Clinical Use of Karyotype and Molecular Markers In Curing Acute Myeloid Leukemia

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THE OHIO STATE UNIVERSITY

NCI CCC
A Comprehensive Cancer Center Designated by the National Cancer Institute

Adult Acute Leukemia was essentially incurable 45 years ago

- Survey of hematologists worldwide for data on any patients with acute leukemia who had survived ≥ 5 years

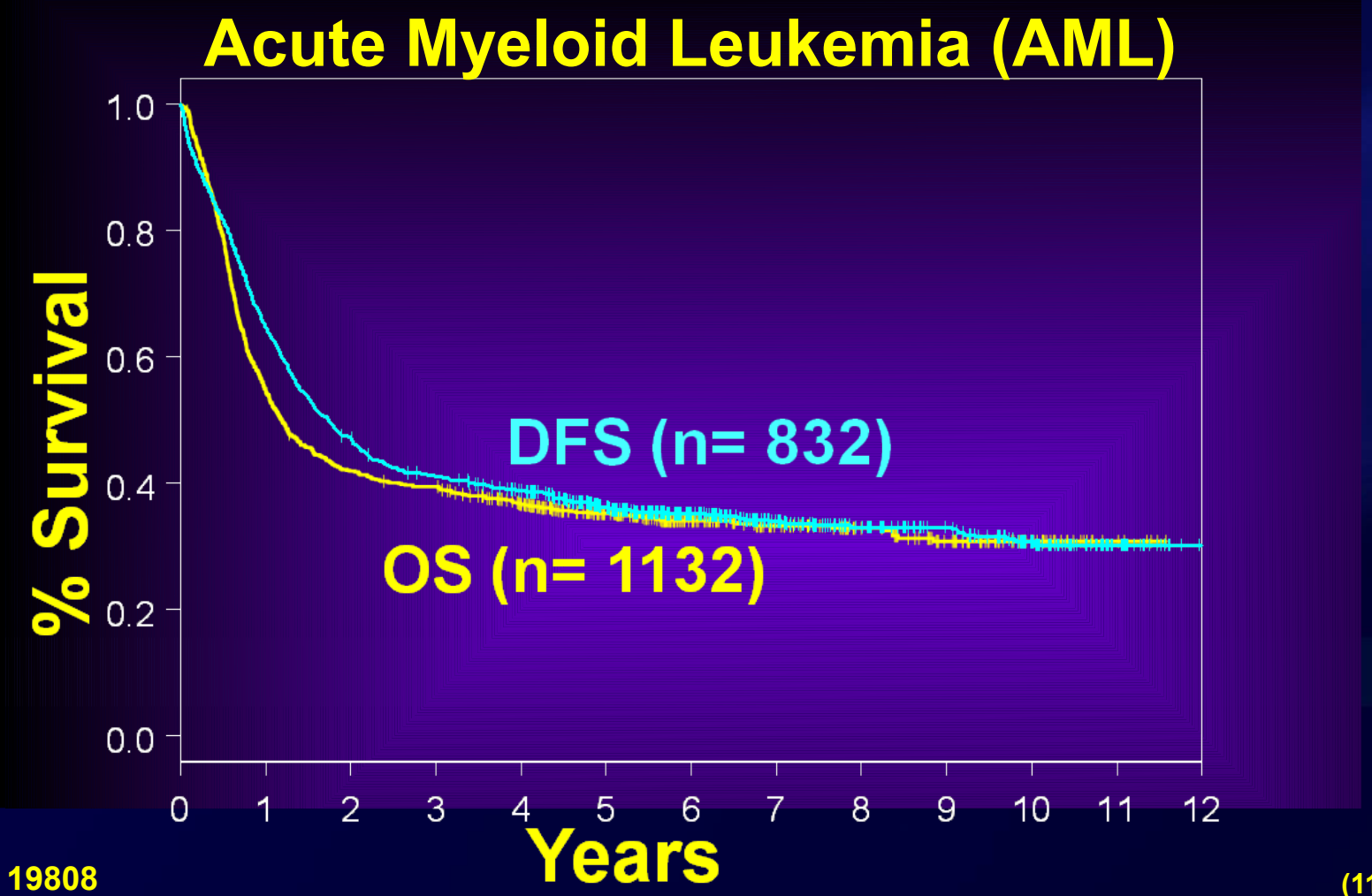
(Burchenal JH, Murphy ML. Cancer Res 25:1491-4,1965)

  - 18 adults found who survived Í 5 yrs
  - 6 were still alive without leukemia for 5-9 years

2011: Long-term Survivors of Adult Acute Myeloid Leukemia <60 years

36% (n=421) alive ≥ 5 years

36% (n=303) continuously disease-free ≥ 5 years

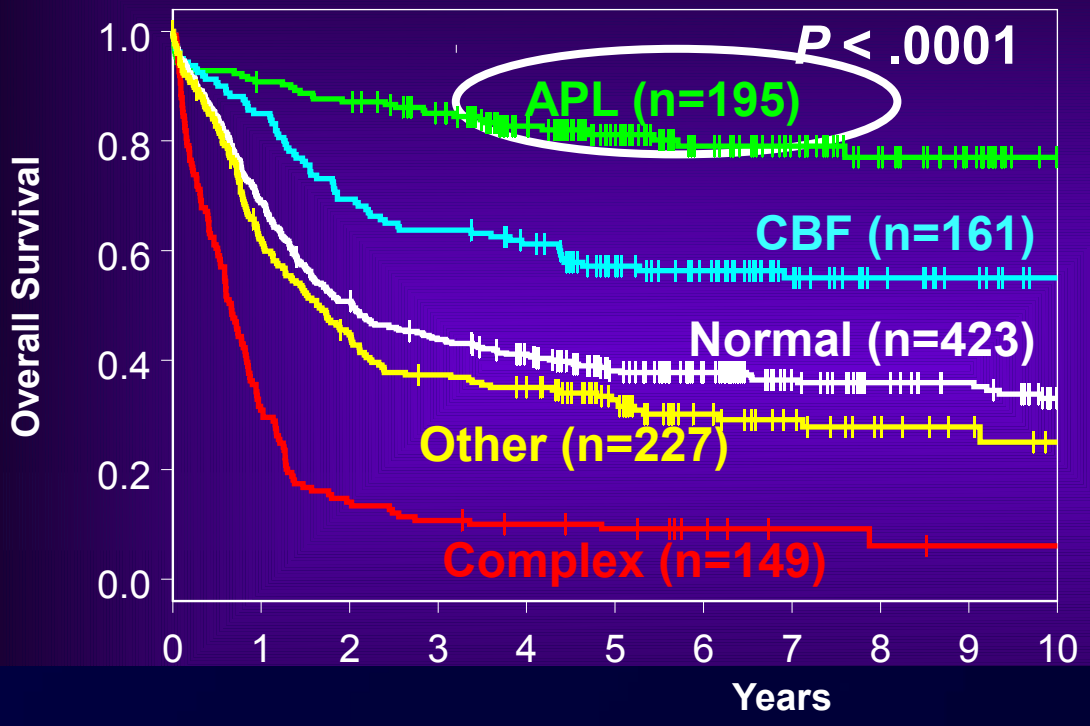


CALGB 9621, 19808

(11/3/10)

Adult Acute Myeloid Leukemia 2011

- By selecting correct therapy, in some cytogenetic types of AML [e.g., acute promyelocytic leukemia (APL) with t(15;17)(q22;q12)] > 80% of adults are now cured as shown by current CALGB data



