- Serum copper level, undetectable
- Copper replacement given, CBC normal within 3 weeks (neuropathy not improved…)

Case 2

- 53 year old female presented with fatigue, anemia, abdominal discomfort
- Hemoglobin 8.8g/dl, mild leucopenia, mild thrombocytopenia
- Bone marrow biopsy “consistent with refractory anemia”; cytogenetics normal
- Responded briefly to Procrit (recombinant erythropoietin) but began to feel worse, RBC transfusion dependent
- Referred to OSU for bone marrow transplantation

Case 2

Dx: Pernicious anemia

Plan: Replace B12

Outcome: Patient immediately began to feel better after B12 shots and returned to work soon after

Take home messages:

1. **Non-hematologic disorders must be considered**
2. **Morphology alone may not be enough to make a diagnosis, increasingly we must rely on cytogenetic/ molecular tools**

Primary hematologic conditions in the differential diagnosis of low blood counts

- **Paroxysmal Nocturnal Hemoglobinuria (PNH)**
- **Acute Myeloid Leukemia (AML)**
- **Pure Red Cell Anemia (PRCA)**
- **Large Granular Lymphocyte (LGL) Leukemia**
- **Aplastic Anemia (AA)**
- **Myeloproliferative Neoplasm (MPN)**
- **Hairy Cell Leukemia (HCL)**
- **Hereditary sideroblastic anemias**
- **Myelodysplastic syndromes (MDS)**

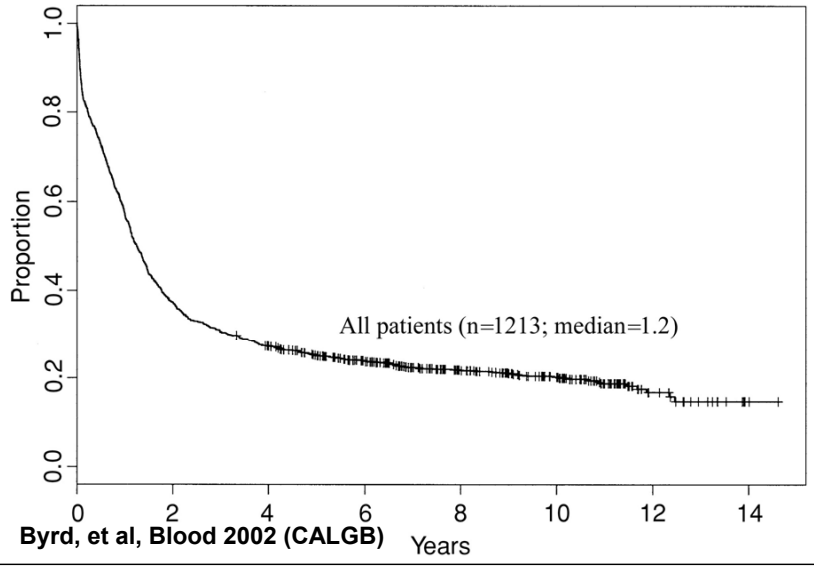
Common Presentations of AML

- vague history of chronic progressive lethargy
- 1/3 of patients acutely ill, usually with infection
- Petechiae with or without bleeding
- Splenomegaly and hyperuricemia (lymphoid?)
- Organ inflitration (of CNS, gums, skin, chloromas, retinal infiltration)

Lab Findings in AML

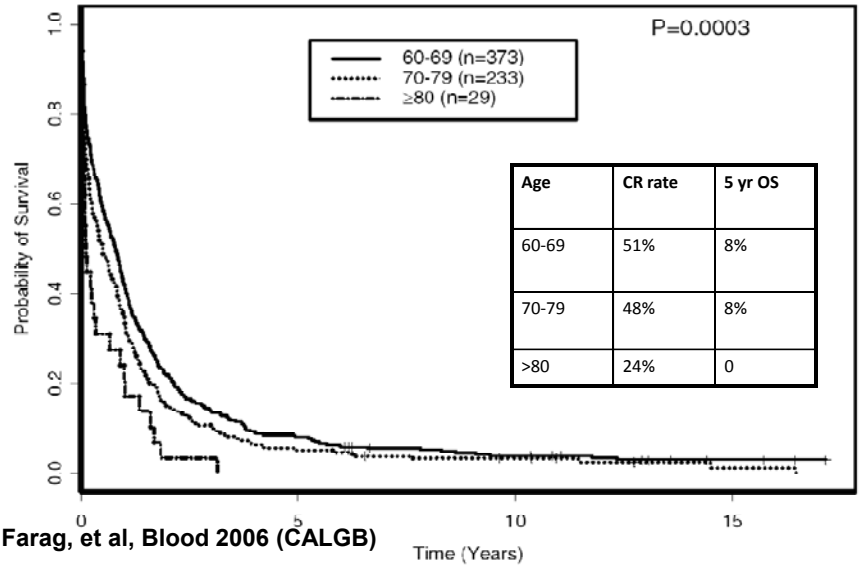
- Hemoglobin generally low, severe anemia uncommon
- WBC may be increased, decreased, or normal
  - 35% of all AML patients will have ANC < 1,000/uL; circulating blast cells may be absent 15% of the time
- Disseminated intravascular coagulation is common
  - especially in acute promyelocytic leukemia
- Thrombocytopenia is frequently observed--platelet counts <20,000/uL are common, often leads to bruising or bleeding (gums)

