Integrated Diagnosis of Acute Myeloid Leukemia

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Integrated diagnosis of AML: objectives

- Recognition of biological & clinically relevant entities
- Prognostic value
- Therapeutic guide
- Providing tools for minimal residual disease assessment
- Platform for research purposes

Tools for an integrated diagnosis in AML

Standard – currently required

Cytomorphology

Cytochemistry

Immunophenot/flow cytometry

Cytogenetics

Molecular cytogenetics – FISH

Molecular biology –

gene rearrangement & mutations

(PML/RARA, RUNX1/RUNXT1, CBFB/MYH11, NPMmut, CEBPA, FLT3-ITD,…)

Novel techniques – research

Molecular cytog - SNP arrays

Epigenetics – DNA & histone methylation

Gene expression analysis

miRNA profiling

....

Whole DNA sequencing

....

Acute Myeloid Leukemia (AML): WHO classification

I. AML with recurrent cytogenetic abnormalities

II. AML with myelodysplasia-related changes

III. Therapy-related AML/MDS

IV. AML, not otherwise specified

V. Myeloid sarcoma

VI. Myeloid proliferations related to Down syndrome

VII. Blastic plasmacytoid dendritic cell neoplasms

WHO Classification, 2008

AML pathogenetic events: what's in an AML?

- Maturation arrest / differentiation block
- Proliferation
- Leukemia-stem cell model: mimicking normal hematopoietic hierarchy
- Disruption of normal hematopoietic niche

Gene mutations in AML: trying to systematize them

MATURATION ARREST
(type II)

Initial leukemogenic events

Involve transcription factors

Mutually exclusive

Fusion transcripts

PML/RARA

AML1(RUNX1)/ETO(RUNX1T1)

CBFbeta/MYH11

MLL/partners

Uncommon types (DEK/CAN, MOZ/CBP,HOXA9/NUP98)

Other mutations

NPM

CEBPA

PROLIFERATION
(*type I*)

Adcquired, evolutive events

Involve cell signalling pathways

Usually associated with diverse type II mutations

Gene mutations

FLT3 (ITD, TKD)

Kit

Ras

…

Correlation between type II mutations & cytogenetics

MATURATION ARREST
(type II)

Fusion transcripts
PML/RARA
AML1(RUNX1)/ETO(RUNX1T1)
CBFbeta/MYH11
MLL/partners
Uncommon types (DEK/CAN, MOZ/CBP,HOXA9/NUP98,…)

Chromosomal translocations
t(15;17)
t(8;21)
inv(16)/t(16;16)
t(6;9)
t(11q23;x)
t(8;16)
t(7;11)
…

Other mutations
NPM
CEBPA
MLL-PTD

Normal Karyotype AML

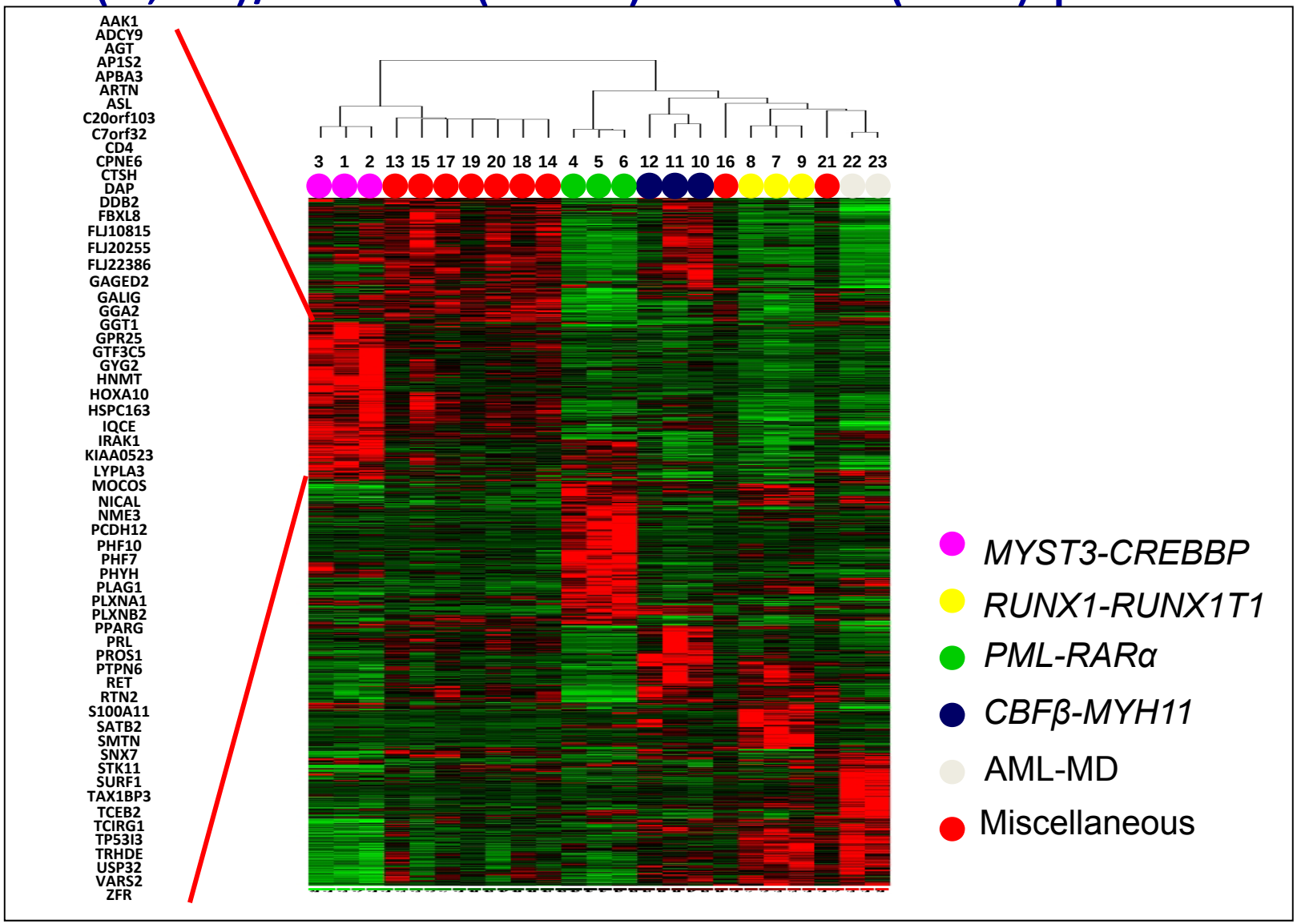
WHO classification of AML (II): an increasing repertoire of molecularly-defined entities

I. AML with recurring genetic abnormalities

- AML with t(8;21)(q22;q22)/RUNX1-RUNXT1
- AML with inv(16) or t(16;16)(p13;q22)/CBFβ-MYH11
- Acute promyelocytic leukemia [t(15;17) & PML-RAR-α]
- AML with t(9;11)(p22;q23)/AF9(MLLT3)-MLL
- AML with t(6;9)(p23;q34)/DEK-CAN(NUP214)
- AML with inv(3) or t(3;3)(q21;q26)/RPN1-EVI1
- Megakaryoblastic AML with t(1;22)(p13;q13)/RBM15-MKL1
- AML with mutated NPM
- AML with normal karyotype and CEBPA mutation

WHO Classification, 2008

Supervised analysis: AML with t(8;16)/MYST3(MOZ)-CREBBP(CBP) profile

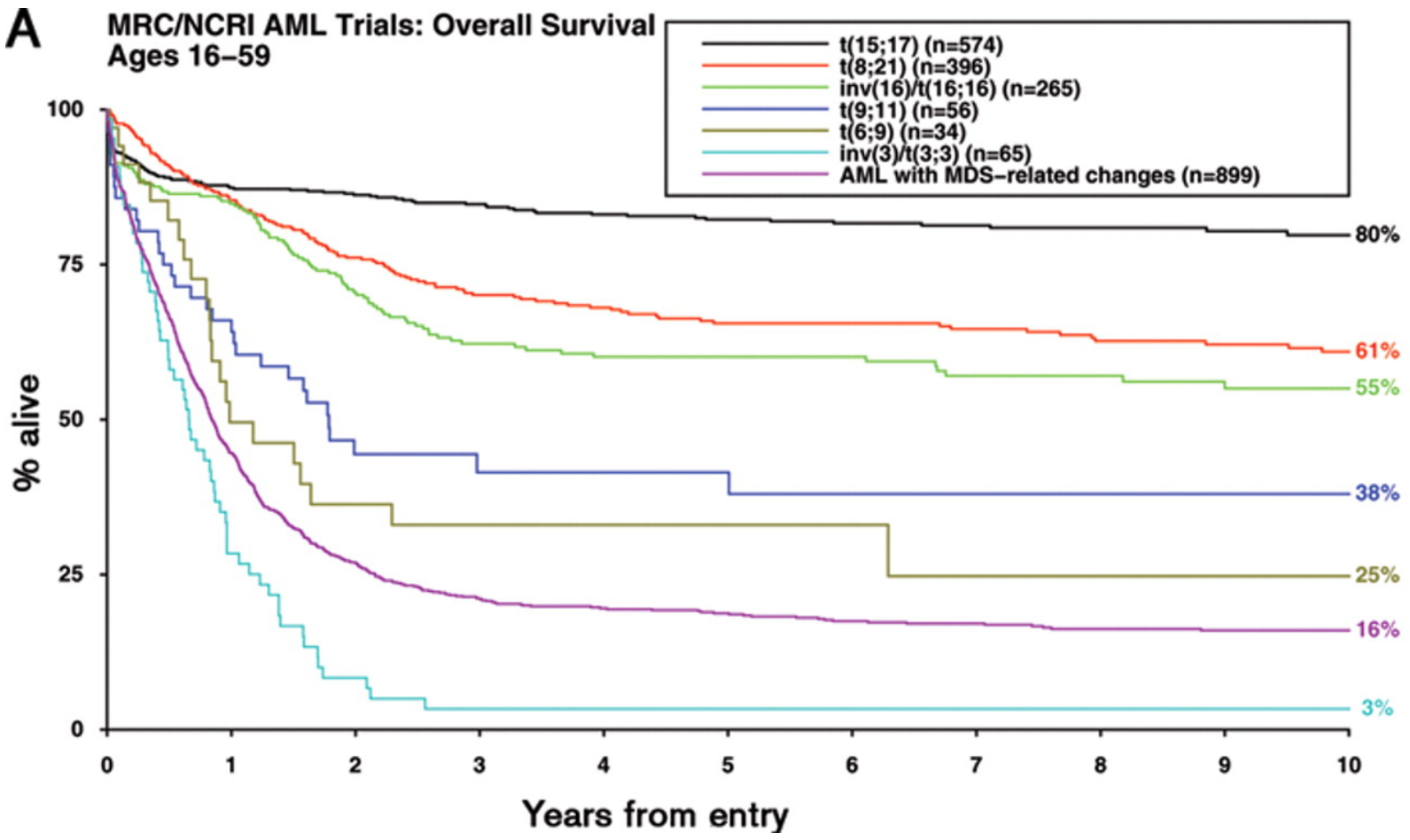


Camos M et al, Cancer Research 2006

Prognosis of AML: light & shade of WHO classification

- Cytogenetic categories
- Molecular markers: *refining* cytogenetics
- *Life is complex*: prognosis as the result of interaction between diverse mutations
- Non-molecularly defined categories: clinically homogenous?
- Prognosis & prediction: is allogeneic HSCT useful to improve poor prognosis of all adverse AML subtypes?

