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Contribution of microarrays in Acute Myeloid Leukemia diagnostics

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Contribution of microarrays in Acute Myeloid Leukemia diagnostics

1. Current molecular diagnostics of acute myeloid leukemia (AML)

   Cytogenetics
   Mutations
   Expression markers

2. Genome-wide molecular approaches and molecular diagnostics of AML

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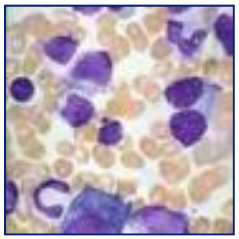
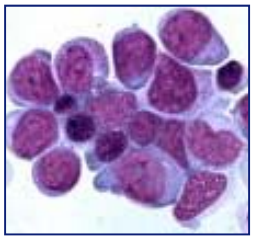
AML diagnostics

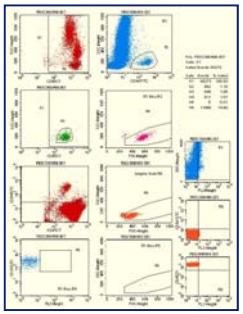
Morphology



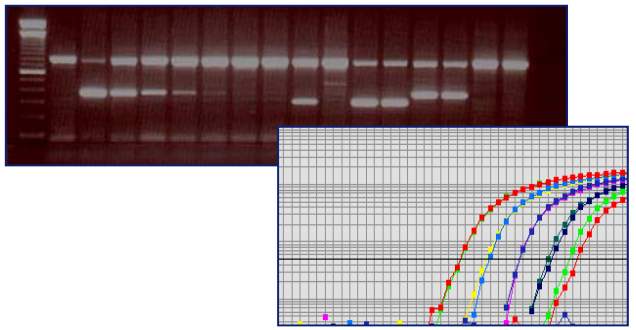


Cytogenetics

Immunophenotyping



Molecular diagnostics



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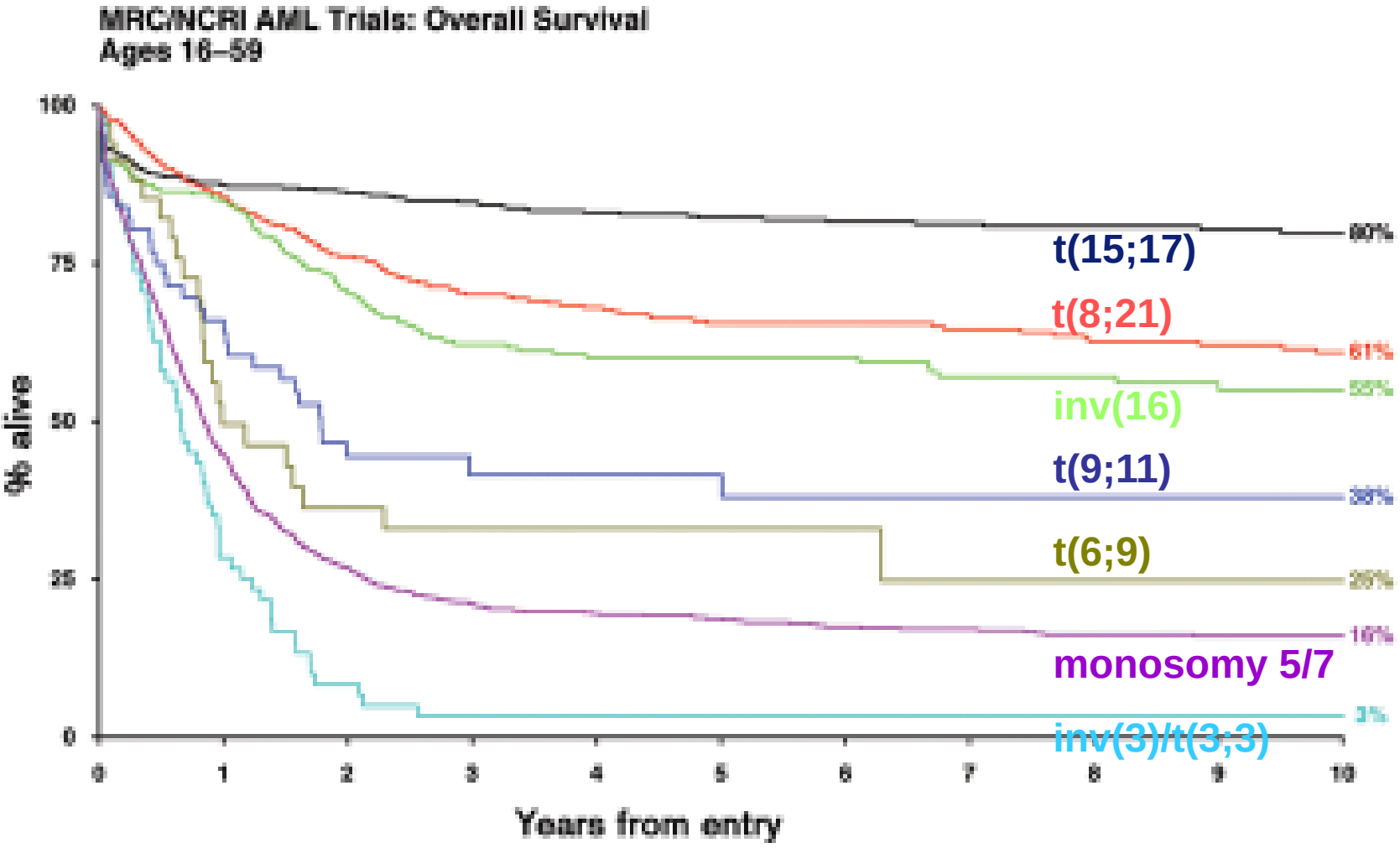
AML survival and cytogenetics