Outcomes in a cohort of younger AML patients (median age 52 years)



Byrd, et al, Blood 2002 (CALGB)

Outcomes in AML patients over 60 years of age…terrible



Age	CR rate	5 yr OS
60-69	51%	8%
70-79	48%	8%
>80	24%	0

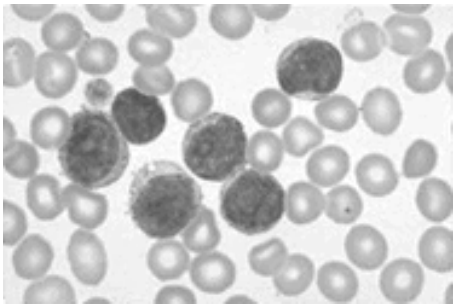
Farag, et al, Blood 2006 (CALGB)

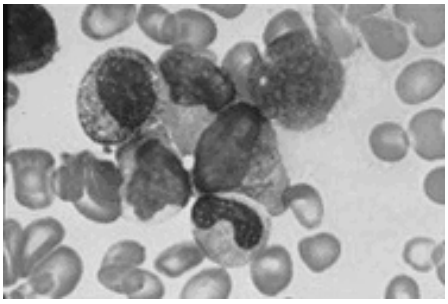
Outcomes worse if:

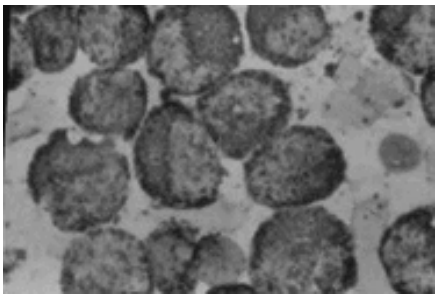
- Older age (>60)
- High WBC (over 20,000/uL)
- Prior hematologic disorder like myelodysplastic syndrome
- Leukemia caused by prior chemotherapy
- Poor initial response to chemotherapy
- Poor performance status

- ADVERSE RISK CYTOGENETICS

Morphology as prognostic? *Not really…*







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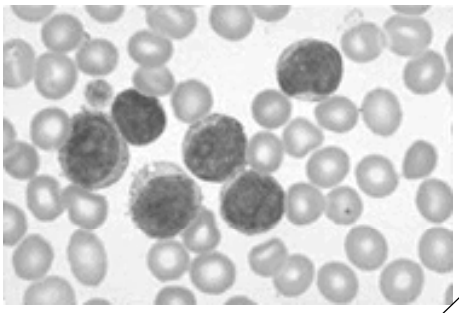
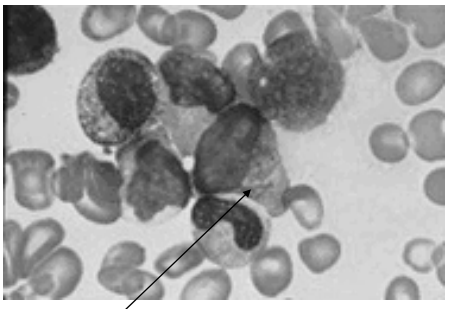
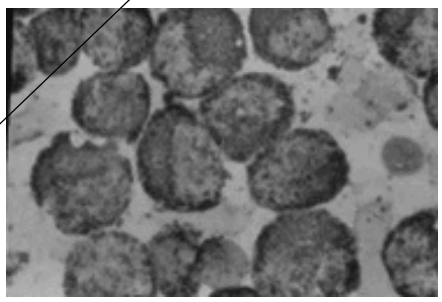
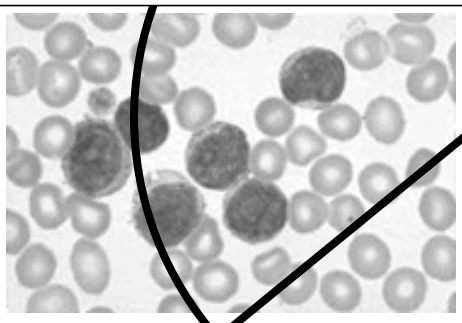


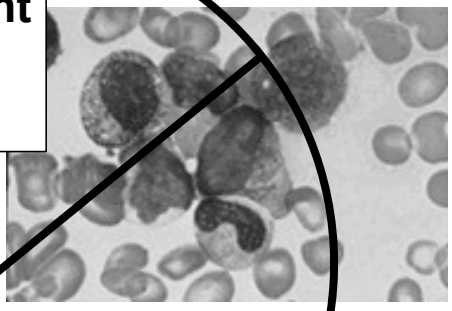


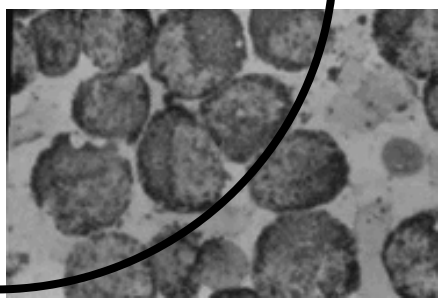


Note the presence of Auer rods in the cytoplasm

From a risk assessment standpoint, appearances deceive







French-American British (FAB) Classification for AML

FAB subtype	Name	% of adult AML patients	Prognosis compared to average for AML
M0	Undifferentiated acute myeloblastic leukemia	5%	Worse
M1	Acute myeloblastic leukemia with minimal maturation	15%	Average
M2	Acute myeloblastic leukemia with maturation	25%	Better
M3	Acute promyelocytic leukemia	10%	Best
M4	Acute myelomonocytic leukemia	20%	Average
M4 eos	Acute myelomonocytic leukemia with eosinophilia	5%	Better
M5	Acute monocytic leukemia	10%	Average
M6	Acute erythroid leukemia	5%	Worse
M7	Acute megakaryoblastic leukemia	5%	Worse