Prognostic impact of WHO classification in younger patients

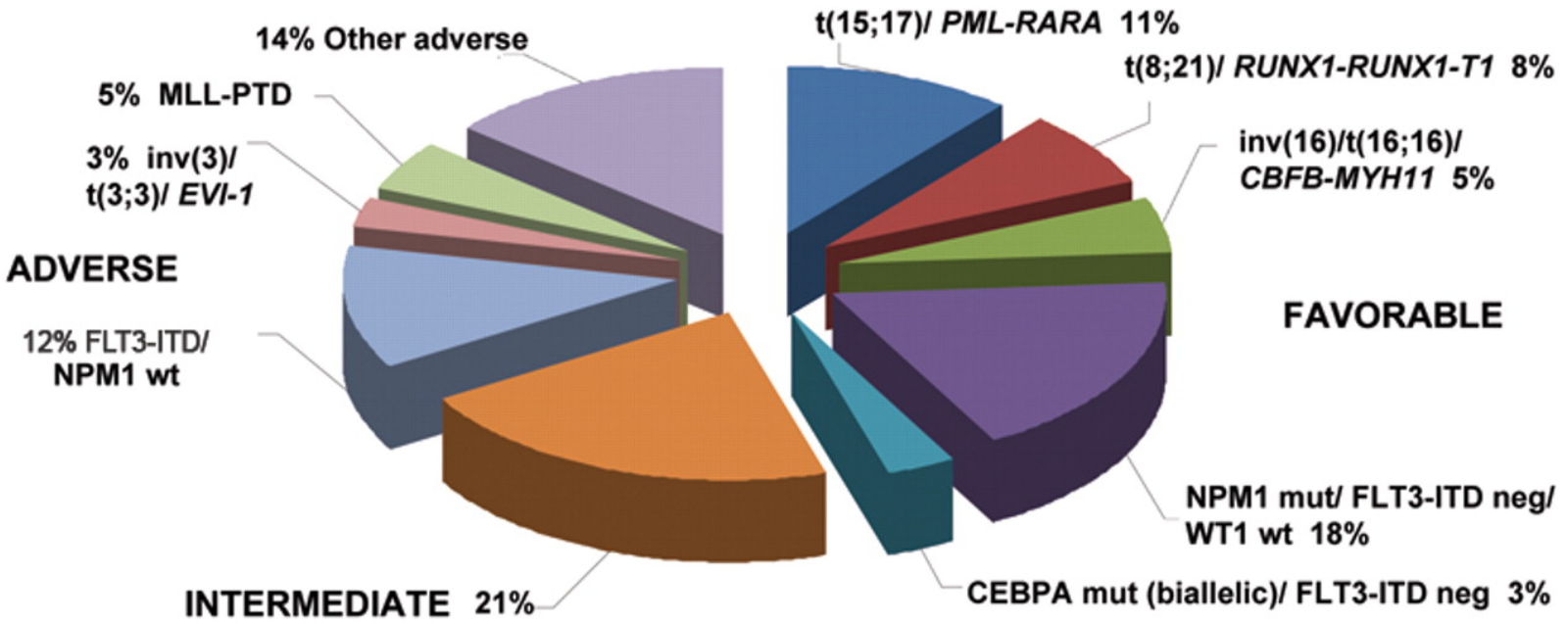


Grimwade D, et al. Hematology 2009

Prognostic value of molecular markers in AML – *reasonable* statements (I)

- ✓ Cytogenetic characterization allows distinction of well-defined prognostic categories
- ✓ Molecular genotyping adds relevant prognostic information in cytogenetic intermediate-risk AML
- ✓ This “biological categorization” may be useful to guide therapy in CR1: basis for risk-adapted strategies

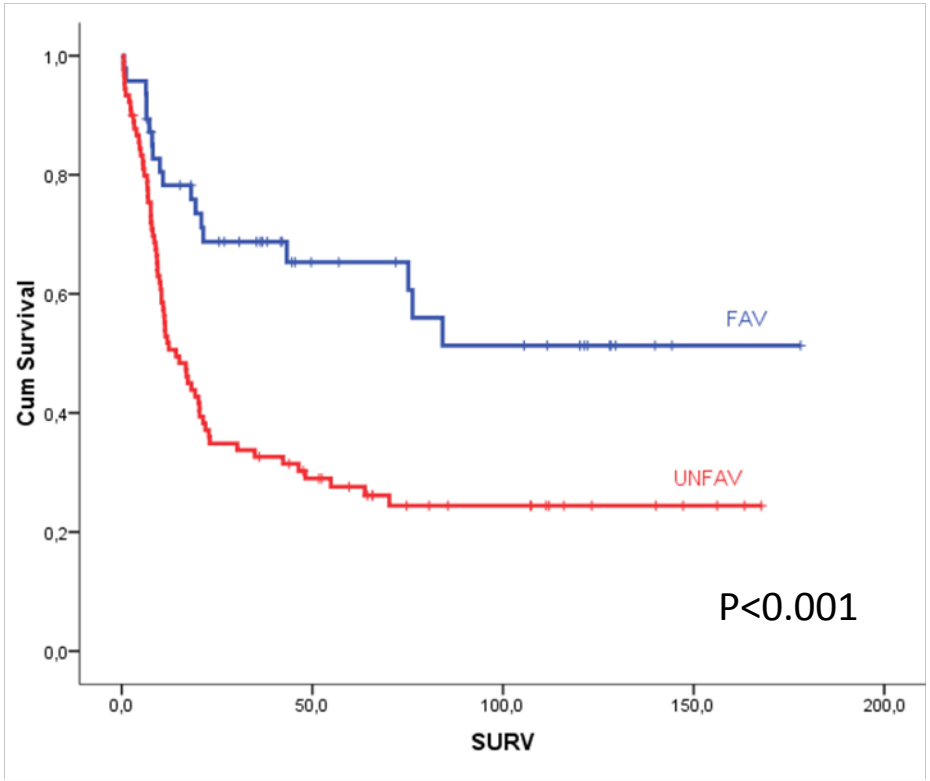
Relevant molecular and cytogenetic subgroups of AML arising in younger adults

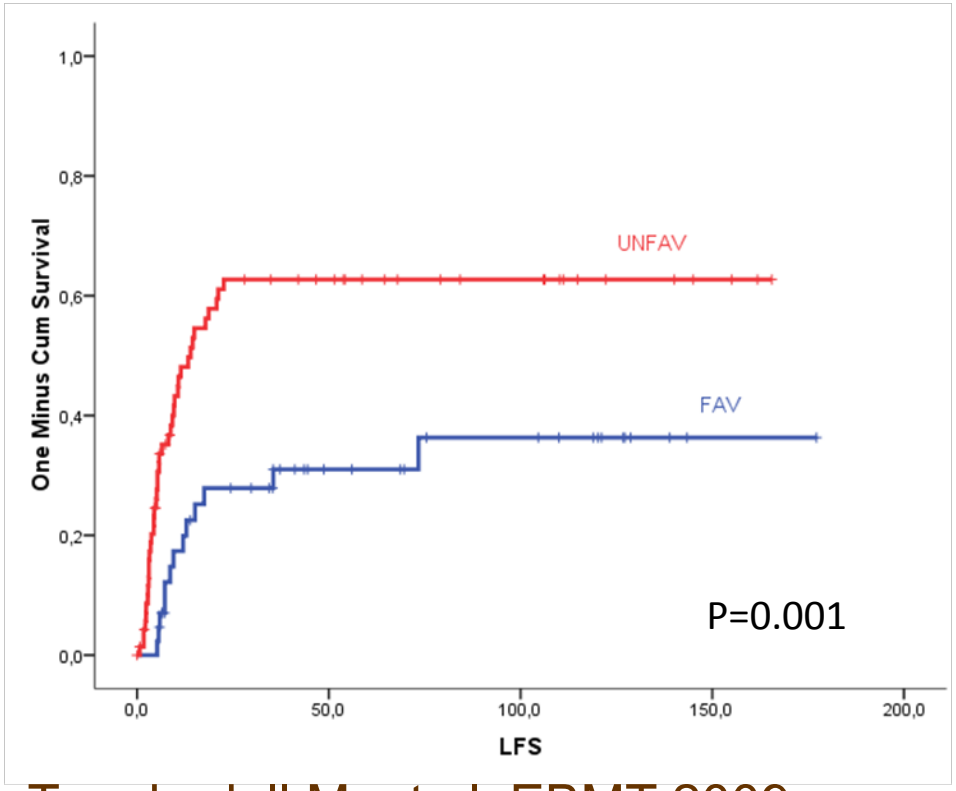


Grimwade D, et al. Hematology 2009

Cytogenetic intermediate-risk AML: molecularly–defined prognostic categories

Favorable genotype	*Unfavorable genotype*
NPMmut/FLT3-ITDneg or CEBPAmut	NPMwt and/or FLT3-ITD