Grimwade en Hills, 2009

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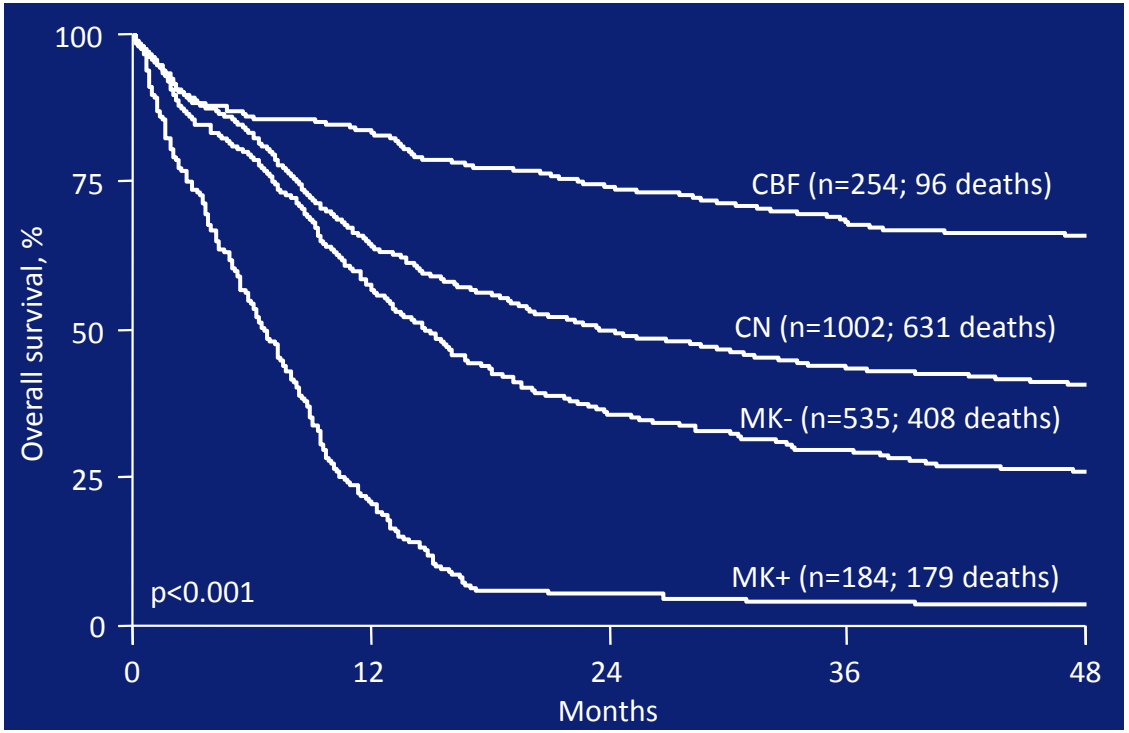
AML with monosomal karyotype