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Morphology does not tell you who will get cured and who will not

How we understand risk in AML is finally changing…

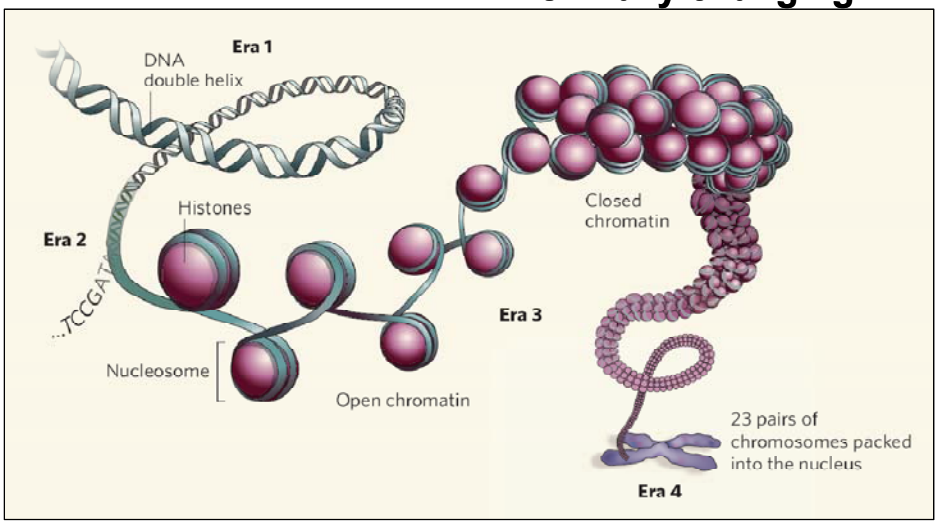

Figure from Baylin and Schuebel, Nature 2007

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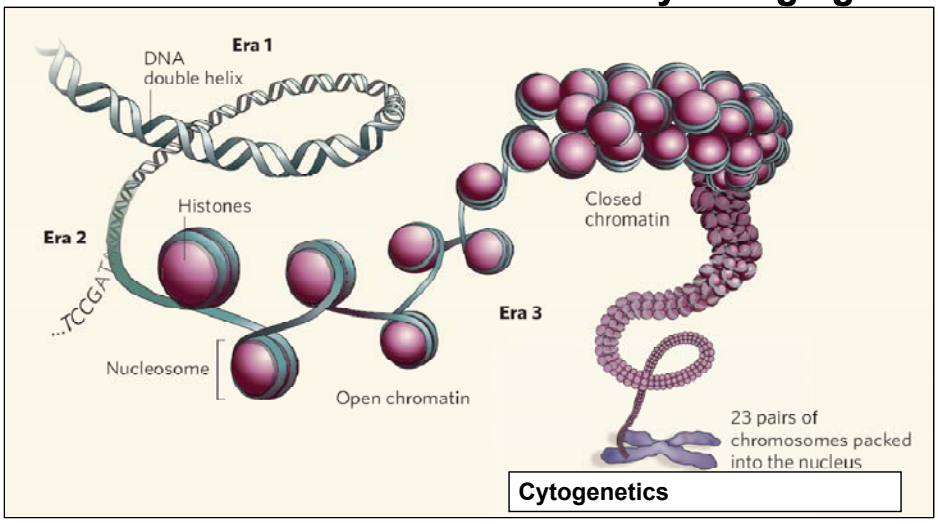

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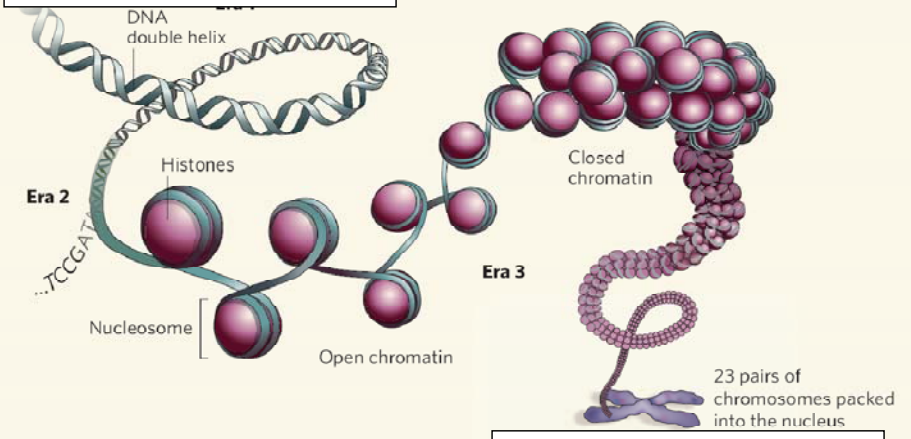
How we understand risk in AML is finally changing…

Molecular diagnostics—what mutations are relevant to outcome and (hopefully) treatment selection?