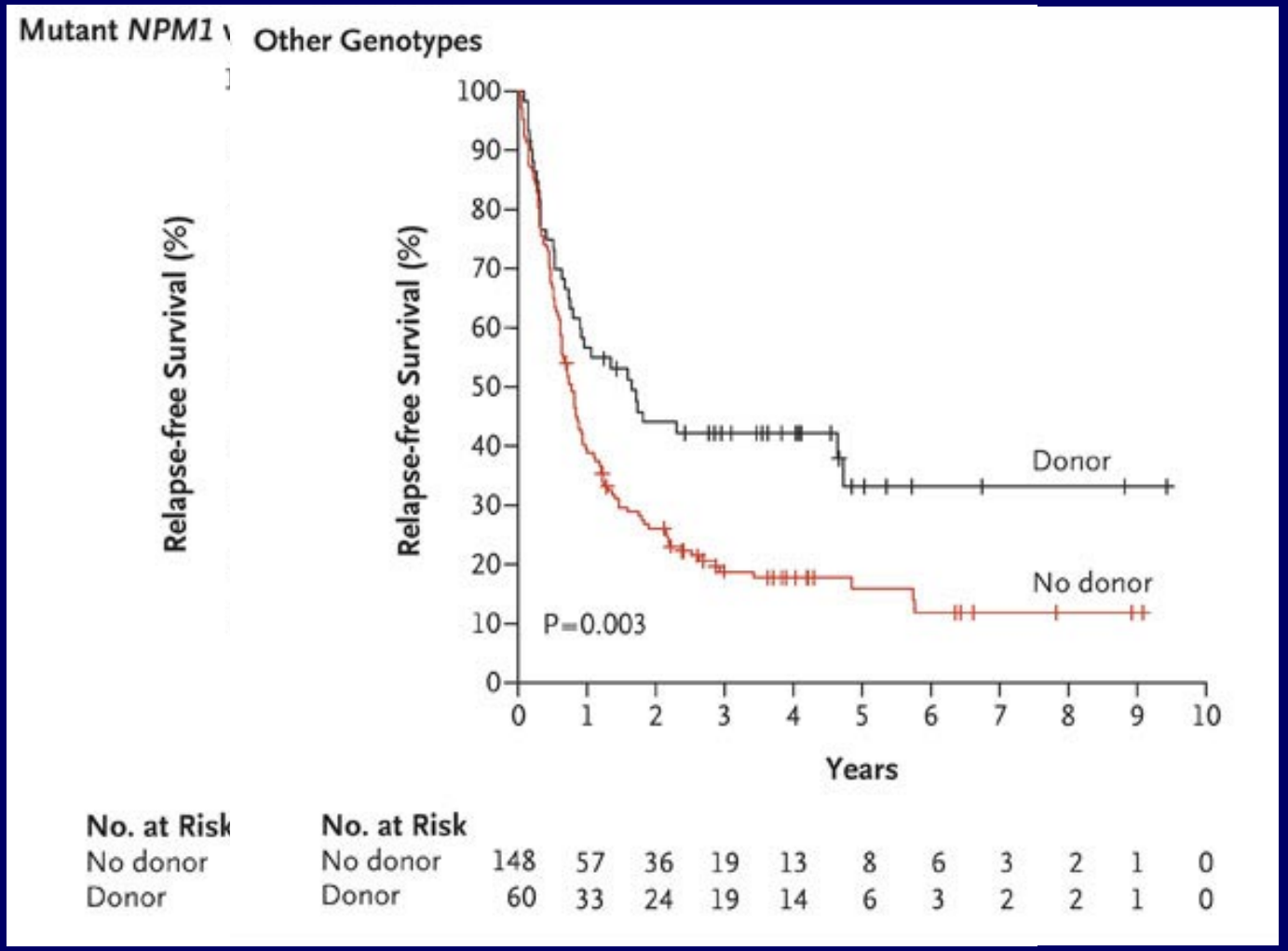




Torrebadell M, et al. EBMT 2009

Pratcorona et al, ASH 2009

Benefit of alloHSCT in CR1 normal karyotype AML might depend on underlying genotype



Schlenk R, et al. NEJM 2008

Risk-adapted therapy in AML: proposed strategy according to molecularly defined *subgroups*

Risk group	*Proposed postCR strategy*
CBF-AML *High-risk subsets*	HiDAC-based chemotherapy *AutoHCT* *AlloHCT?* *Experimental (TKIs,…)*
Normal karyotype with fav markers (NPMmut/FLT3(-), CEBPAmut,…) **Unfavorable markers** (FLT3-ITD, MLL-PTD,…)	HiDAC AutoHSCT AlloHCT Experimental (FLT3inh,…)
Adverse-risk cytogenetics	AlloHCT Experimental therapy (demethylating,…)

Prognostic value of molecular markers in AML – a complex world (II)

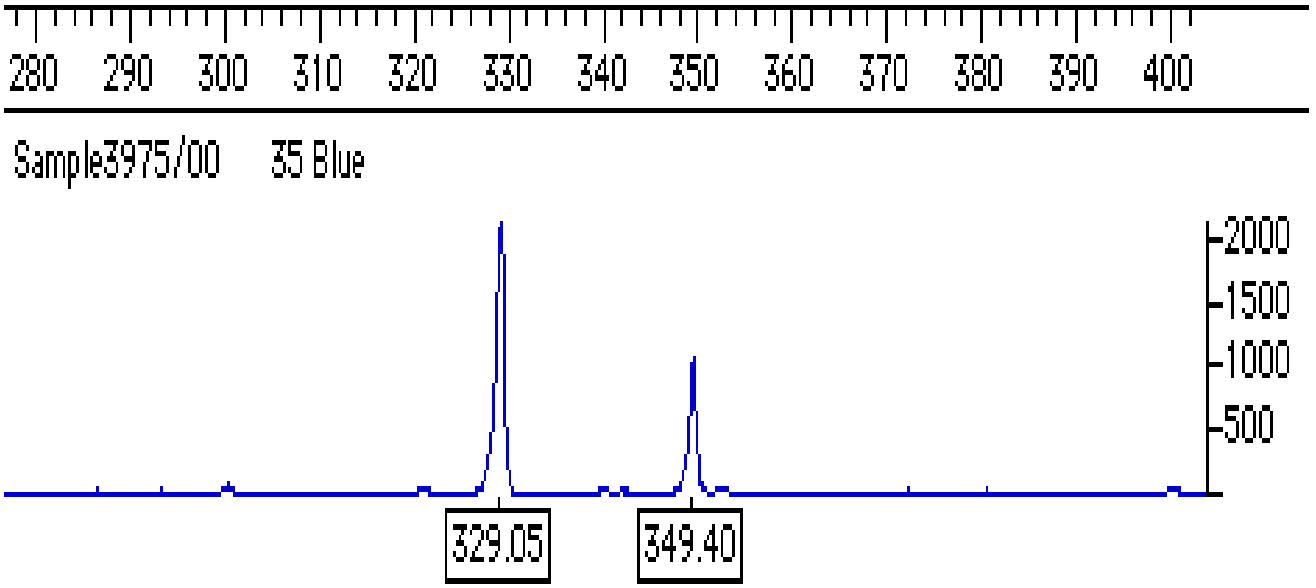
- ✓ Interaction between cytogenetic & molecular features
  - Poor-risk molecular lesions within favorable cytogenetics (e.g., c-kit mutations in CBF-AML)
  - FLT3-ITD among NPMmut AML
- ✓ Several mutational characteristics might modulate its prognostic value
  - FLT3-ITD: relapse risk depends on allelic burden
  - IDH1 in NPMmut

Background: prognostic impact of FLT3-ITD

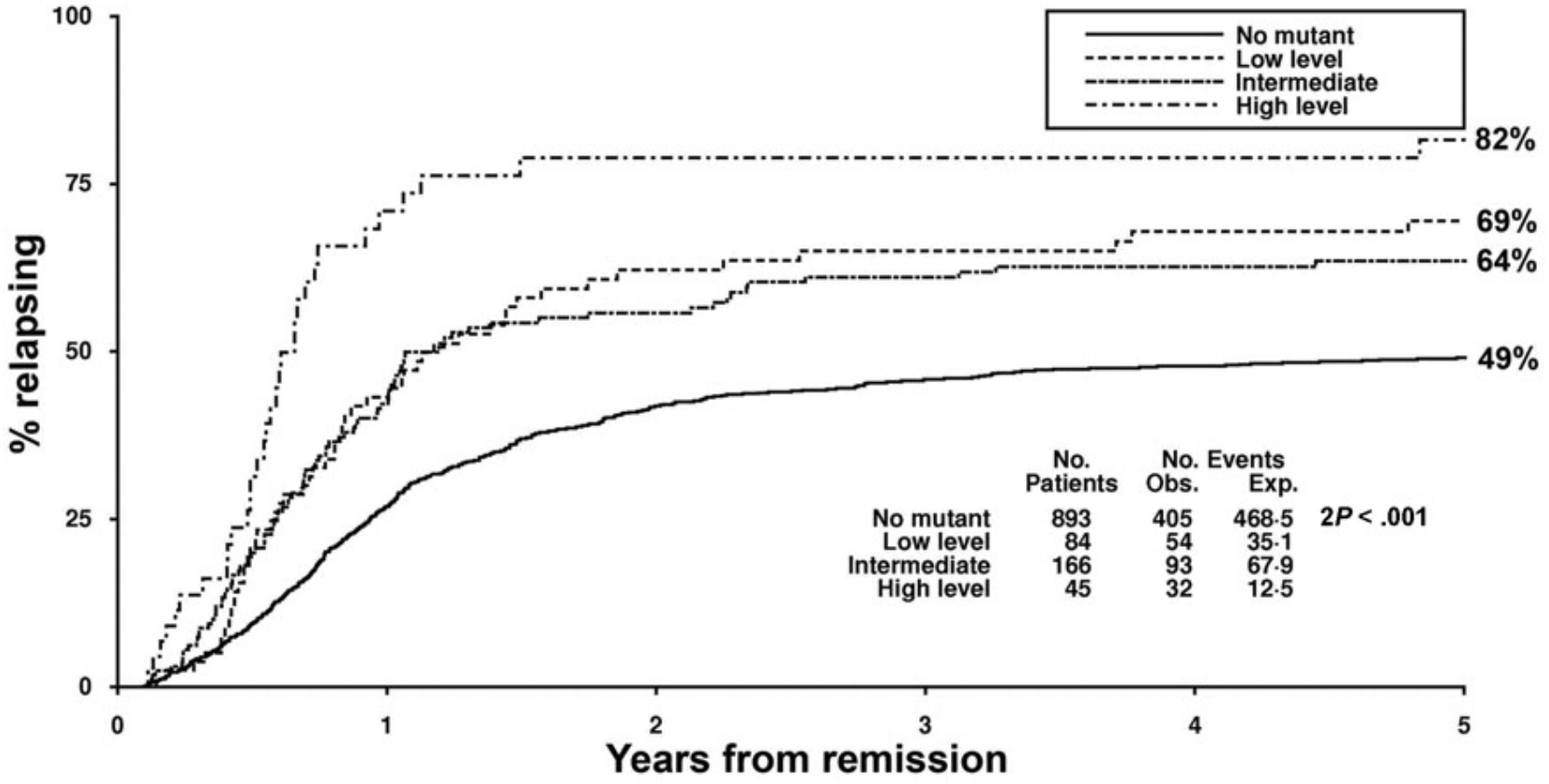
- ✓ FLT3-ITD is associated to a higher relapse risk & poor prognosis in AML
- ✓ FLT3-ITD allelic burden might modulated this effect (Gale et al., Blood 2008)
- ✓ Moreover, the underlying mutational status of NPM1 might interact with the effect of FLT3-ITD allelic burden:
  - Lower risk in NPMmut AML with low FLT3-ITD/wt ratio? (Schnittger et al, ASH 2009)