CALGB 9621/19808/9191(ATRA)/9710 10/2010 update N=1155

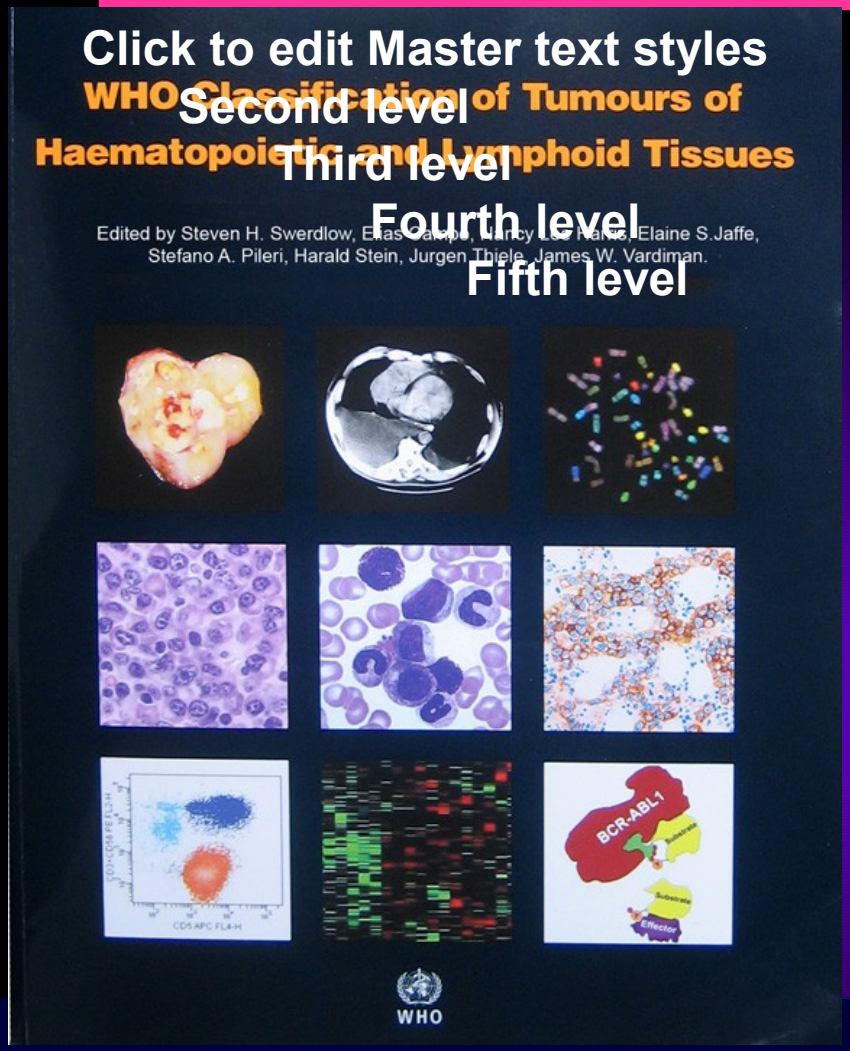
Adult Acute Myeloid Leukemia 2011

- AML is now a curable disease
  - 35-40% of *de novo* AML <60 years are cured
  - In older patients (≥60 years), 5-15% are cured
- Most important factors used to select therapy are karyotype and molecular findings
- Therapies are now being developed that target the genetic aberrations

Karyotype and molecular findings most important factors for selecting therapy in AML in 2011

- Allow improved diagnosis, prognosis and therapy
- Translation to clinic increasing
  - Current (2008) World Health Organization (WHO) classification for *de novo* AML primarily based on genetic findings
  - Inclusion in clinical practice guidelines (ELN, NCCN)
  - Routine use of molecularly targeted therapies for APL
  - Clinical trials targeting patients with mutant tyrosine kinases or epigenetic abnormalities on-going

2008 WHO Classification of AML increasingly based on cytogenetics/molecular genetics



- 2001 WHO first used genetic findings for diagnosis
- First time *de novo* AML first classified by specific cytogenetic/molecular findings
- For some groups diagnosis AML regardless of % blasts
- 2008 WHO increases % of AML genetically classified from ~30% to >75%

IARC: Lyon 2008

WHO 2008: AML with recurrent genetic abnormalities

- Acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARA*
- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*

CBF*

- ----------
  - Above AML regardless of blast count

- AML with t(9;11)(p22;q23); *MLLT3-MLL*
- AML with t(6;9)(p23;q34); *DEK-NUP214***
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1***
- AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1***
- *Provisional entity: AML with mutated NPM1***
- *Provisional entity: AML with mutated CEBPA***

*CBF = Core-binding factor AML

**New group added since WHO 2001

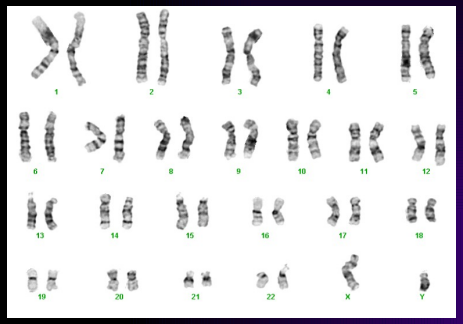
Clinical Practice Guidelines: New Recommended Standardized Reporting for Correlation of Cytogenetic & Molecular Genetic Data with Clinical Data in AML*

Genetic 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	Mutated *NPM1* and *FLT3*-ITD (normal karyotype) Wild-type *NPM1* and *FLT3*-ITD (normal karyotype) Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
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;11q23); *MLL* rearranged -5 or del(5q);-7;abn(17p); complex karyotype (≥3)

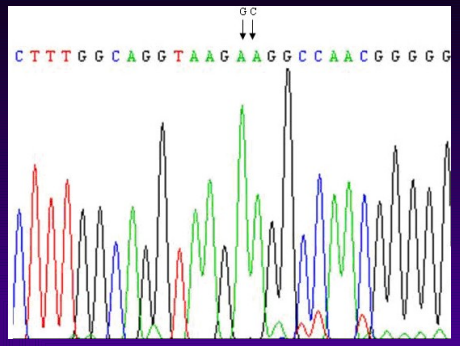
*International expert panel recommendations on behalf of ELN (Blood 115:453-74, 2010. Epub 2009 Oct 30)

Integration of cytogenetic and molecular information leads to identification of novel molecular subsets of AML allowing individualized therapy