Cytogenetics

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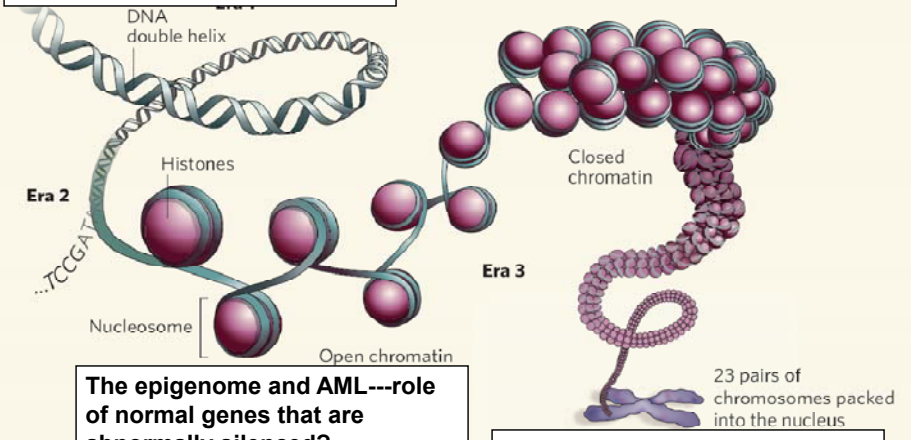

The epigenome and AML---role of normal genes that are abnormally silenced?

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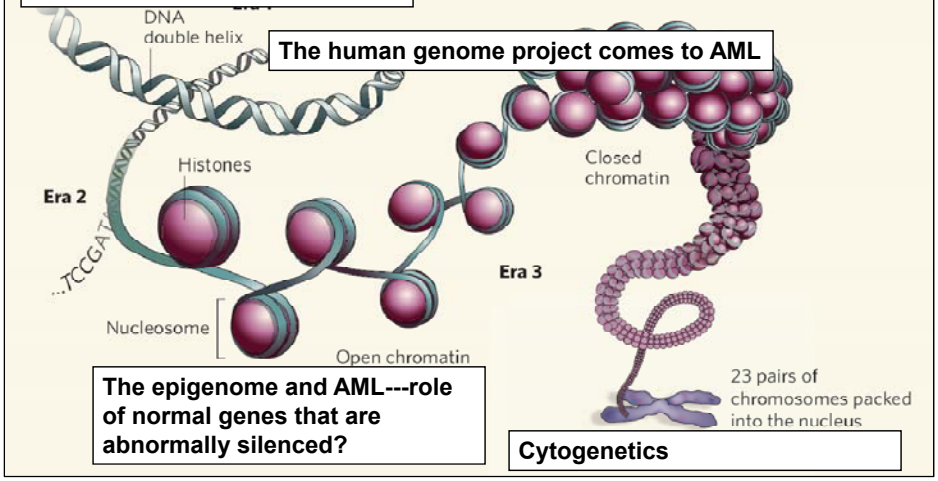

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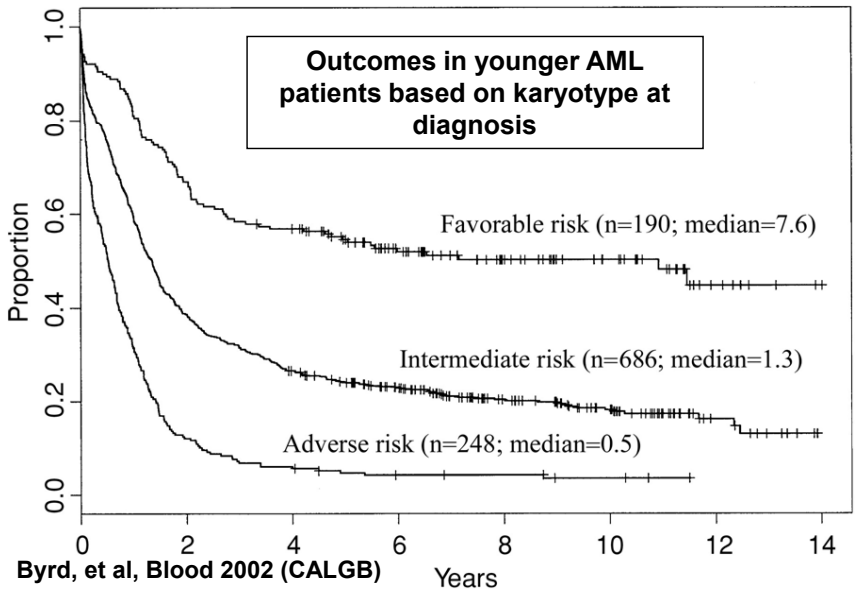
Chromosome analysis provides the most important disease specific prognostic information



Byrd, et al, Blood 2002 (CALGB)