Assessment of FLT3 internal tandem duplication (FLT3-ITD)

- PCR of exons 11-12 of FLT3 gene & Genescan analysis (Thiede et al, 2002)
- AUC between mutated & wild-type alleles (ratio FLT3-ITD/wt)

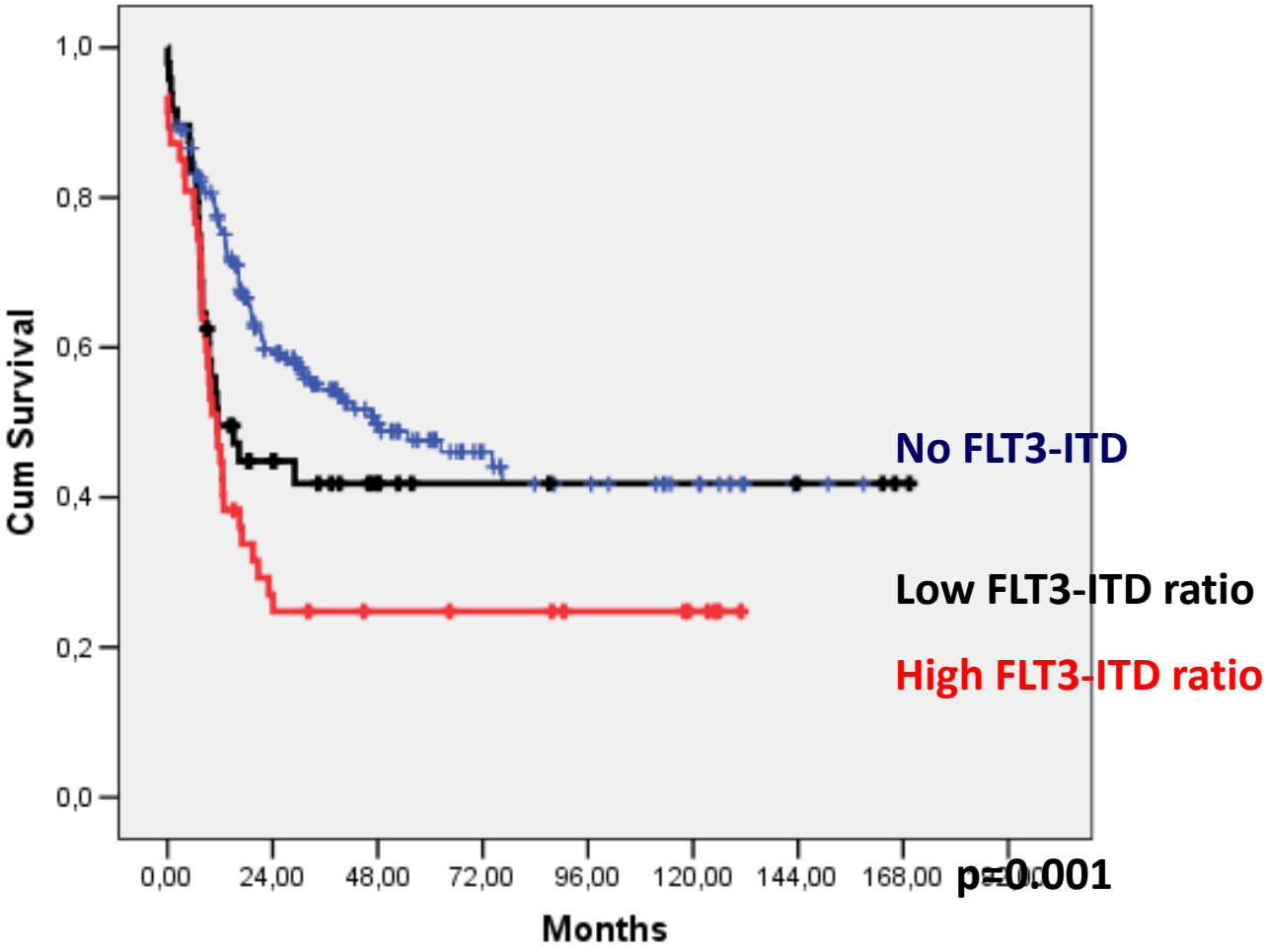


Relapse risk according to FLT3-ITD allelic burden (MRC AML8 & AML10)



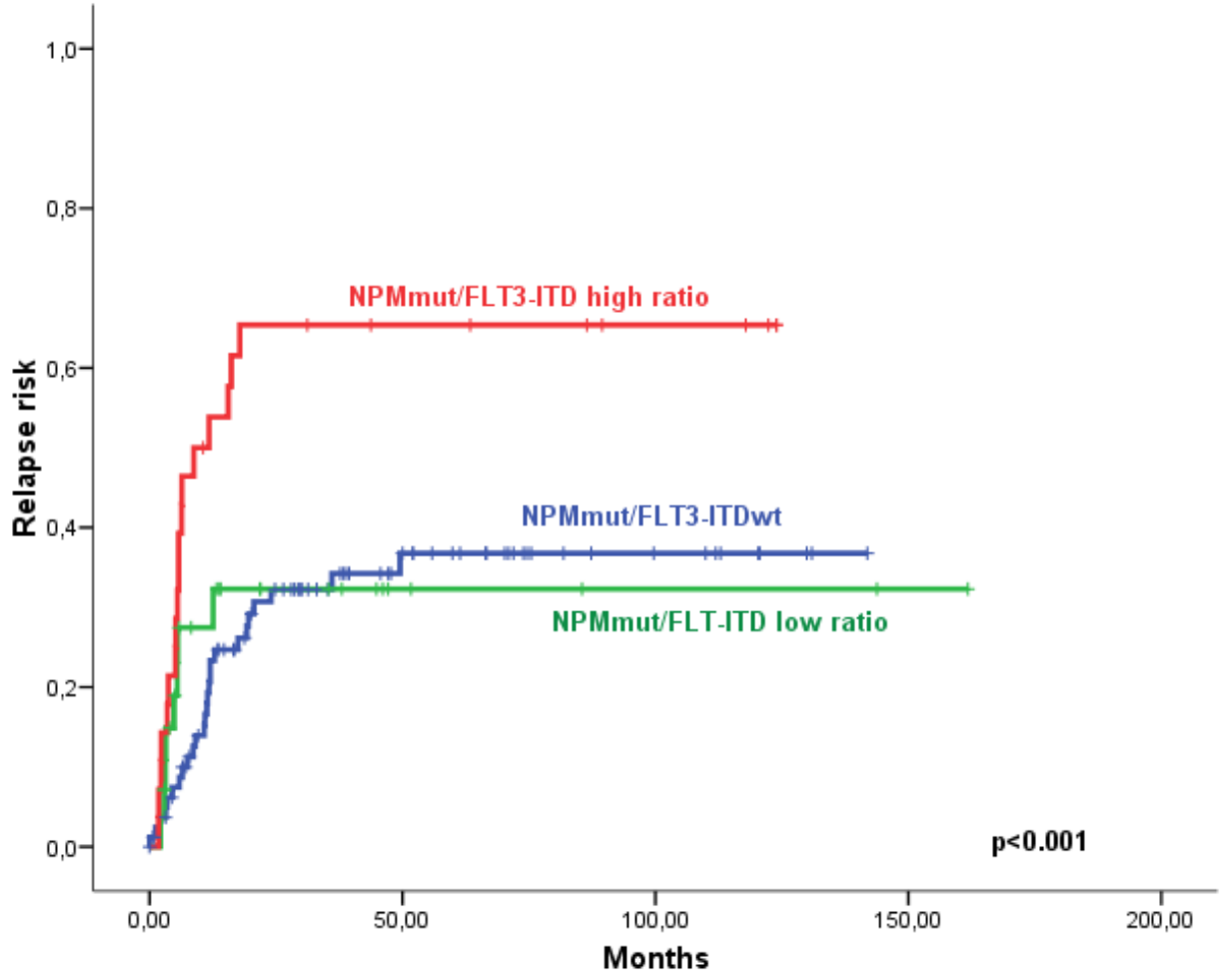
Gale RE et al, 2008

Prognostic impact of FLT3-ITD allelic burden: survival



Pratcorona M (CETLAM), EHA 2010

Value of FLT3-ITD/wt ratio in NPMmut AML: relatively favorable outcome in pts with low ratio (<median: 0.6)



Pratcorona M (CETLAM), EHA 2010

AML – a *myriad* of potentially relevant genetic & epigenetic lesions

"Consolidated" mutations

FLT3 ITD

NPM1

CEBPα

MLL-PTD

"Emerging" mutations

IDH1 (R132)

IDH2 (R172)

IDH2 (R140)

Mutations of uncertain value

C-kit

WT1

RUNX1

N-ras, k-ras

….

Altered gene expression

EVI-1

ERG

BAALC

PI3K/Akt pathway

Gene expression signatures

WHO classification AML: not *molecularly* defined entities

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II. AML with myelodysplasia-related changes

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IV. AML, not otherwise specified

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VII. Blastic plasmacytoid dendritic cell neoplasms

WHO Classification, 2008

Redefinition of AML with multilineage dysplasia (WHO 2001): AML with myelodysplasia-related changes (WHO 2008)