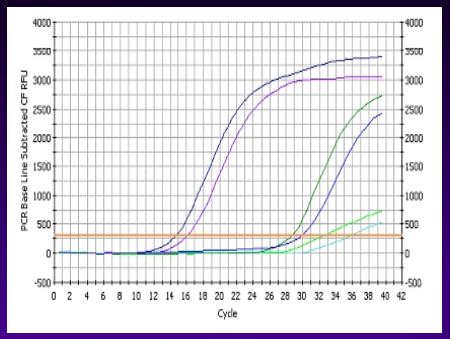
Cytogenetics



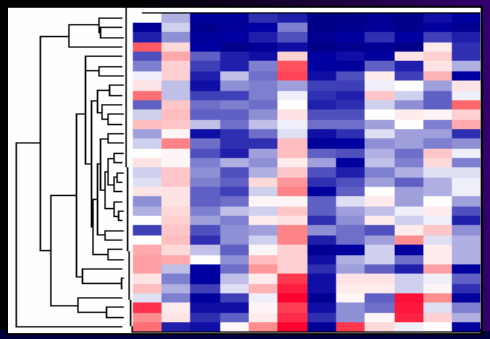
Gene Mutations



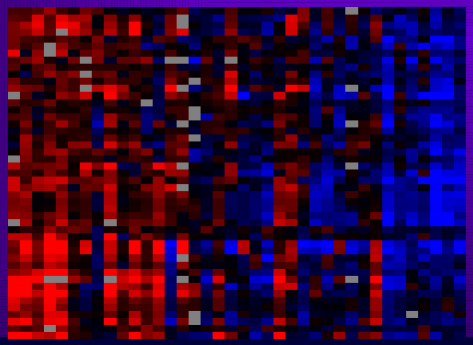
Gene Expression



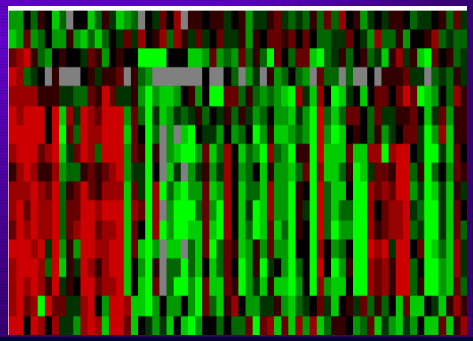
SNP Profiling



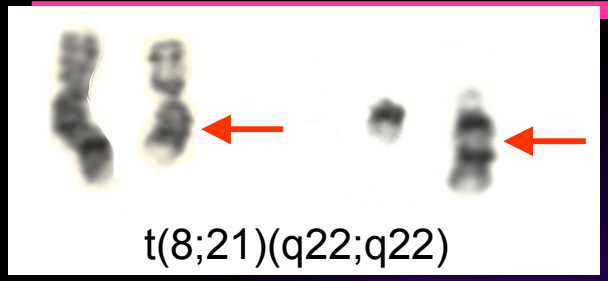
Gene profiling



miR Profiling

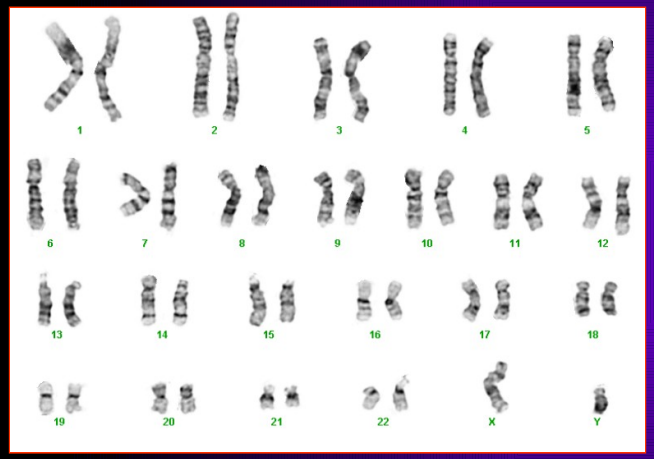


Importance of Cytogenetic and Molecular Heterogeneity in Curing Adult AML


t(8;21)(q22;q22)

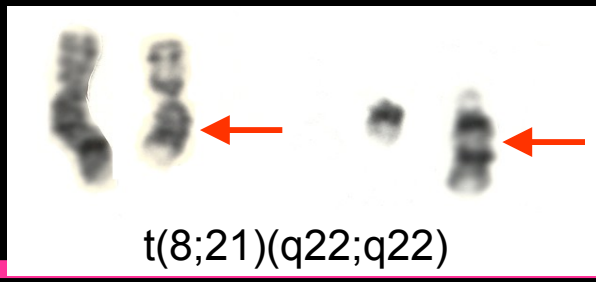

inv(16)(p13q22)

Core-binding factor (CBF) AML



Cytogenetically normal AML (CN-AML)

CBF AML in 2011


t(8;21)(q22;q22)


inv(16)(p13q22)

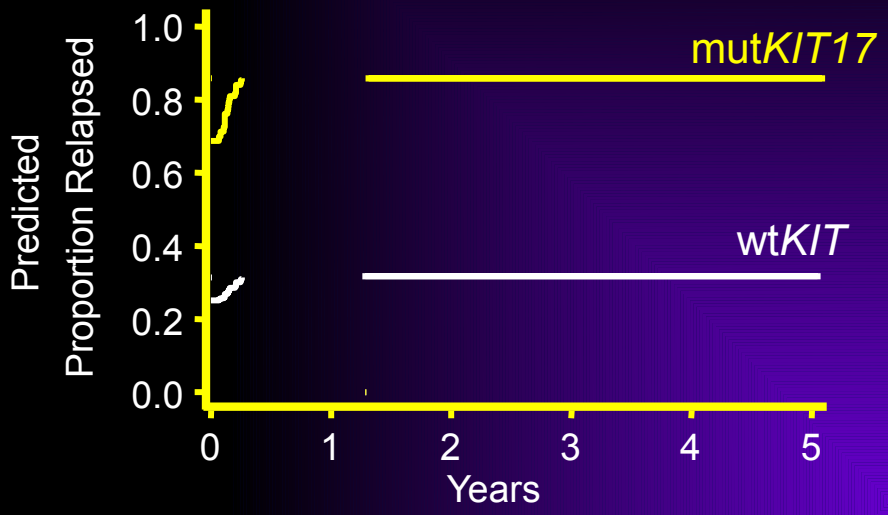
- 13% of adult *de novo* (primary) AML
- t(8;21)(q22;q22) and inv(16)(p13q22) **usually treated alike and differently from other AML**
- With high-dose cytarabine cure increased from <10-25% to 55-60% of patients (→**ELN Favorable Genetic Group**)
- However, even among adults <60 yrs, 40% are not cured
- Can molecular markers help us increase cure rate?
  - The *KIT* gene may help

KIT in CBF AML

- KIT protein is a cell surface receptor (CD117) with tyrosine kinase (TK) activity that promotes cell proliferation and survival
- *KIT* gene overexpressed in CBF AML
- Activating mutations in *KIT* gene occur in CBF AML
- *KIT* mutations activate a complex protein-microRNA network (*miR-29b*/Sp1/NFkappaB/HDAC) that upregulates mutated *KIT* expression and increases TK activity (Marcucci/Bloomfield group Cancer Cell 17:333-47, 2010)
- We showed the adverse prognostic impact of *KIT* mutations in adult *de novo* CBF AML treated on CALGB protocols and assigned to post-remission high-dose cytarabine (Paschka et al. Plenary Session ASCO 2006; JCO 24:3904-11, 2006)

CBF AML: Mutations in *KIT* exon 17 independently predict higher relapse risk

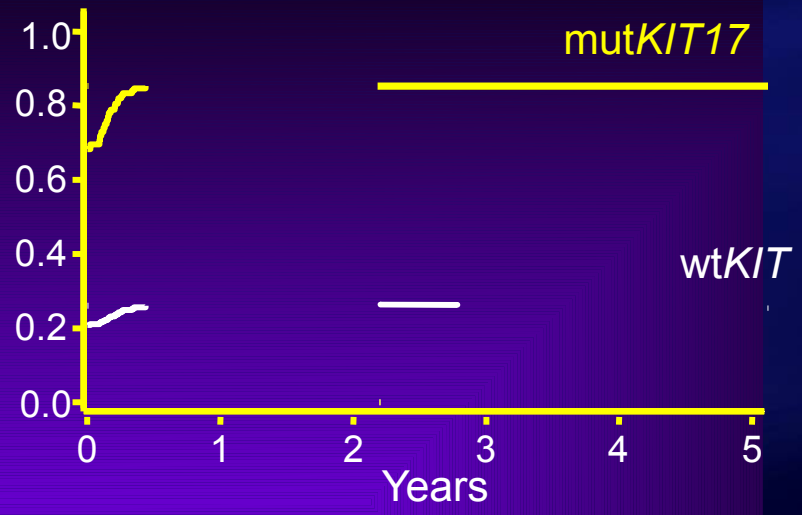
Frequency of *KIT* mutations in t(8;21): 22%