HOVON



Breems et al., 2008

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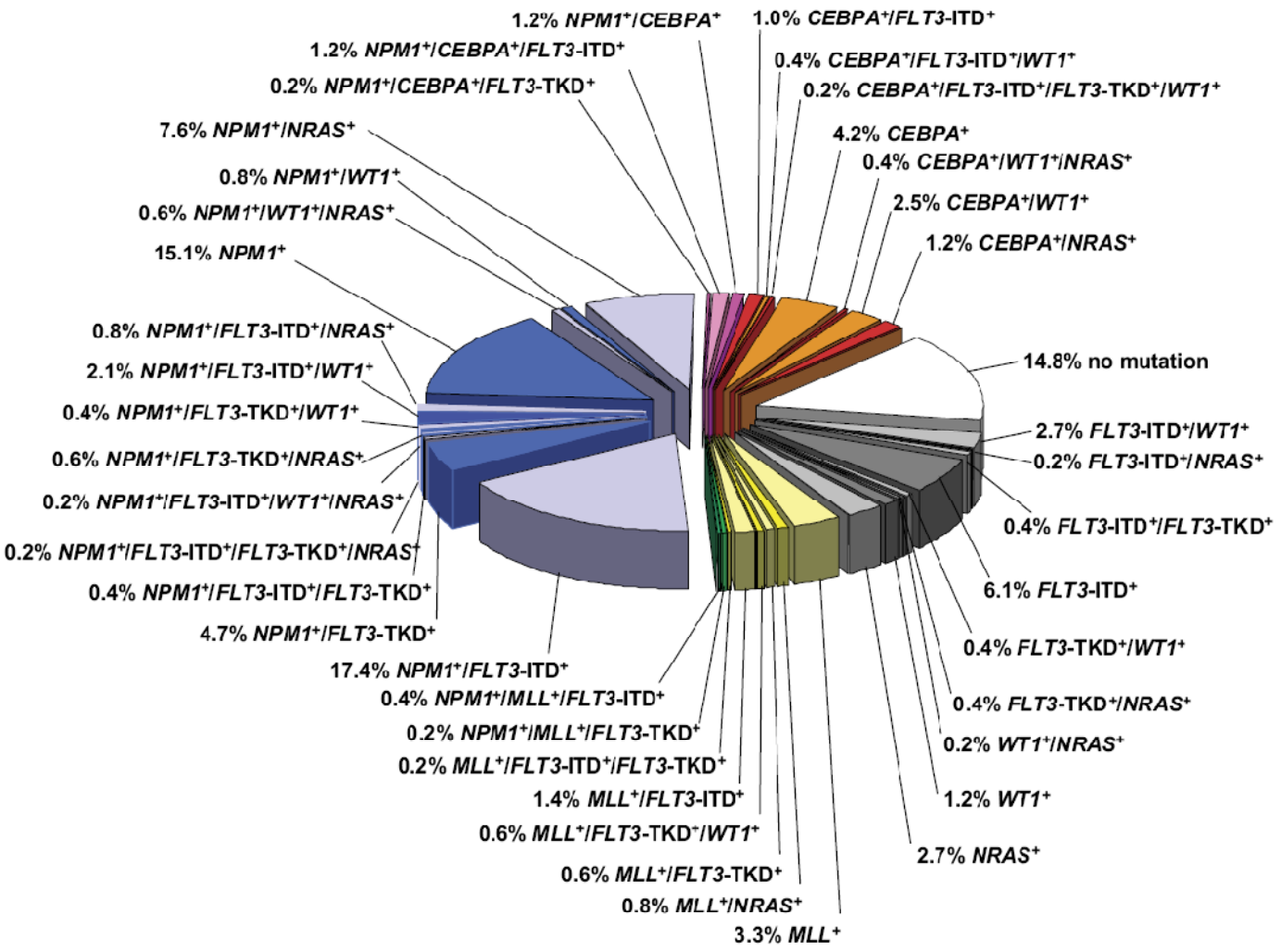
Heterogeneity AML – molecular aberrations