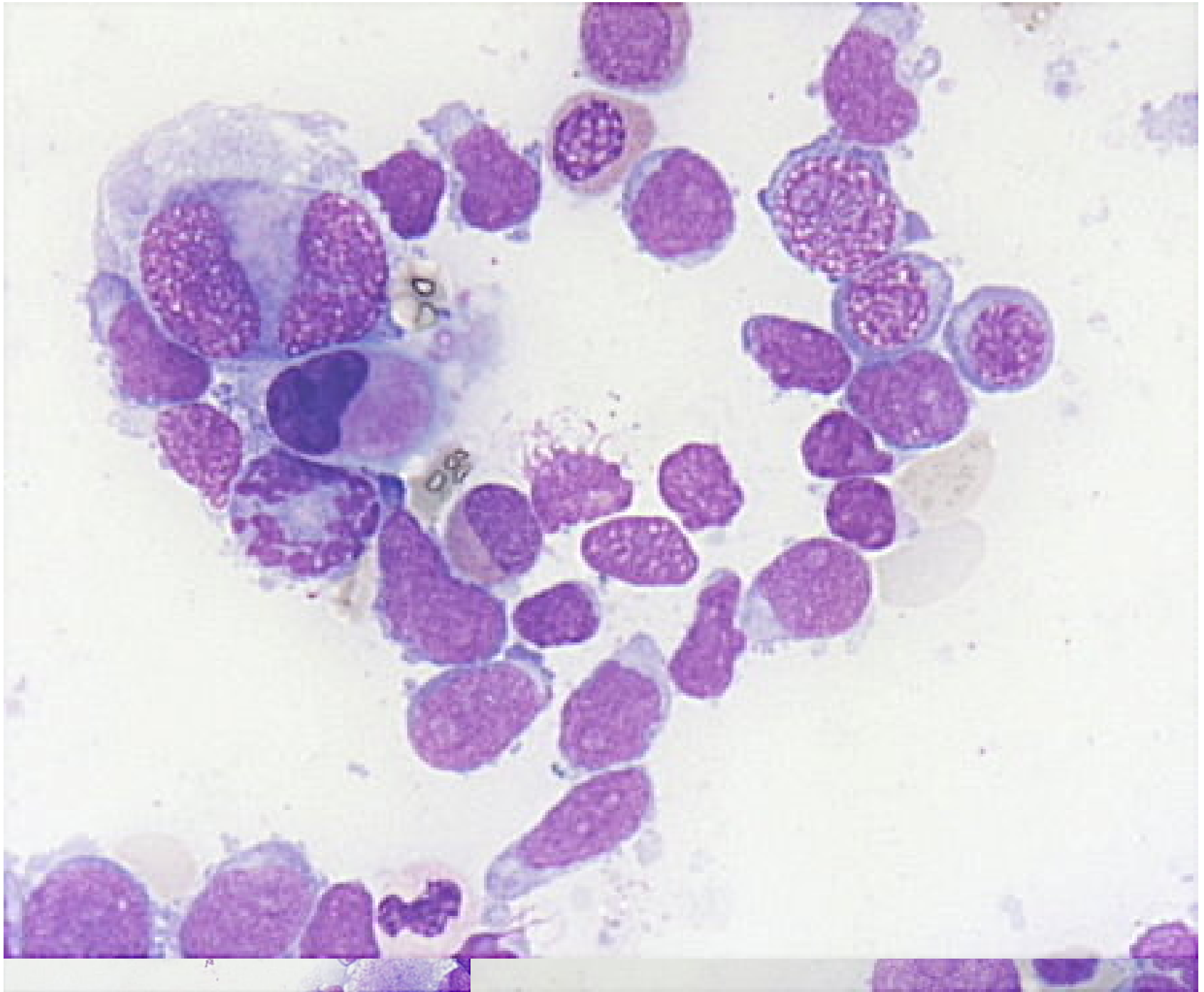
≥20% blast cells

+

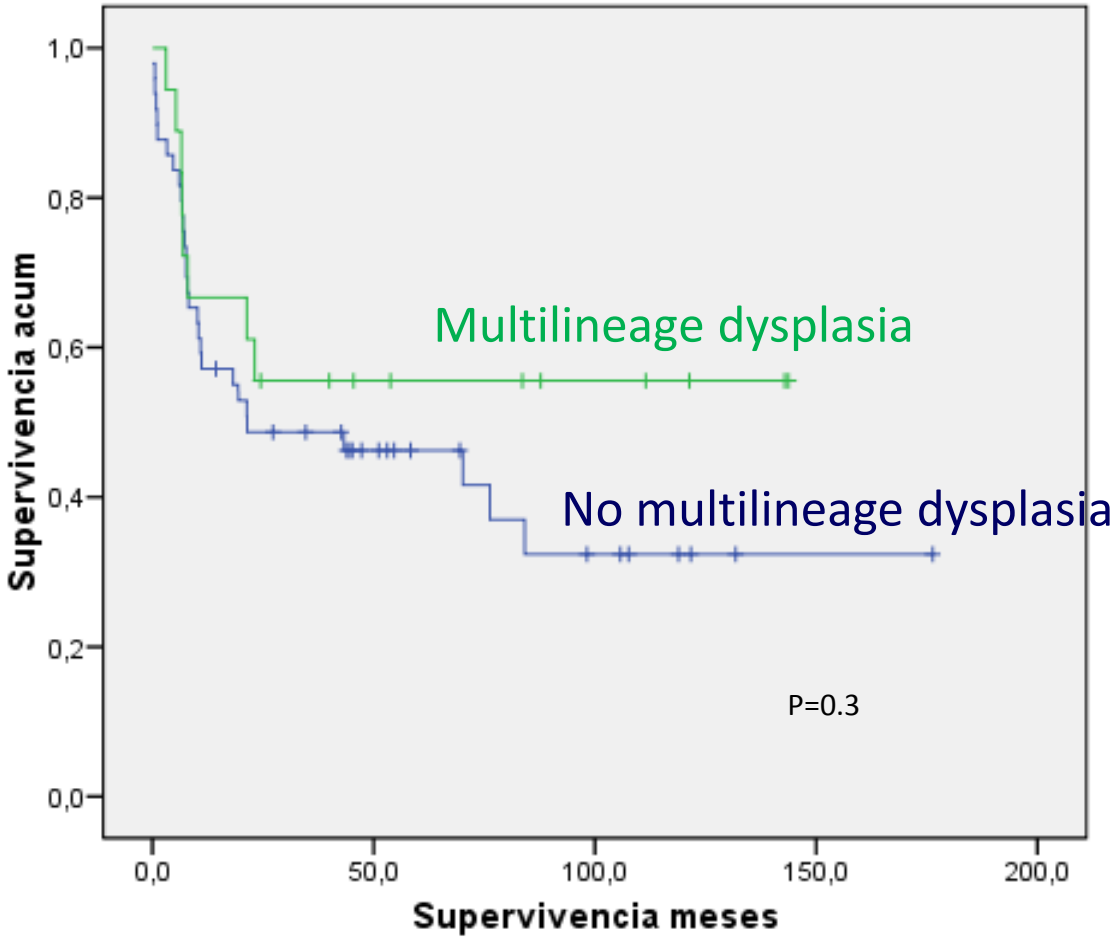
- Preceding MDS and/or
- Cytogenetic abnormality related to MDS and/or
- Multilineage dysplasia

AML with myelodysplasia-related changes: subtype-defining cytogenetic findings

Non-balanced changes	Translocations	Complex karyotype
-7/del(7) -5/del(5q) i(17q)/t(17p) -13/del(13q) del(11q) del(9q) del(12p)/t(12p) Idic(X)(q13)	11q23 (*MLL*): t(11;16) & t(2;11) 5q32: t(5;12) / t(5;7) / t(5;17) / t(5;10) / t(3;5)	>3

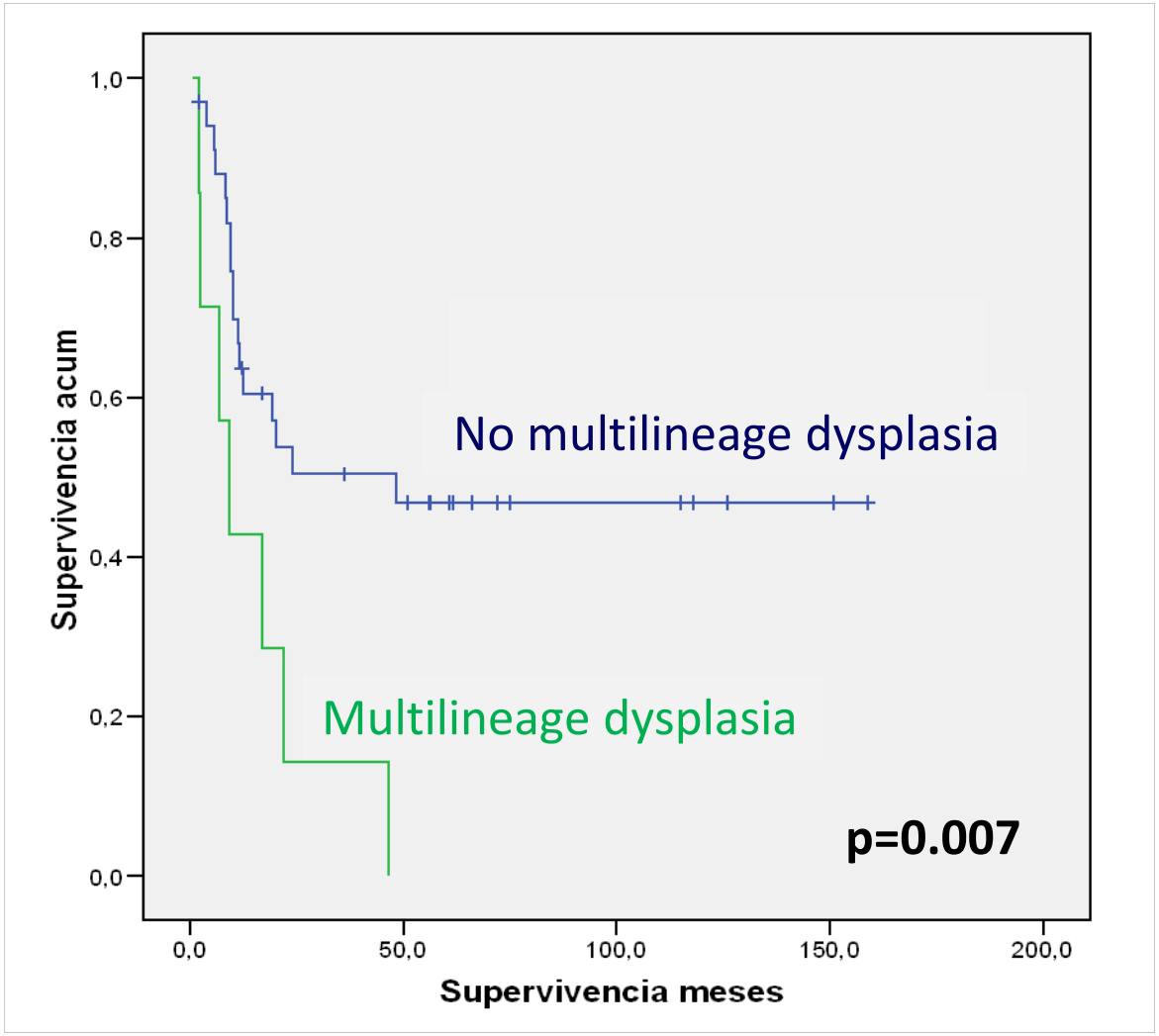


Multilineage dysplasia: absence of prognostic value in AML with mutated NPM1



Díaz Beyá M, submitted to AEHH 2010

Multilineage dysplasia defines porgnostic subgroups in pts with wild-type NPM1 & intermediate-risk cytogenetics