Multivariable analysis HR 5.2, *P* = .005*

***Adjusted for % blood blasts**

Frequency of *KIT* mutations in inv(16): 30%



Multivariable analysis HR 6.4, *P* < .001**

****Adjusted for sex**

ASCO Plenary Session 2006; Paschka et al. JCO 24:3904-11, 2006

Clinical Guidelines: NCCN CBF-AML Recommended Therapy*

Better Risk	5-yr CIR**	Intermediate Risk	5-yr CIR
KITwt inv(16)	29%	*KITmut* inv(16)	56%
KITwt t(8;21)	36%	*KITmut* t(8;21)	70%

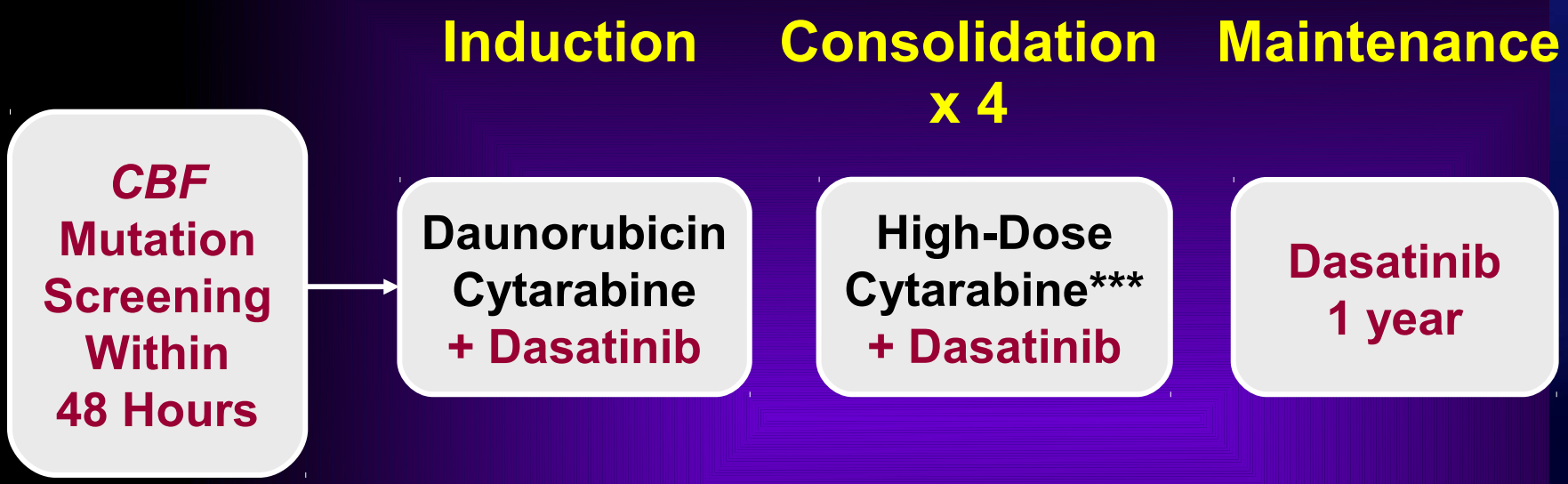
Postremission Therapy

Better Risk	Intermediate Risk
High-dose cytarabine (HiDAC) x 3-4 (1***) → maintenance (2B) or HiDAC x 1-2 → autologous stem cell transplant (HSCT) (2B) or Clinical trial (2A)	Matched sibling or unrelated donor HSCT or HiDAC x 1-2 → autologous HSCT (2A) or HiDAC x 3-4 (2A) or Clinical trial (2A)

*version 2.2011 ** JCO 24:3904-11, 2006 ***NCCN category of evidence & consensus

CIR=cumulative incidence of relapse

Phase II Study of Chemotherapy + the Tyrosine Kinase Inhibitor (TKI) Dasatinib in Patients with Newly Diagnosed Core Binding Factor (CBF) AML: AMLSG 11-08 (Germany)* CALGB 10801**



All adult patients eligible for intensive therapy, no upper age limit

Cytarabine: 18-60yrs: 3g/m2, q12hr, d1-3; >60yrs: 1g/m2, q12hr, d1-3

*PI: H. Döhner, AMLSG [ClinicalTrials.gov Identifier: NCT00850382]