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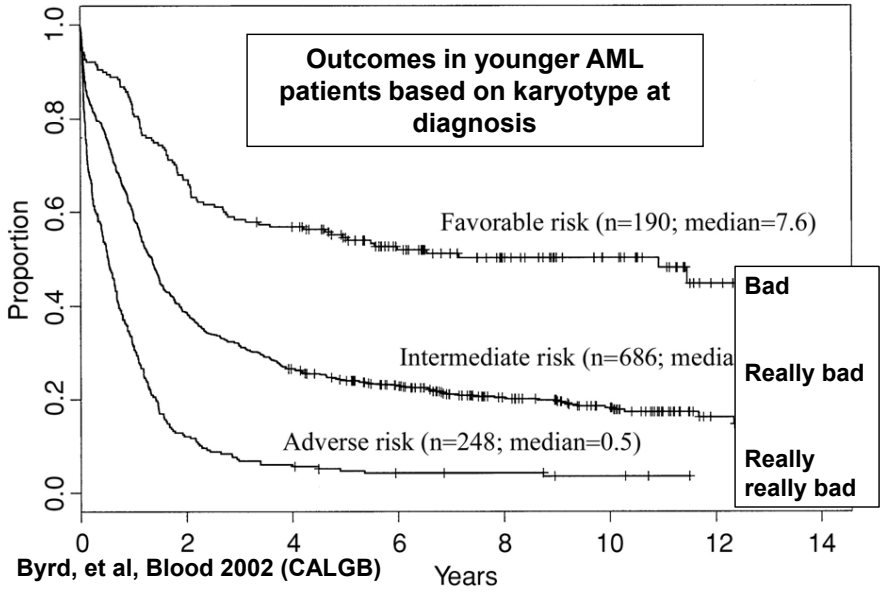

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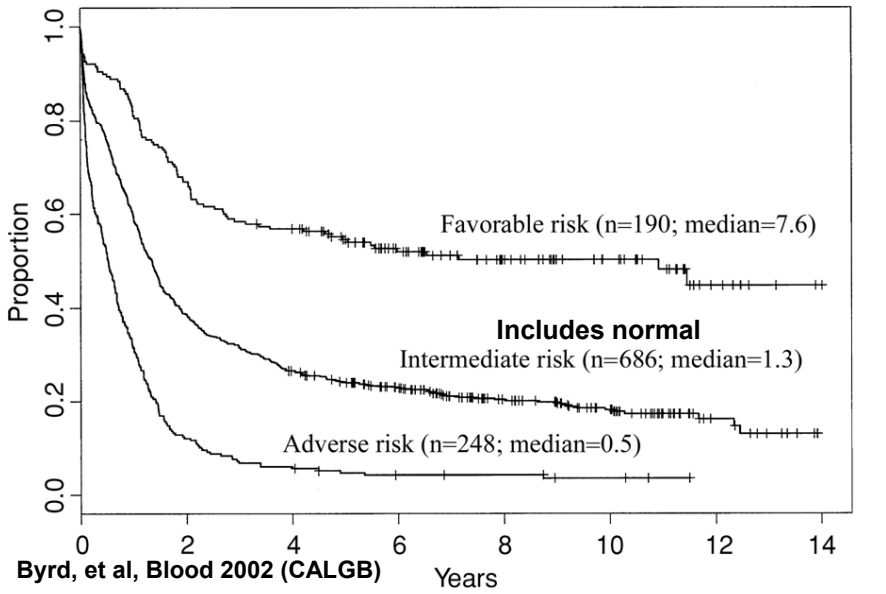
Outcomes in younger AML patients based on karyotype at diagnosis



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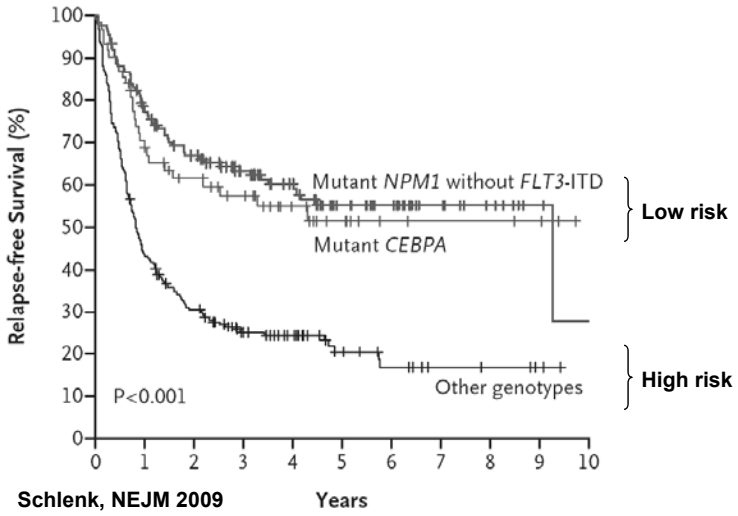
Impact of most common mutations on survival in cytogenetically normal AML



Schlenk, NEJM 2009

Unfortunately, the problem of assigning risk in AML is getting more and more complex…

nature Vol 456|6 November 2008|doi:10.1038/nature07485

ARTICLES nature

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley[1,2,3,4]*, Elaine R. Mardis[2,3]*, Li Ding[2,3], Bob Fulton[3], Michael D. McLellan[3], Ken Chen[3], David Dooling[3], Brian H. Dunford-Shore[3], Sean McGrath[3], Matthew Hickenbotham[3], Lisa Cook[3], Rachel Abbott[3], David E. Larson[3], Dan C. Koboldt[3], Craig Pohl[3], Scott Smith[3], Amy Hawkins[3], Scott Abbott[3], Devin Locke[3], LaDeana W. Hillier[3,8], Tracie Miner[3], Lucinda Fulton[3], Vincent Magrini[2,3], Todd Wylie[3], Jarret Glasscock[3], Joshua Conyers[3], Nathan Sander[3], Xiaoqi Shi[3], John R. Osborne[3], Patrick Minx[3], David Gordon[8], Asif Chinwalla[3], Yu Zhao[1], Rhonda E. Ries[1], Jacqueline E. Payton[5], Peter Westervelt[1,4], Michael H. Tomasson[1,4], Mark Watson[3,4,5], Jack Baty[6], Jennifer Ivanovich[4,7], Sharon Heath[1,4], William D. Shannon[1,4], Rakesh Nagarajan[4,5], Matthew J. Walter[1,4], Daniel C. Link[1,4], Timothy A. Graubert[1,4], John F. DiPersio[1,4] & Richard K. Wilson[2,3,4]