Dohner et al., 2010

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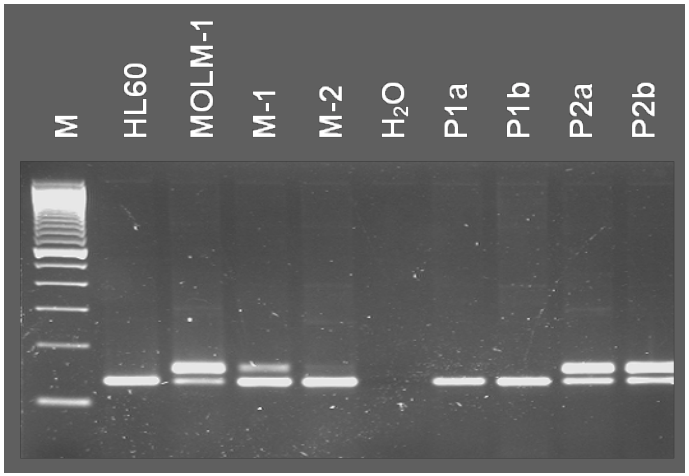
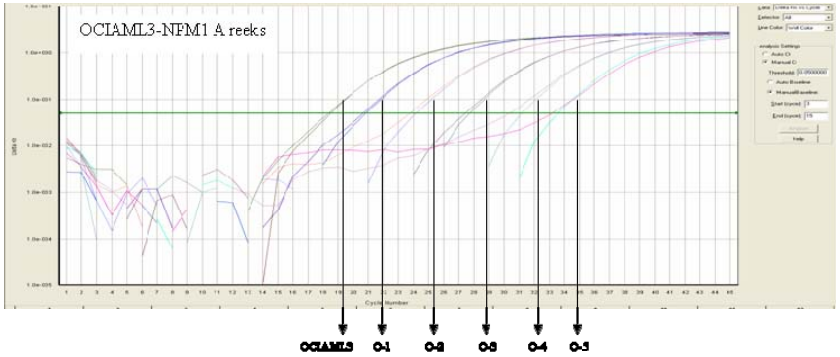
Molecular diagnostics *FLT3* ITD and *NPM1* mutation