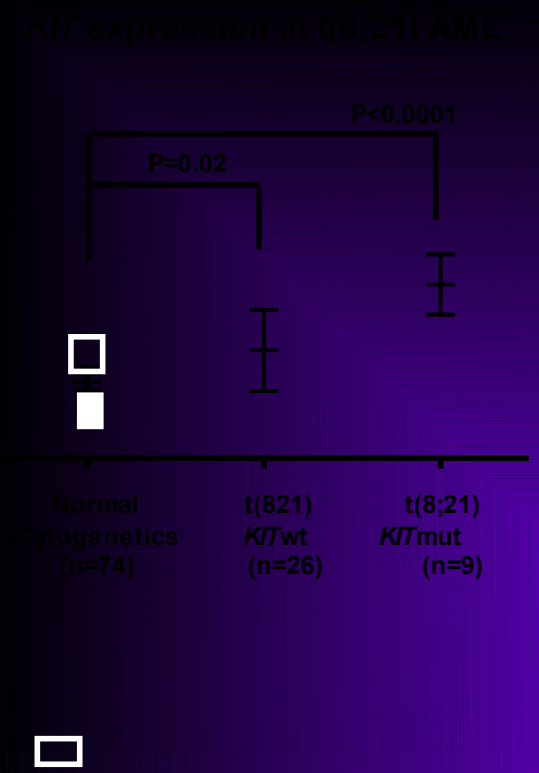
**PI: G. Marcucci, CALGB

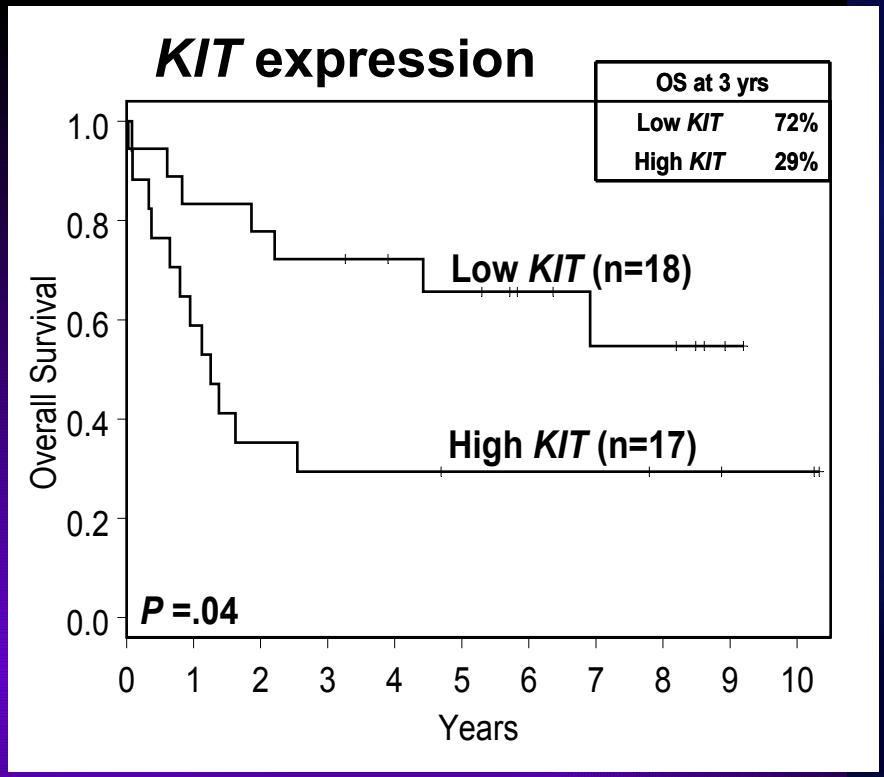
STUDY AML GROUP CALGB

Shortcomings of TKI therapies in KIT-driven AML

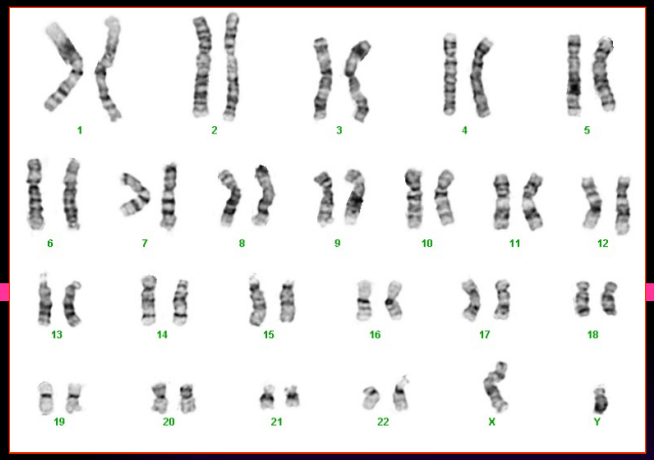
- Clinical response to tyrosine kinase inhibitors depends mostly on the nature of *KIT* mutations
- The type of the *KIT* mutation needs to be identified at the time of initial diagnosis
- Acquisition of secondary *KIT* mutations is a mechanism of resistance to tyrosine kinase inhibitors
- A potential “one-fits-all” strategy is to suppress *KIT* expression

KIT expression in t(8;21) AML patients





(Liu/Marcucci/Bloomfield group Cancer Cell 17:333-47, 2010)

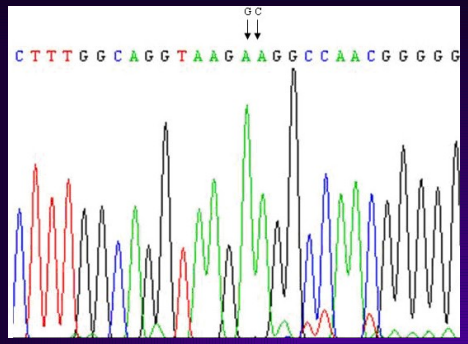


Cytogenetically Normal AML (CN-AML)

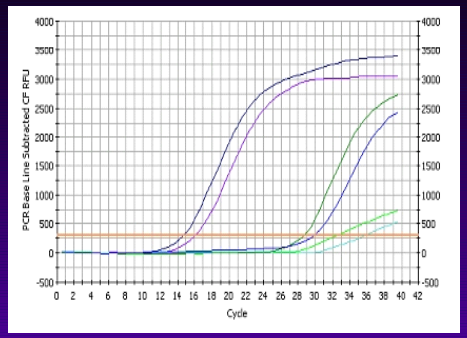
- 40-45% of adults with *de novo* AML are CN-AML
- Approximately 40% of CN-AML patients <60 yrs are cured with autologous hematopoietic stem cell transplantation (auto HSCT) or 3-4 cycles of high-dose cytarabine given in first complete remission (CR1)
- CN-AML is very heterogeneous molecularly

How might we use molecular information to identify the 40% of CN-AML patients cured with current therapy and develop better treatment for the rest?

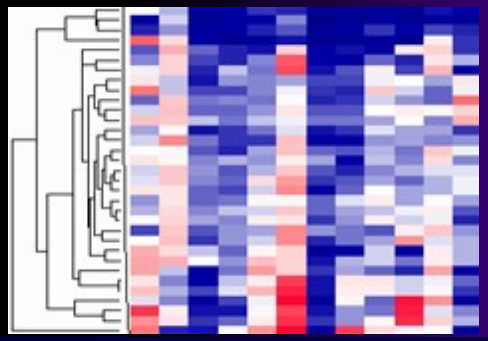
Gene Mutations



Gene Expression



SNP Profiling



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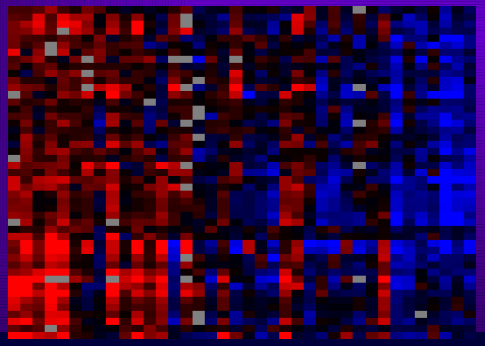
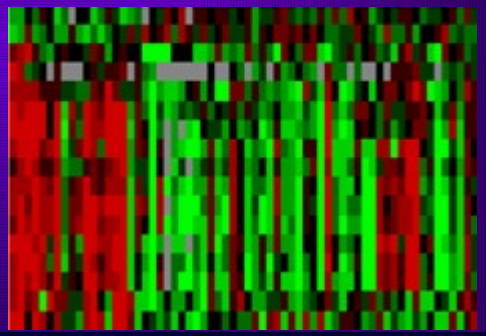


miR Profiling



Prognostic Single-gene Markers in *de novo* Adult CN-AML*

Gene Symbol	Location	Frequency	Prognostic Impact
MLL-PTD**	11q23	5-10%	Adverse→Neutral
FLT3-ITD***	13q12	25-35%	Adverse
CEBPA mutations***	19q13.1	10-20%	Favorable
NPM1 mutations***	5q35	45-65%	+/-Favorable
WT1 mutations	11p13	~10%	Adverse
FLT3-TKD	13q12	~10%	? Adverse
IDH mutations (*IDH1* & *IDH2*)	2q33.3/15q26.1	31%	+/- Adverse
TET2 mutations	4q24	23%	+/- Adverse
BAALC overexpression	8q22.3		Adverse
ERG overexpression	21q22.3		Adverse
MN1 overexpression	22q21.1		Adverse
miR-181a overexpression	1q32.1 and 9q33.3		Favorable

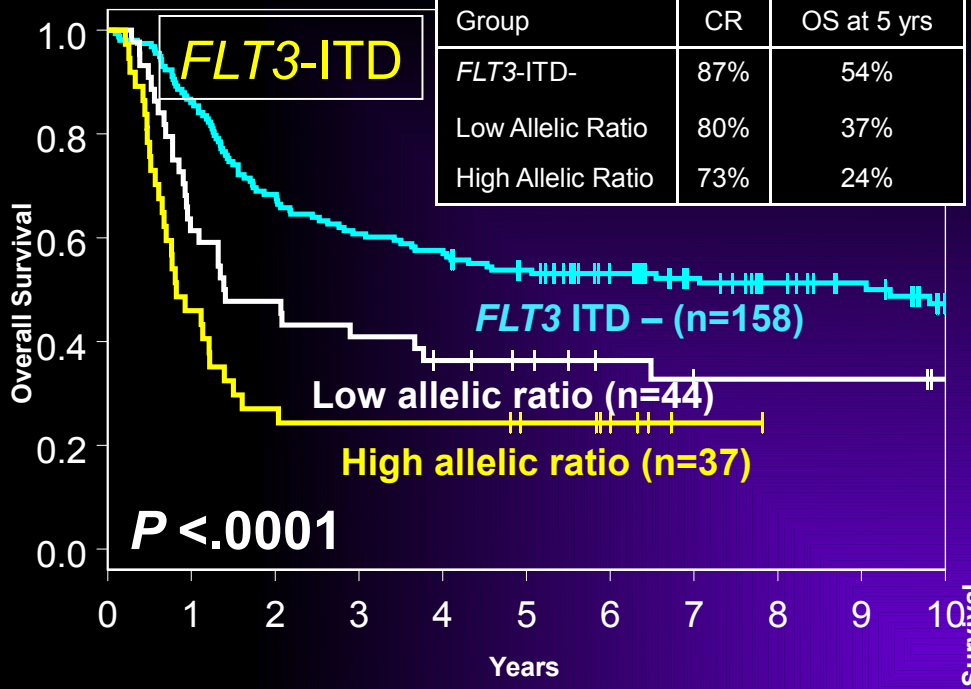
* Gene mutations reported in relatively large studies by more than one group