Sequencing the AML genome

- **Using next generation technology and the apparatus previously harnessed for the Human Genome Project, the authors sequenced the entire genome in two ways from the same AML patient, examining both**
  1) **leukemia cells**
  2) **normal germline cells (skin)**

- **By comparing the two results, they found 10 genes that were mutated in the leukemia cells and normal in the skin cells. 8 of these genes had never before been found to be associated with leukemia.**

10 genes were mutated in the patient's AML cells but were normal in skin

Gene	Type of mutation	Mutations in other AML cases
* *CDH24*	Nonsense	**0/187**
* *SLC15A1*	Nonsense	0/187
**KNDC1*	Missense	0/187
**PTPRT*	Missense	0/187
**GRINL1B*	Missense	0/187
**GPR123*	Missense	0/187
**EB12*	Missense	0/187
* *PCLKC*	Missense	0/187
FLT3	Indel	51/185
NPM1	Indel	43/180

*** in pathway known to be associated with cancer pathogenesis**

*** in pathway that suggests potential mechanism in cancer pathogenesis**

Sequencing the genome

- Is it practical?
  - First case took 8 months, second case only 8 weeks
  - Will every AML patient be fully sequenced in 10 years?
    - Now it takes only a week, costs $ 35K
- What is the function of the mutated genes?
  - Of the 8 new genes, 4 affect well described pathways related to cancer pathogenesis
- Role of epigenetics?
  - Can metabolic pathways important in leukemogenesis be disrupted even if the genes involved are NOT mutated?
  - Does screening for mutations actually tell the whole story?...
- Technology still advancing--authors went back with better DNA coverage and found
  - DNMT3a mutation, which is recurring, with negative prognosis

Is there an Achilles heel of AML? No…

There will not be a single unifying anomaly to explain "why" AML (cancer??) developed,

or to predict outcome,

or that can be targeted in all (or even most) AML patients.

Personalized medicine truly takes the stage.

Cytogenetic/Molecular Risk Stratification for 2011

Risk group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I	All other combinations of *FLT3* and *NPM1*
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	-5 or del(5q); -7; abnl(17p);
	complex karyotype; monosomal karyotype

Adapted from Dohner et al, Blood 2010

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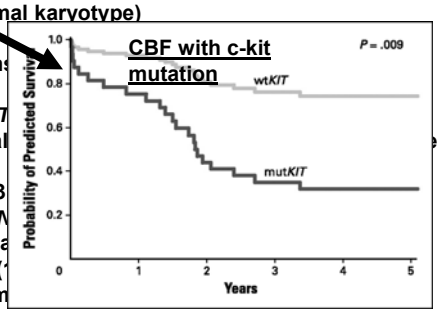

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If you were the patient, what would sound better?

"History tells us that you are likely to do poorly with conventional treatment, but its all we have so let's give it a try anyway."

"History tells us that you are likely to do poorly with conventional treatment, so we have altered your treatment plan to mitigate this risk and improve your chances."

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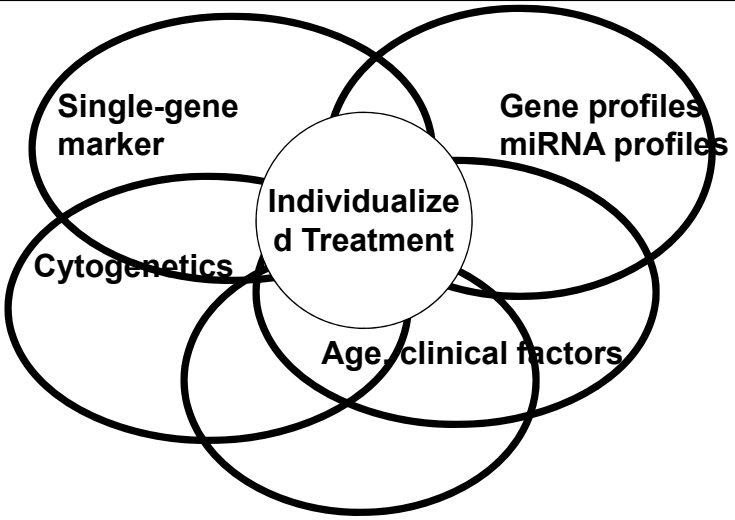
How do we integrate the prognostic/ predictive information in AML?



How can we use prognostic information more effectively in 2010?