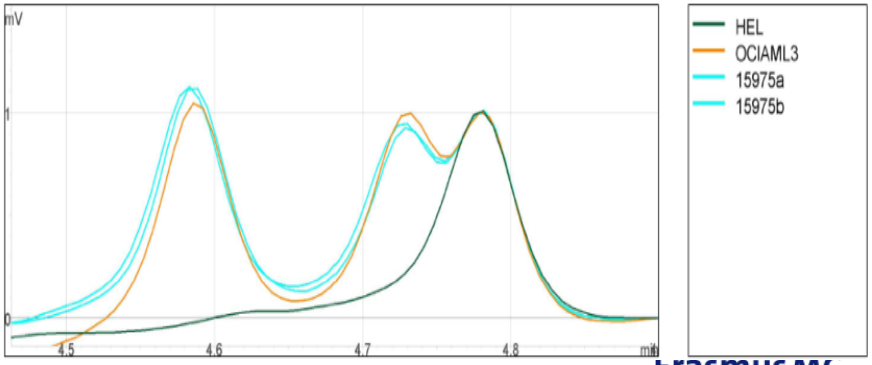
RT-PCR *FLT3* ITD



RQ-PCR *NPM1* mutation ABD



dHPLC WAVE– *NPM1* mutation



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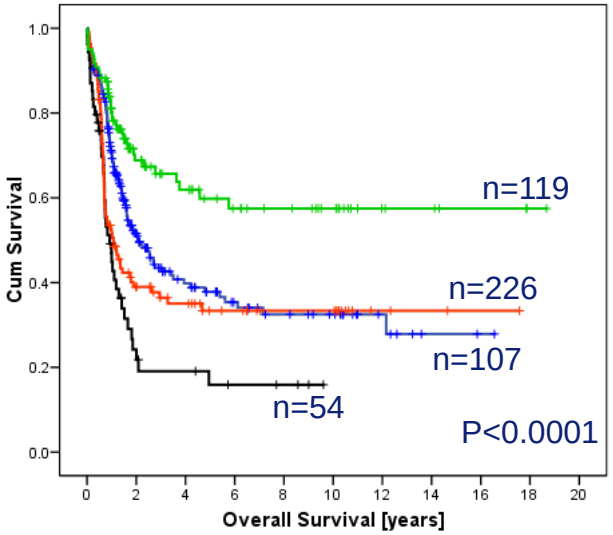
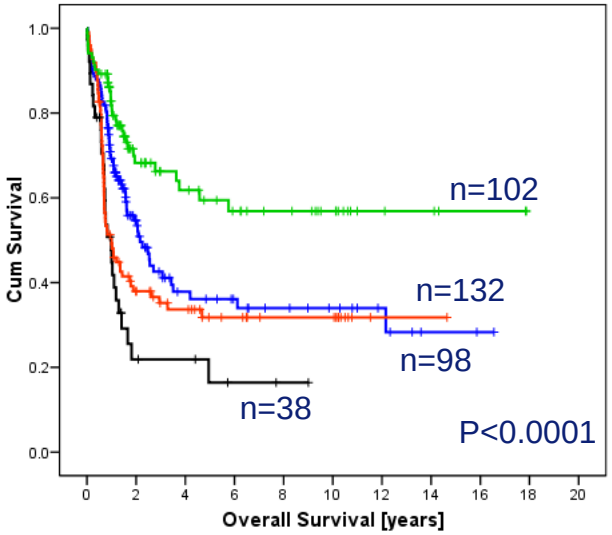
AML survival in *FLT3* ITD en *NPM1* mutation subgroups
HOVON4(A), -29, 42(A)

Intermediate karyotype



Normal karyotype



- ***FLT3* wt, *NPM1* wt**
- ***FLT3*-ITD, *NPM1* mut**
- ***FLT3* wt, *NPM1* mut**
- ***FLT3*-ITD, *NPM1* wt**

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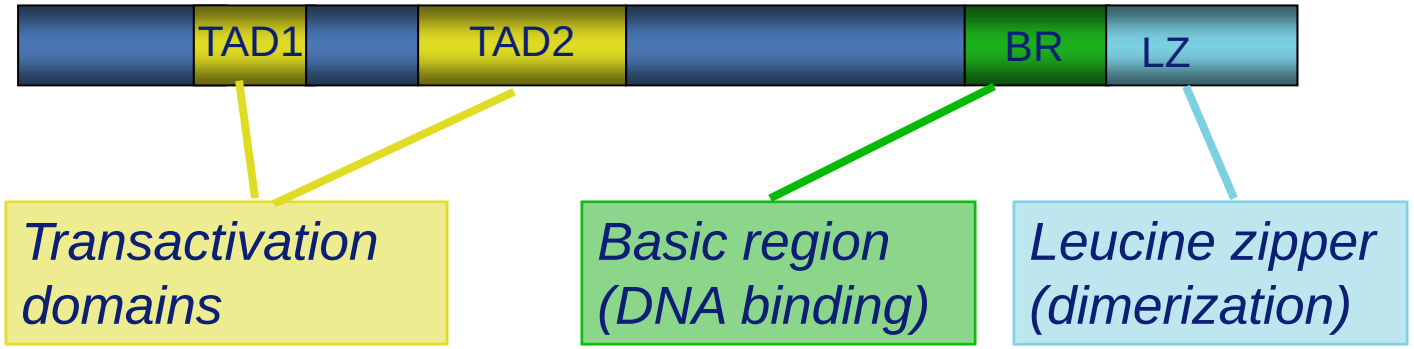
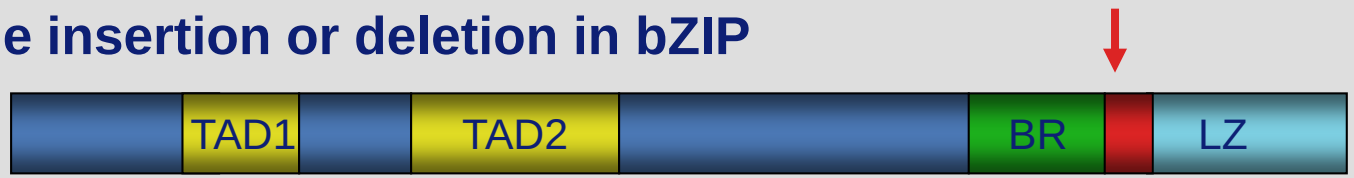
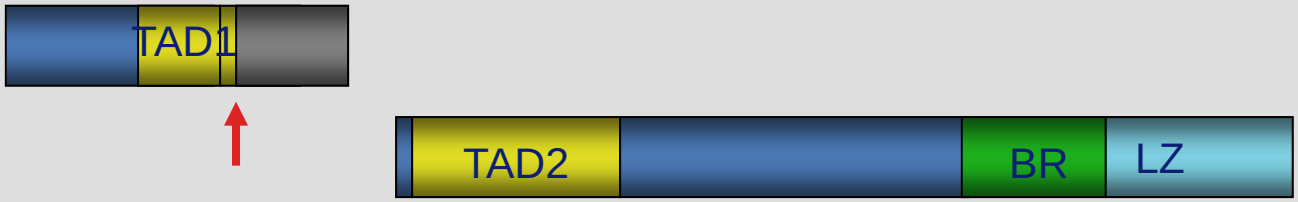
Most common types of *CEBPA* mutations in AML