** First molecular prognostic marker in CN-AML

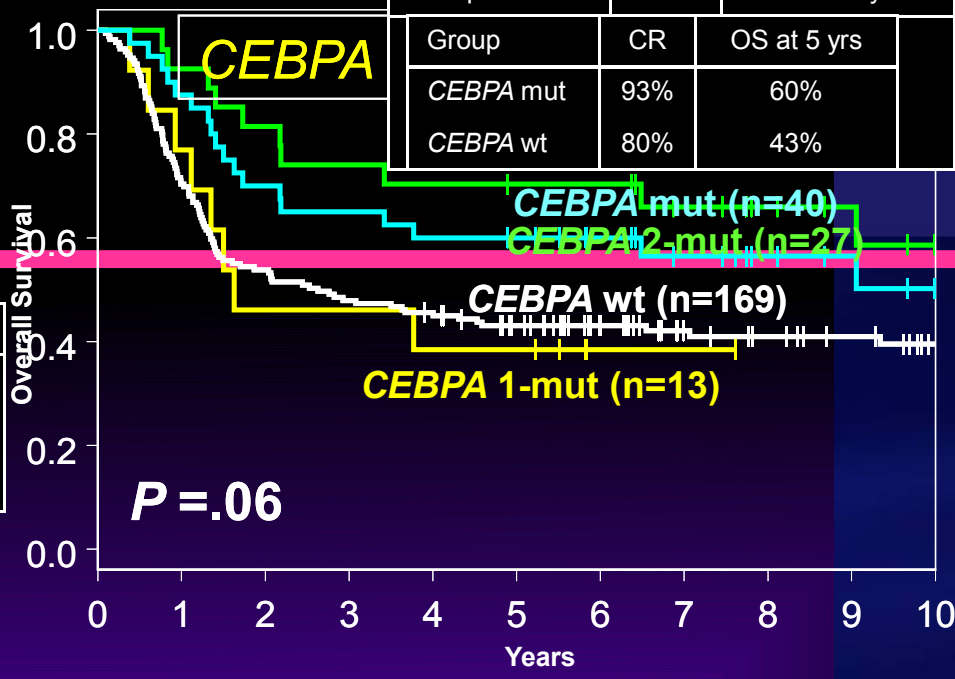
*** Recommended for study in CN-AML by WHO 2008, ELN 2009, NCCN 2011

Prognostic significance of *FLT3*-ITD, *CEBPA & NPM1* mutations as single markers in CN-AML (adults <60 yrs old*)

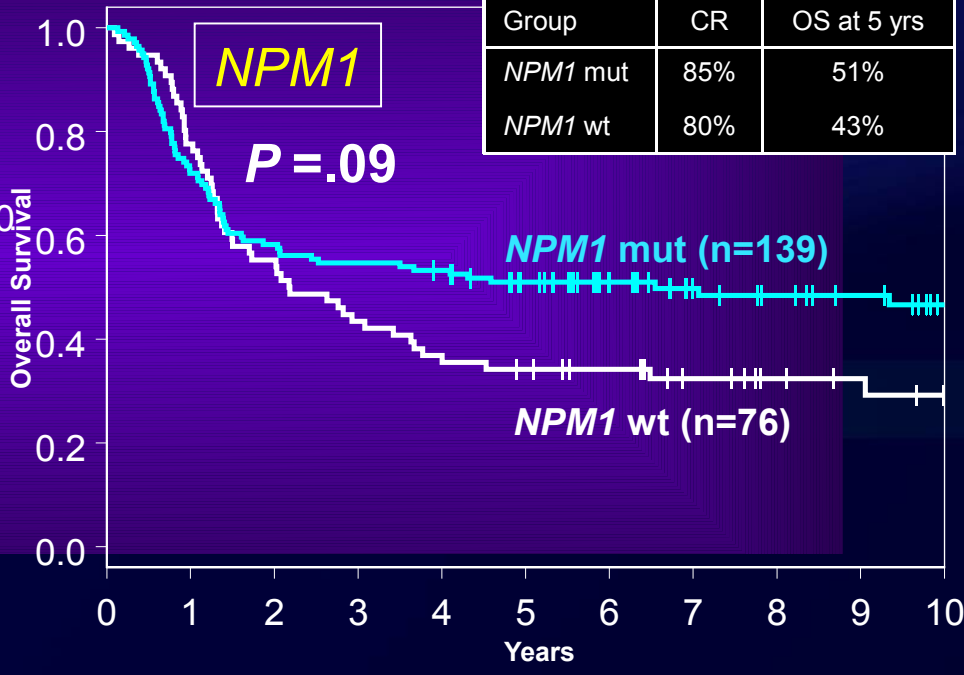
Group	CR	OS at 5 yrs
FLT3-ITD-	87%	54%
Low Allelic Ratio	80%	37%
High Allelic Ratio	73%	24%



Group`	CR	OS at 5 yrs
Group	CR	OS at 5 yrs
CEBPA mut	93%	60%
CEBPA wt	80%	43%



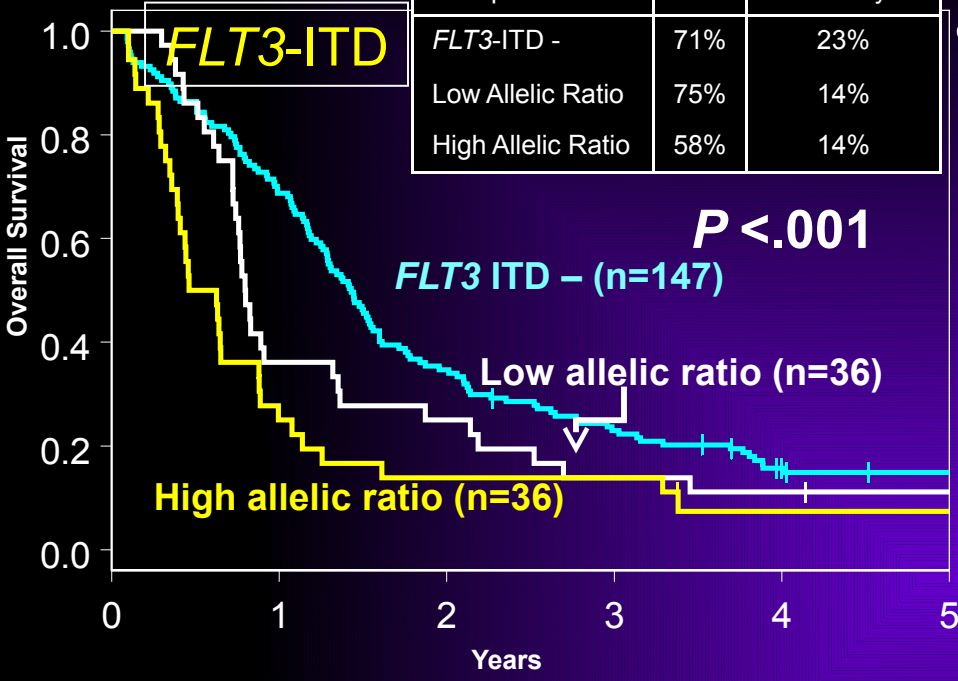
Group	CR	OS at 5 yrs
NPM1 mut	85%	51%
NPM1 wt	80%	43%