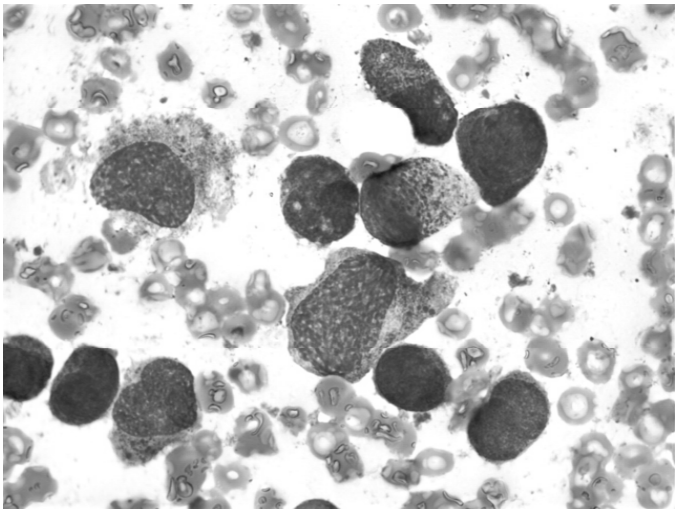
Use cytogenetics and molecular risk to guide current therapy

- Chemotherapy selection
- Role of allogeneic stem cell transplantation for patients in first complete remission (CR1)
- Targeted therapies?

Case 3 and 4

- #3--79yo female, WBC 1.0, Hgb 9.7, Plt 97
- #4--79 yo female, WBC 1.2, Hgb 10.7, Plt 141
- Predominant symptom in both is fatigue
- Morphologically, both patients appear to have acute promyelocytic leukemia (APL)

Acute Promyelocytic Leukemia



Peter Maslak, ASH Image Bank 2011; 2011-2043

Case 3 and 4

- Case #3—t(15;17) negative and molecular studies negative, 5 year survival rate for AML>60 years close to 0
  - Patient elected hospice care
- Case #4—t(15;17) +, 40-50% cured
  - Patient elected retinoic acid/arsenic based induction

Treating the "older" AML patient

- Who should be treated "intensively"? Subsets who are likely to do better than most…
  - Core binding factor AML , APL
  - NPM1 mutations +
- Consider alternative (experimental) Rx if
  - Comorbid disease
  - Age >70
  - Borderline functional status
  - Cytogenetic adverse risk

Case 5

- At the urging of her husband who is "worried about her cholesterol" (and progressive fatigue…), a 63-year-old mutual fund manager presents to her internist for her first evaluation in 5 years.
- Review of systems: Fatigue, worsening for about a year, otherwise negative
- Past medical history: In good health otherwise, no significant past medical history

Exam: pallor, slight decreased BS, mild systolic murmur,

Laboratory findings:

Hgb:	9.2g/dL with an MCV of 101
WBC:	2.3 with an ANC of 690/uL
Platelet:	64,000/uL
Retic count:	0.3%
Peripheral blasts:	None
B12:	Normal
RBC folate:	Normal
Erythropoietin:	254

Marrow Biopsy: Trilineage dysplasia, 11% blasts

Cytogenetics: 4 abnormalities including monosomy 7

Myelodysplastic syndromes (MDS)

MDS: Characteristics

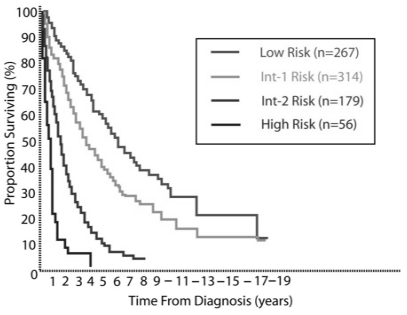
- Clonal bone marrow disorders
- Typically with hypercellular marrow, low counts, and cell function abnormalities
  - "ineffective erythropoiesis"
- Natural history is highly variable
  - Typical presentation is older patient with fatigue and anemia, often patients are treated inappropriately for iron deficiency initially
  - Can progress to AML, often fatal even if it does not due to high risk of infection over time
- No cure except for allogeneic transplantation, but most patients are elderly and not candidates

International Prognostic Scoring System (IPSS) for MDS

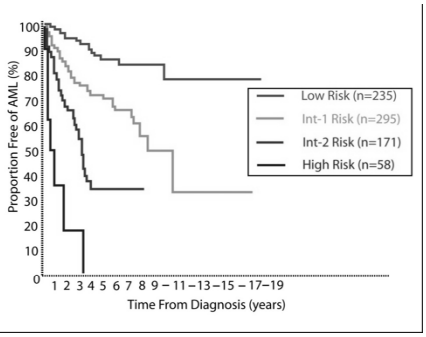
Cytopenia	**Neutrophils**	**<1,800/ µl**
	Hemoglobin	**<10 g/dl**
	Platelets	**<100,000/ µl**
Cytogenetics		
Good:	**Normal or -5q, -Y, -20q as sole abnormalities**	
Intermediate:	**Other abnormalities**	
Poor:	**-7, complex (>3 abnormalities)**	

Greenberg P, et al. *Blood.* 1997:89(6):2079-88.

IPSS Predicts Overall Survival and AML Evolution In *De Novo* MDS



Death



AML

(n=816)

Greenberg P, et al. *Blood.* 1997:89(6):2079-88.

MDS: Median Survival by IPSS and Age

Age, yr (n)	Median Survival, yr			
	Low	Int-1	Int-2	High
All ages (n = 816)	5.7	3.5	1.2	0.4
≤60 (n = 205)	11.8	5.2	1.8	0.3
>60 (n = 611)	4.8	2.7	1.1	0.5

- From diagnosis in untreated patients

Adapted from Greenberg P, et al. *Blood.* 1997:89(6):2079-88.