Díaz Beyá M, submitted to AEHH 2010

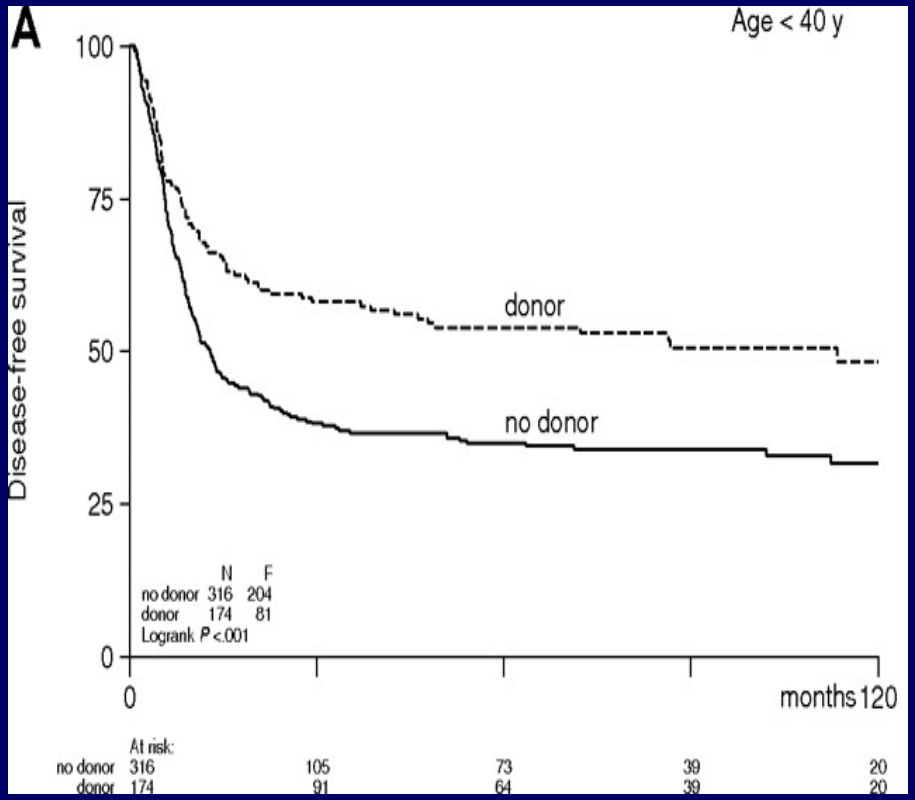
Prognosis of AML: light & shade of WHO classification

- Cytogenetic categories
- Molecular markers: *refining* cytogenetics
- *Life is complex*: prognosis as the result of interaction between diverse mutations
- Non-molecularly defined categories: clinically homogenous
- Prognosis & prediction: is allogeneic HSCT a cure for all poor-prognosis AML subtypes?

AlloHSCT in poor-prognosis cytogenetic AML: neat benefit of donor availability

	n	*Group*	*EFS (%)*	*Surv (%)*	*HR*
Burnett, 2002	128	No donor	21	24	
	48	Donor	20	21	NS
Suciu, 2003	441	No donor	18	NR	
	293	Donor	43	NR	0.62
Cornelissen, 2007	193	No donor	17	30	
	116	Donor	33	40	0.59 (EFS)

... But benefit of alloHSCT is restricted to younger patients



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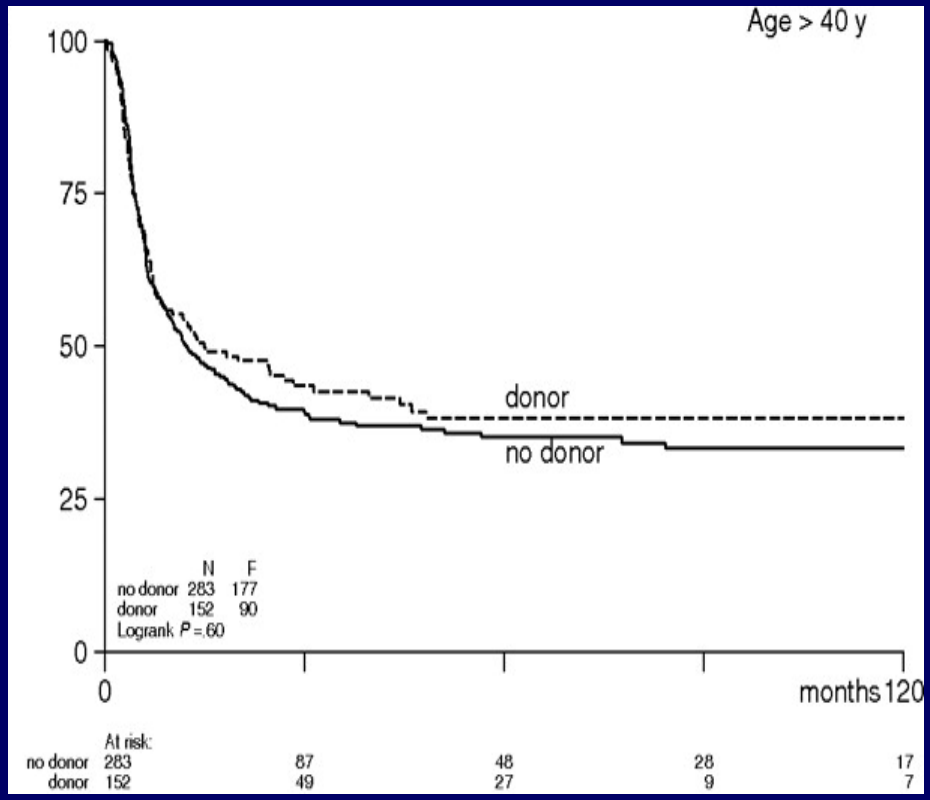

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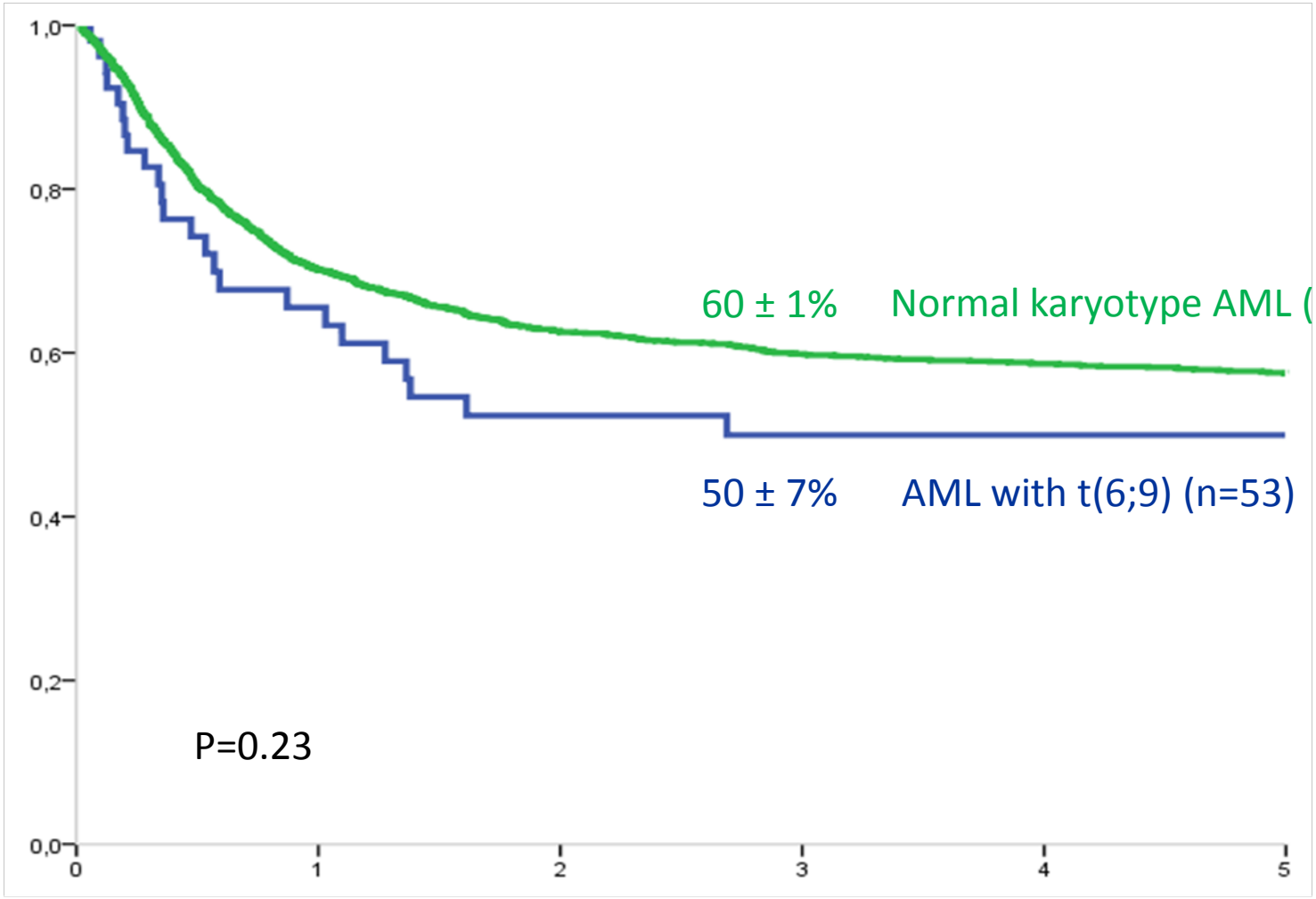
Cornelissen JJ, et al. Blood 2007