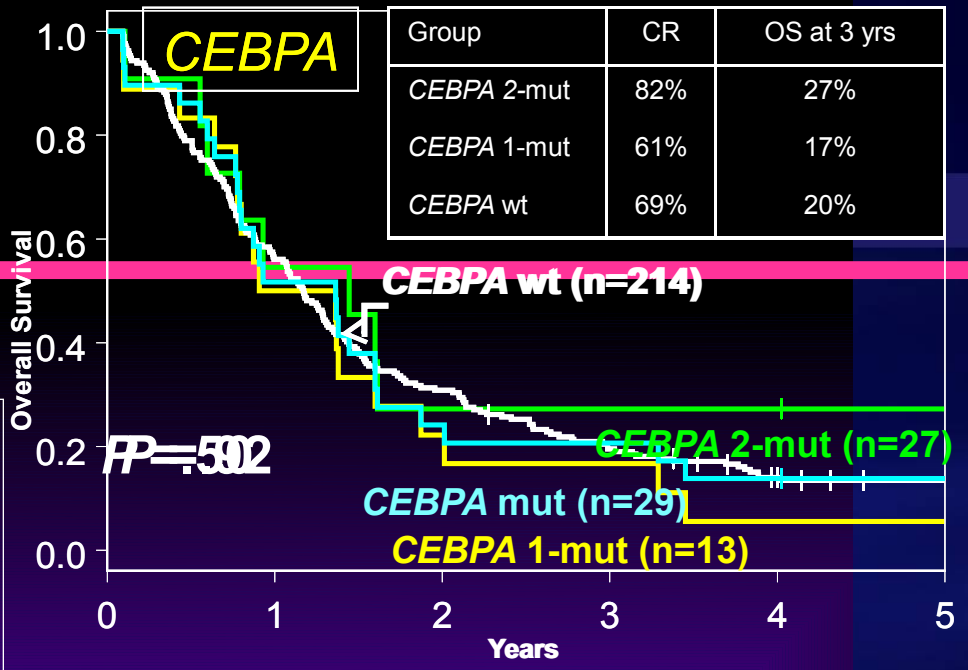


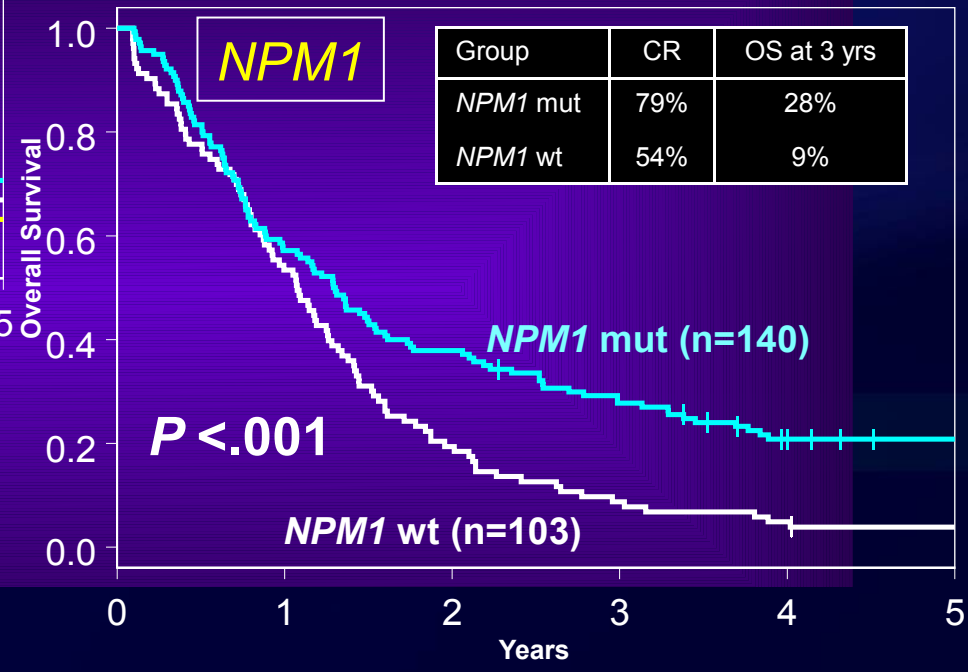
Whitman et al. Cancer Res 61:7233-9, 2001
Marcucci et al. JCO 26:5078-87, 2008
Paschka et al. Haematologica 92(suppl 1):145, 2007

*CALGB 9621, 19808 10/2010 update

Prognostic significance of *FLT3*-ITD, *CEBPA* & *NPM1* mutations as single markers in CN-AML(adults ≥ 60 yrs old**)





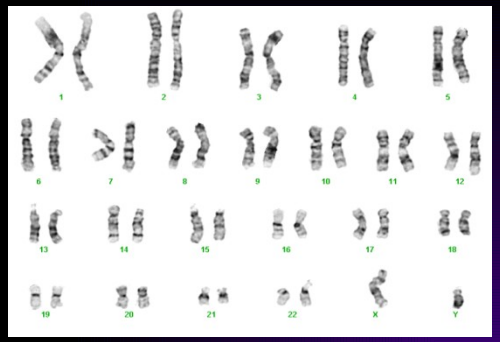


Whitman et al. Blood pre-published online 07/23/2010
Becker et al. Haematologica 95 (suppl 1) abstr 593, 2010
Becker et al. JCO 28:596-604, 2010

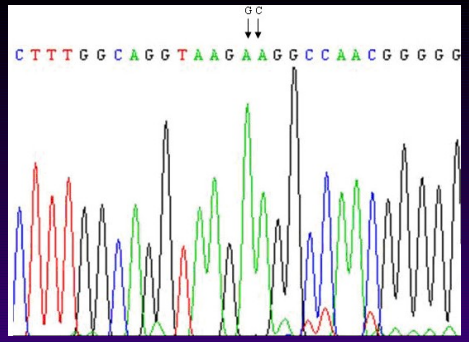
**CALGB 8525, 8923, 9420, 9720, 10201 update 10/2010

Integration of cytogenetic and molecular information leads to identification of novel targets in CN-AML