Case 5
63yo with "high risk" MDS

- Early initiation of hypomethylating agent
  - Azacitidine or decitabine
- Screen for HLA matched donor at diagnosis (sibs, unrelated volunteer adults, cord blood?)
  - Nonmyeloablative conditioning given age
  - Eligibility depends on preserved organ function, response to hypomethylating agent therapy

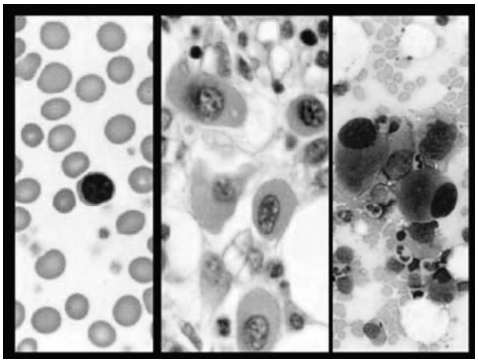
Case 6

- 56 yo female with fatigue. Recently diagnosed with early stage breast cancer.
  - WBC 5.4, Hgb 9.4, Plt 555
- Marrow evaluation shows atypical megakaryotypes, dyserythropoiesis
  - Cytogenetics: partial deletion of 5q

MDS with partial deletion of 5q:

A unique clinical entity serves as a window to the future for "personallized" therapy?

MDS with del (5q)



Vardiman, J. W ASH Image Bank 2001;2001:100197

-Typically a more indolent disease course than most MDS patients

-Anemia, high platelet count; typical marrow findings include normal %blasts and hypolobulated mega-karyocytes

-Unique clinical activity of the drug lenalidomide in del (5q) MDS

Lenalidomide responses in lower risk* MDS patients with or without del (5q)

Lower risk	RBC TI	Time to response	Duration	Cytogenetic CR
del (5q)	67%	4.6 weeks	115 weeks	45%
Non-del (5q)	26%	4.8 weeks	41 weeks	<10%

*lower risk refers to IPSS risk group of low or int-1

List et al, 2006; Raza et al, 2008

Aplastic anemia (AA)

- Pancytopenia and aplastic marrow
  - Commonly present with anemia and hemorrhage
- Typically an acquired autoimmune disorder
- Bimodal distribution with peaks in children and young adults, again in age>60

Aplastic anemia (AA)

Treatment selection

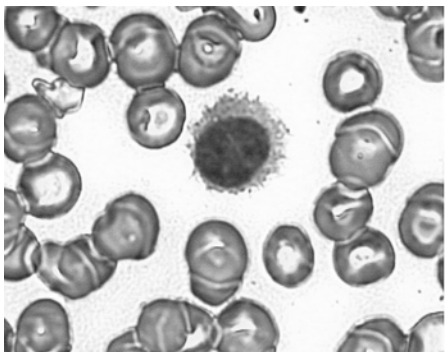
- For younger patients or for severe/very severe AA (defined by lower ANC), first line therapy is BMT if HLA identical sib available
  - 10 year survival 75-85%
- Immunosuppressive therapy
  - ATG, Cyclosporine
  - RR 60-80%, 5 year event free survival 50%

Case 7

- 50 year old male presents for his usual Red Cross donation appointment and is told to see his doctor instead, due to anemia
- WBC 1.1, Hgb 12.5, Plt 80
- Palpable spleen

Hairy cell leukemia

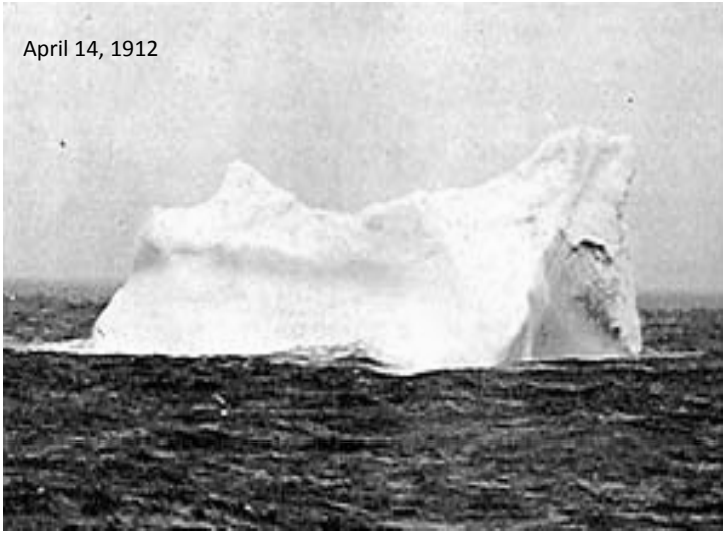
- "Reticuloendotheliosis", Bouroncle at OSU in 1958
- Hypocellular marrow, common misdiagnosis of AA
- Remission rates with cladribine or pentostatin of 85-91%
- Case 7 patient treated with pentostatin and still has normal CBC 7 years later



Peter Maslak, ASH Image Bank 2011; 2011-1503

BRAF mutation in HCL Tiacci, NEJM this week

- Massive parallel whole exon sequencing of a patient with hairy cell leukemia (HCL)
  - 5 mutations identified, one was a BRAF mutation known to be oncogenic in other tumors
  - Next, authors looked for the mutation in 47 additional HCL patients
    - All 47 had the same mutation
    - And none of the 195 "control" patients with B-cell NHL had it



Does this matter for my primary care practice?

Conclusions

- **The heterogeneity of diagnoses in "blood disorders that cause cytopenias" suggests that early hematologic expert consultation is in order**
- **These disorders provide a glimpse of the future of medicine and "personalized therapy"**