AML with t(6;9)/DEK-NUP214(CAN) rearrangement

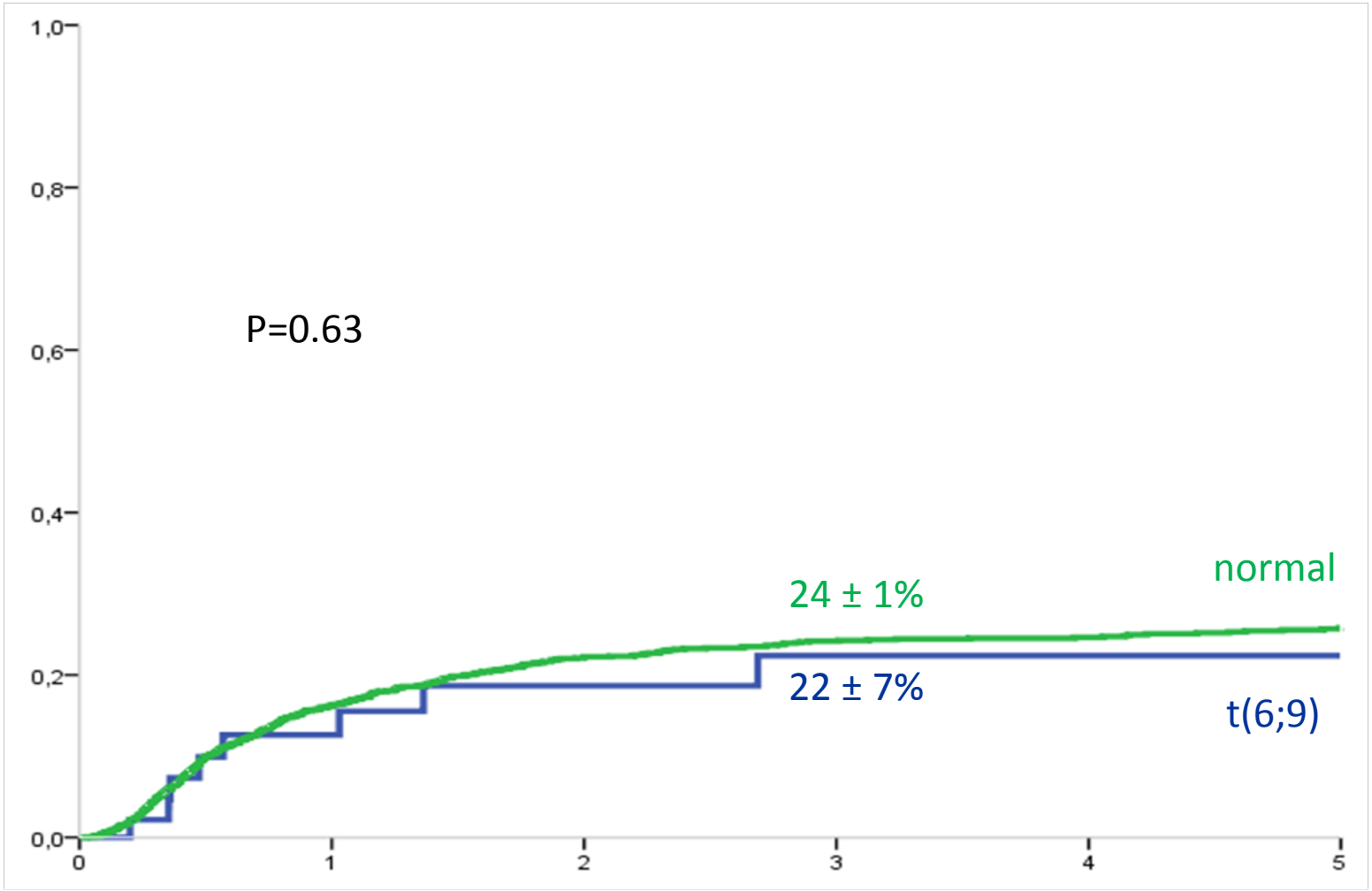
- Rare but well-characterized AML subtype [WHO 2008 classification]
- Young patients (median age≈30)
- Frequent association to FLT3-ITD
- Associated to poor prognosis – included in the unfavorable cytogenetic category (SWOG & CALGB)
- A favorable outcome after alloHSCT has been suggested

AML with t(6;9) vs. AML-normal karyotype: LFS after alloHSCT in CR1



Esteve J et al (ALWP), EBMT 2010

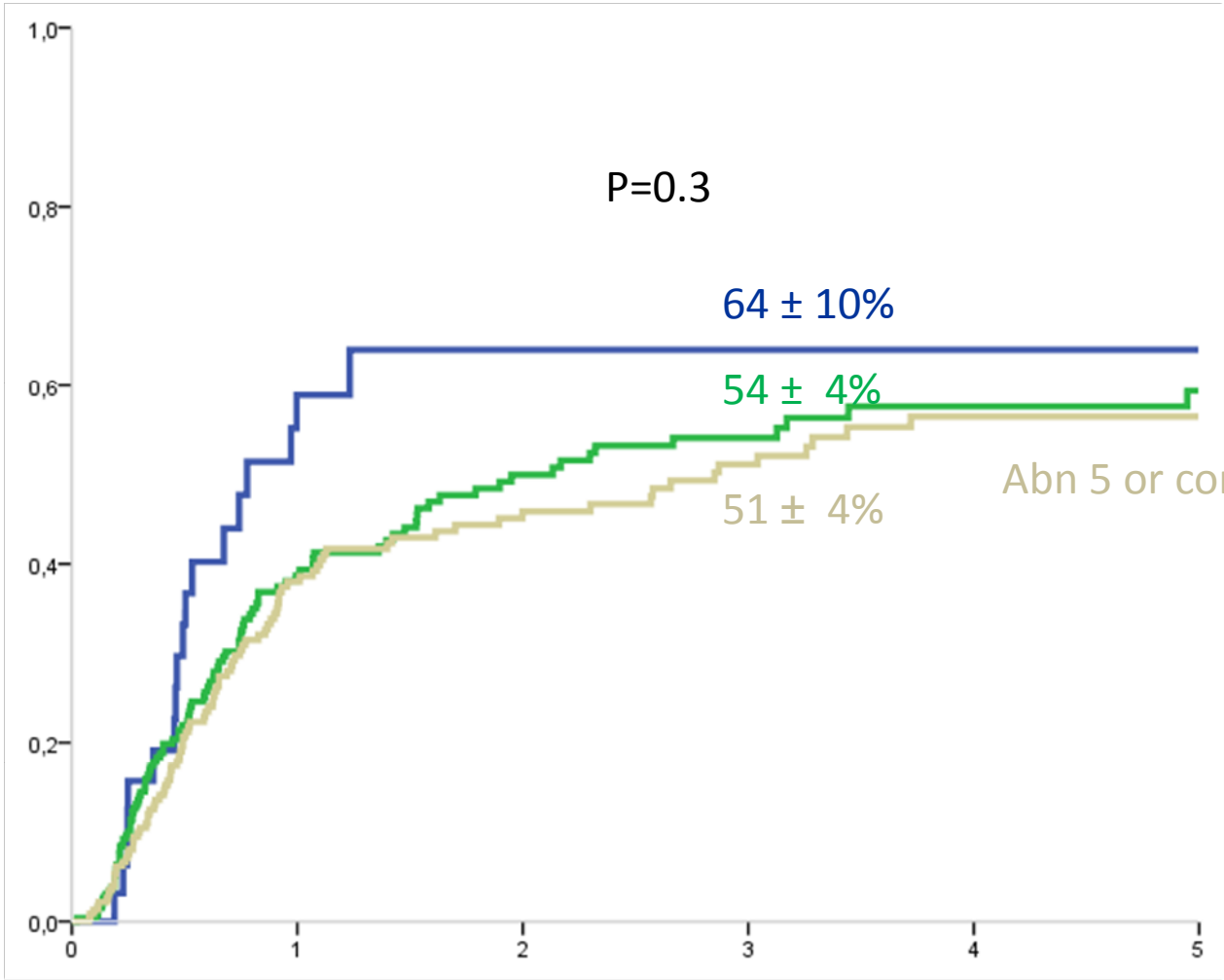
AML with t(6;9) vs. AML-normal karyotype: relapse incidence after alloHSCT in CR1



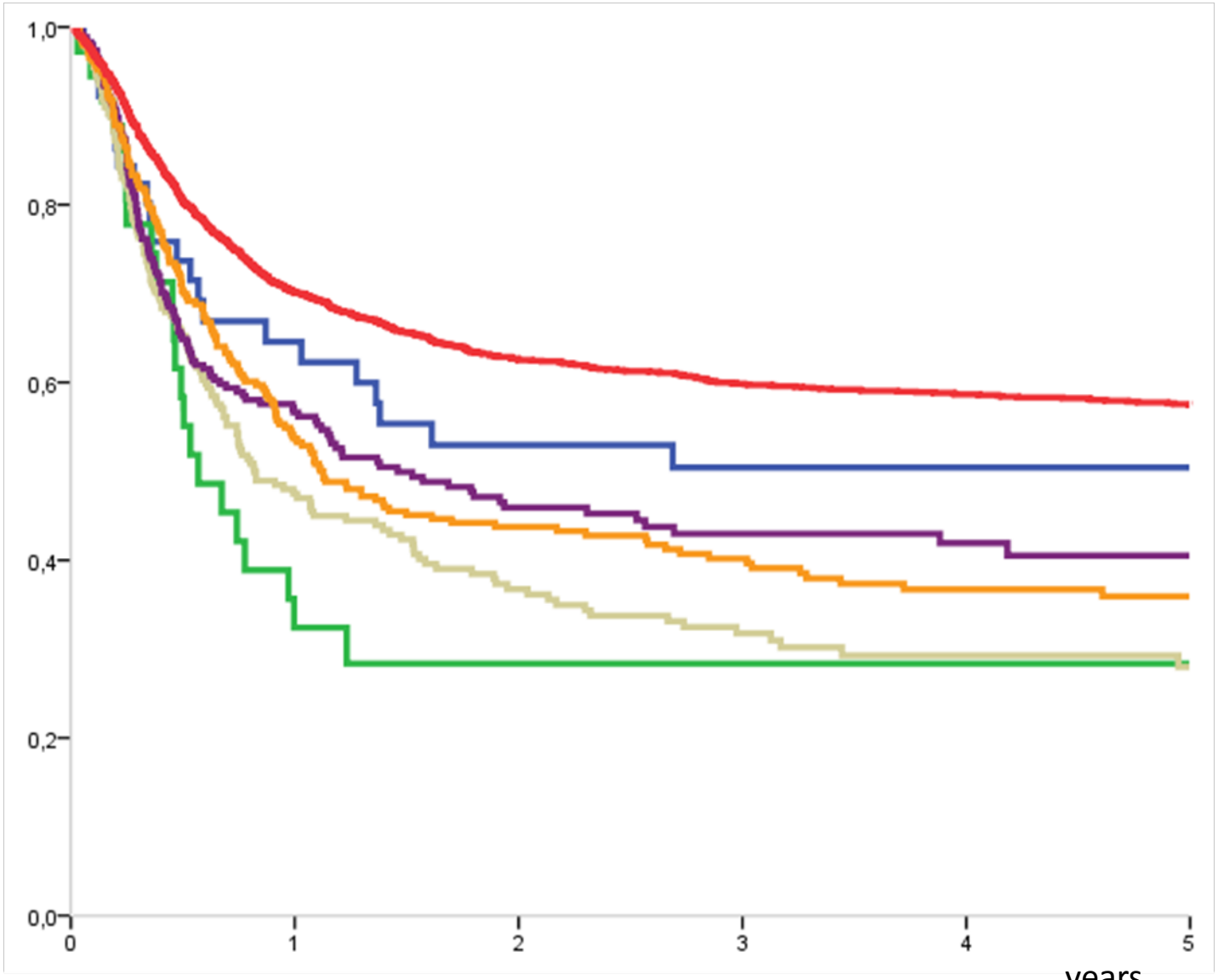
AML with 3q26 abnormalities – a challenging disease

- AML with inv(3)/t(3;3)(q21;q26) is a rare AML subtype with poor prognosis
- Leads to EVI1 overexpression
- Poor response to standard therapy & long-term survival<20%
- As a sole aberration or frequently associated to additional karyotypic abnormalities (-7, del5q, complex karyotype,…)
- Controversial role of alloHSCT in these patients

AML with 3q26 abn vs. AML with 5q abn vs. AML with 7q abn: RI in CR1



High-risk AML: a diverse world



Prognostic value of molecular markers in AML – final considerations

- ✓ Lack of prospective validation of these prognostic markers
- ✓ Studies are based on “genetic” randomization (donor vs. no-donor) – role of alternative donors in CR1?
- ✓ Absence of studies addressed to specific entities (e.g., cytogenetic subtypes within high-risk category)
- ✓ Impact of variables related to transplant procedure (donor, conditioning, SC source) mostly unknown

Beyond WHO 2008 classification: future challenges of diagnosis integration in AML

- *Rationale integration* of new overwhelming knowledge
- Adapting to new molecularly-targeted therapies – urgent screening at diagnosis
- *Individualized* therapy: analysis of genetic polymorphisms
- Characterization of leukemia stem-cell population
- *Dissecting* the hematopoietic niche of AML

Whole-genome sequencing of normal cytogenetics AML (NC-AML) – chapter I

- Comparison of genome from AML cells & skin from a patient with normal karyotype AML (FAB M1)
- Results – adcquired mutations in 10 genes: *NPM1, FLT3-ITD* & 8 genes (*single-base changes*) not previously described in AML
- Several ot these genes reported in other tumors (*PTPRT*, *CDH24*, *PCLKC*, *SLC15A1*)
- Heterozygous mtations
- *Whole-genome sequencing* as a required tool to elucidate leukemogenic transformation process?