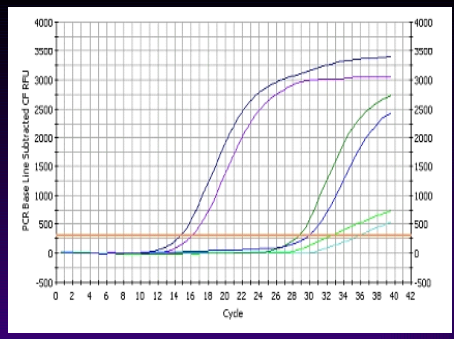
Cytogenetics



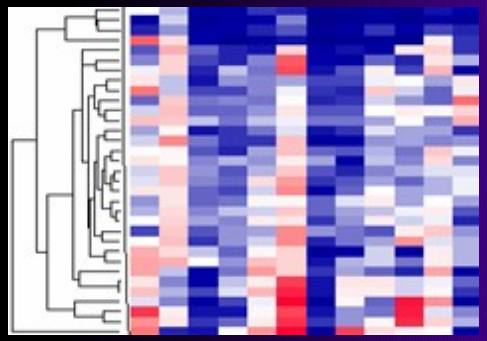
Gene Mutations



Gene Expression



SNP Profiling



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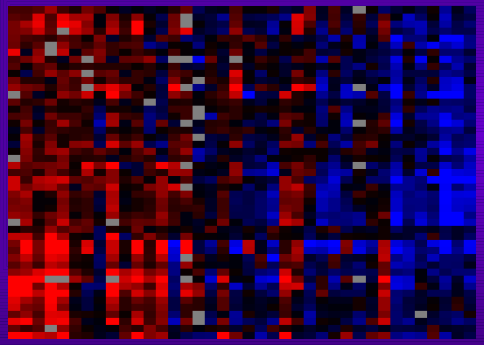
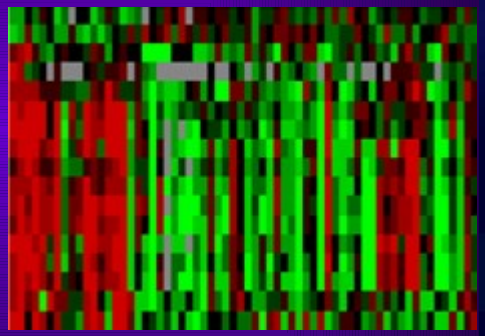
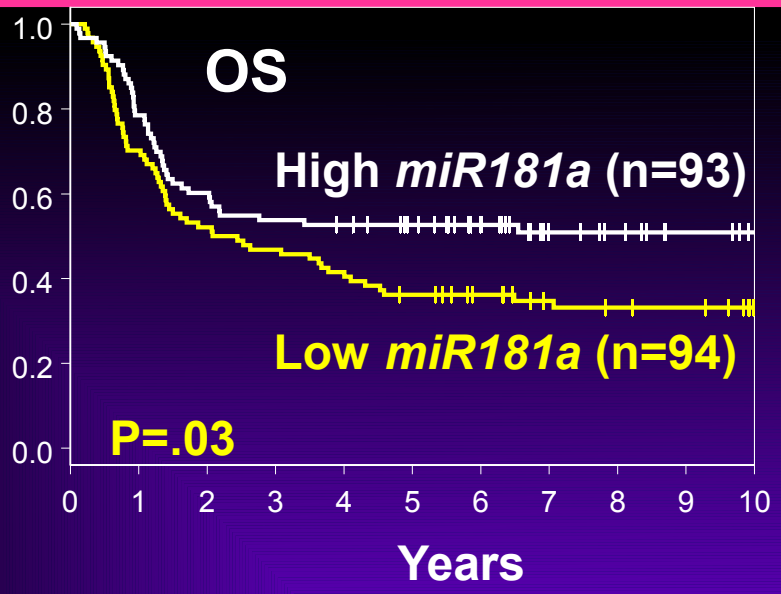


miR Profiling



Higher *miR-181a* expression is associated with higher CR rate, and longer DFS and OS in molecular high-risk (*FLT3*-ITD and/or *NPM1* wt) CN-AML*



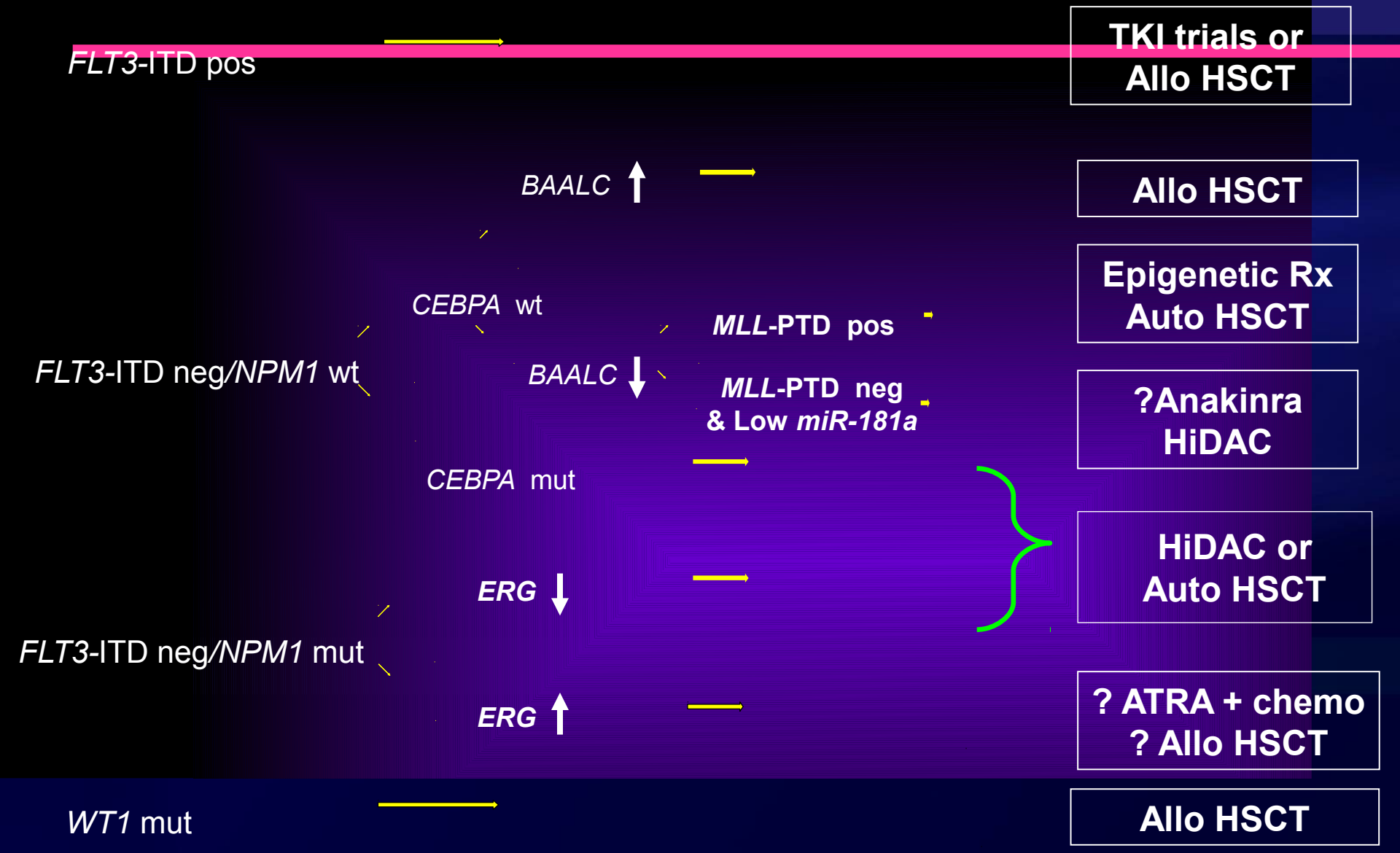
	CR	DFS	OS
Univariable analyses**	*P*=.009	*P*<.001	*P*<.001
Multivariable analyses***	OR: 1.6, *P*=.02	HR: 0.7, *P*=.02	HR: 0.7, *P*=.002

*CALGB 9621,19808 **continuous variable

Schwind et al. JCO 28:5257-64, 2010

***other molecular markers in model: DFS-*CEBPA*, *NPM1*, *FLT3*-ITD; OS-*CEBPA, NPM1, WT1*

Examples of stratification to molecular risk-adapted clinical trials for CN-AML



Curing Adult CN-AML in 2011

- Molecular understanding of CN-AML is increasing at a rapid rate resulting in
  - subgroups with apparent cure rates of >60%
  - new targeted therapies (e.g., TKIs for *FLT3* mutations, ATRA for *NPM1* mutations, epigenetic therapy for *MLL*-PTD)

- Clinical trials in newly diagnosed patients based on the molecular subtype or stratify based on it
- Randomized prospective clinical trials in newly diagnosed patients on-going combining molecularly targeted therapy with chemotherapy
- Use of molecular information appears likely to substantially increase the cure rate for adult CN-AML within next 5 years

Curing AML in Adults in 2011

- 45 years of chemotherapy have changed adult AML from an incurable disease to one where substantial percentages of patients are being cured
- Karyotype and molecular findings now used for diagnosis, prognosis and increasingly for selecting therapy
- Only in APL, using molecularly targeted agents (ATRA + arsenic) with chemotherapy, are almost all patients cured
- New agents targeting specific genetic defects are becoming available and, when combined with chemotherapy and other modalities, promise to substantially increase the cure rate

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