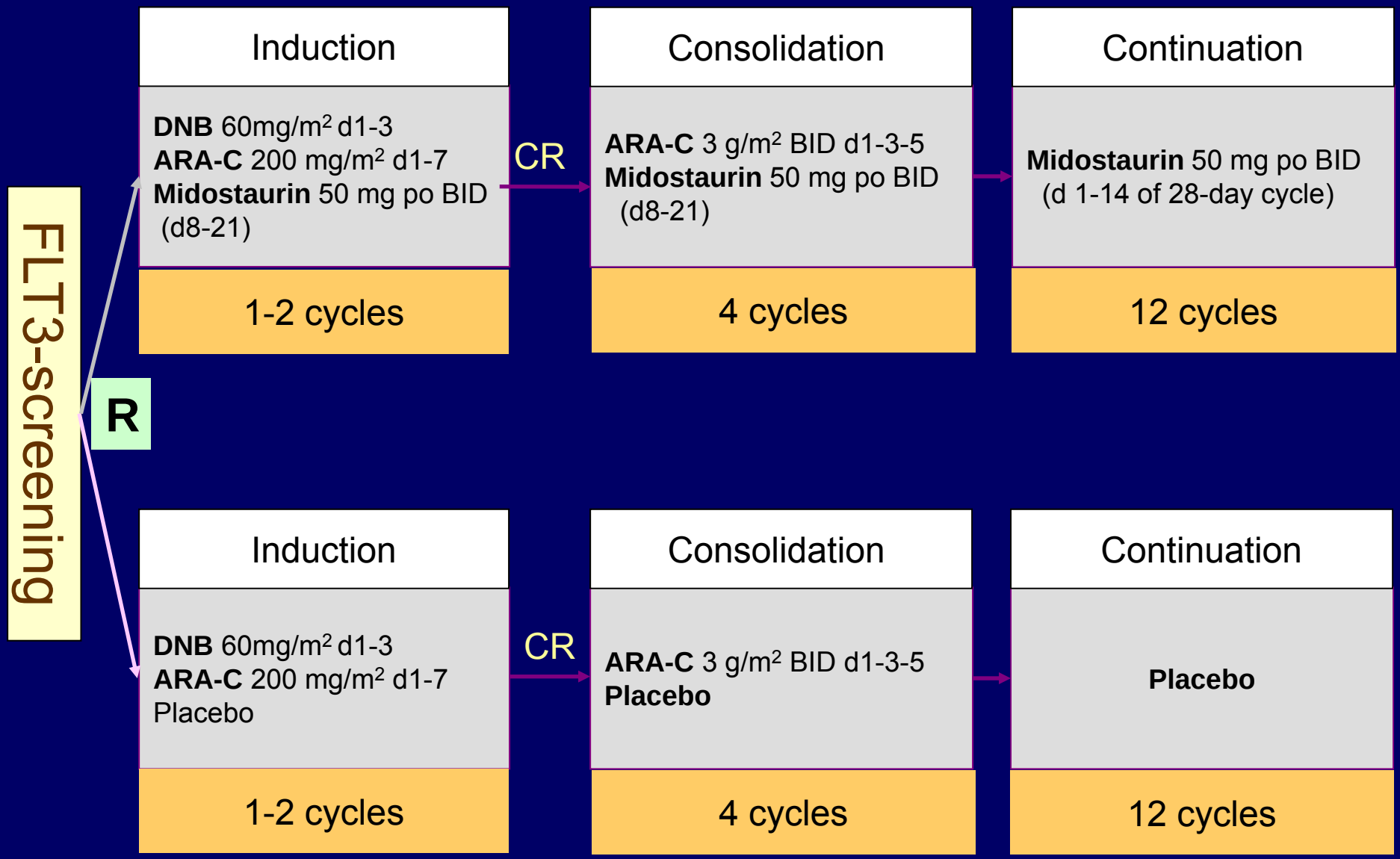
Ley TJ, Nature 2008

Whole-genome sequencing of normal cytogenetics AML (NC-AML) – part II

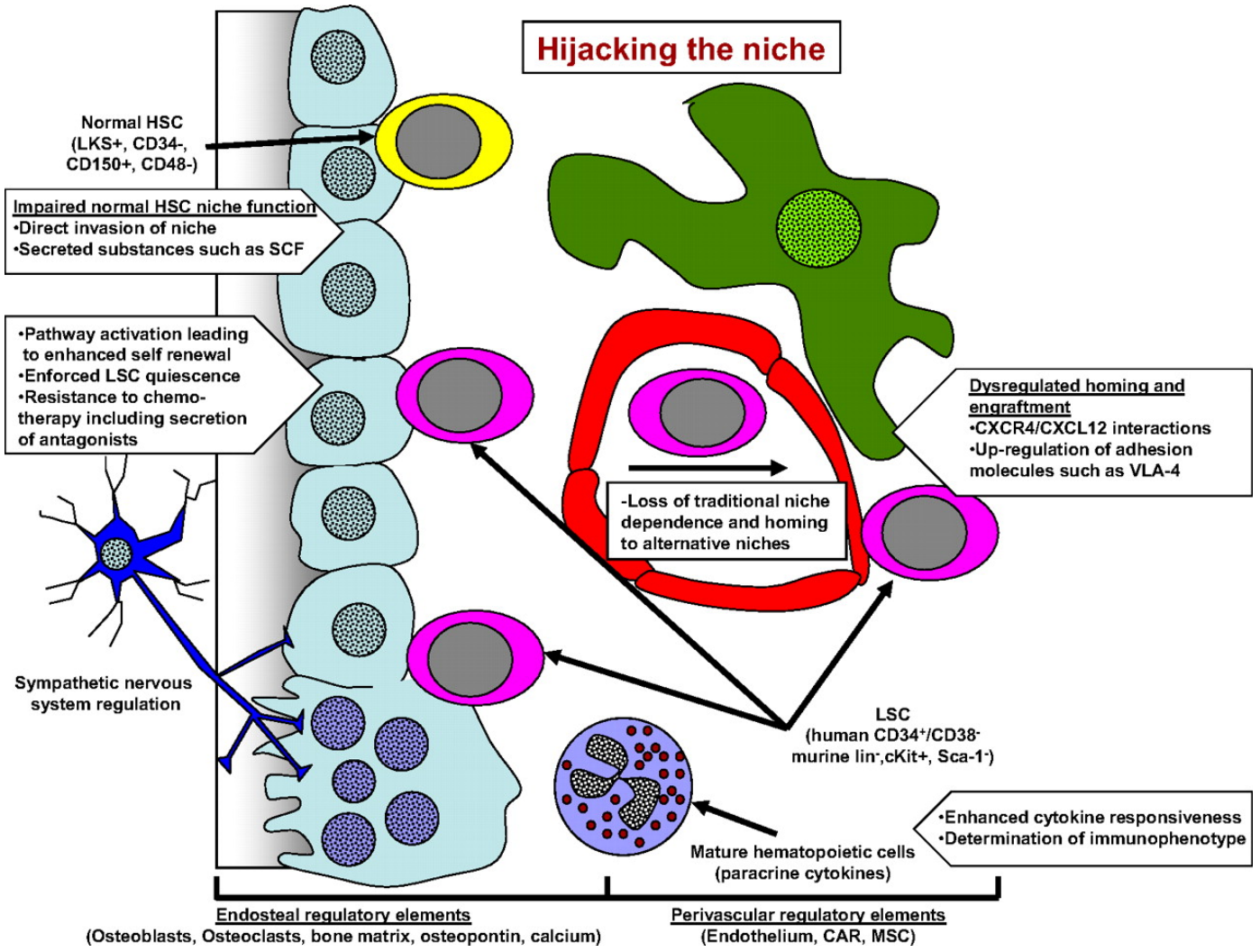
- Massive parallel DNA sequencing of a primary, normal karyotype, AML with minimal maturation (M1) and matched skin genome
- Identification of 12 acquired mutations within coding sequences of genes & 52 somatic point mutations in conserved or regulatory portions of genome:
  - 4/64 are recurrent mutations
  - Two were previously known: *NPM1*, *NRAS*
  - *Two previously unknown: IDH1 (15/187)*

Mardis ER, et al (n=62!) NEJM 2009

Design


FLT3-screening
R
Induction
DNB 60mg/m2 d1-3
ARA-C 200 mg/m2 d1-7
Midostaurin 50 mg po BID (d8-21)
1-2 cycles
CR
Consolidation
ARA-C 3 g/m2 BID d1-3-5
Midostaurin 50 mg po BID (d8-21)
4 cycles
Continuation
Midostaurin 50 mg po BID (d 1-14 of 28-day cycle)
12 cycles
Induction
DNB 60mg/m2 d1-3
ARA-C 200 mg/m2 d1-7
Placebo
1-2 cycles
CR
Consolidation
ARA-C 3 g/m2 BID d1-3-5
Placebo
4 cycles
Continuation
Placebo
12 cycles

AML cells disrupt normal hematopoietic niche



Lane SW et al, Blood 2009

Integrated diagnosis of AML: final considerations

- Sample banking: every AML case is a treasure!
- Universal applicability of highly sophisticated diagnosis – reference labs
- Consensus for molecular response assessment
- *Global integration* – neoplastic population, microenvironment, patient's unique genetic background

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