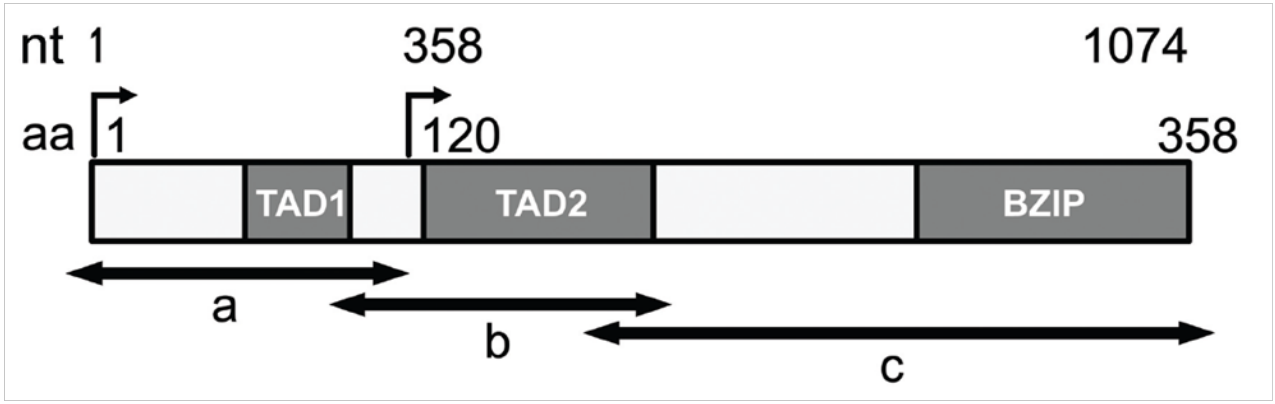
In-frame insertion or deletion in bZIP

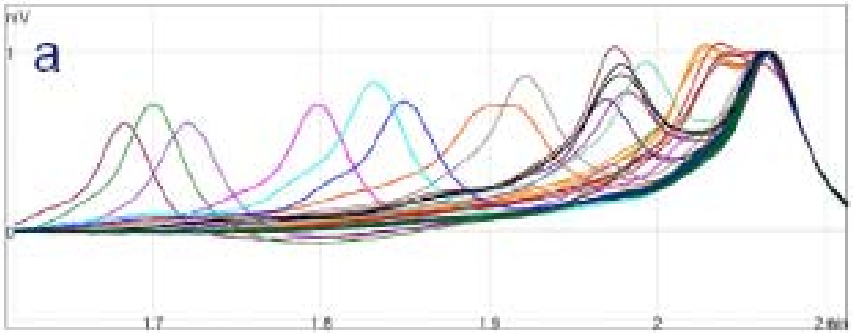


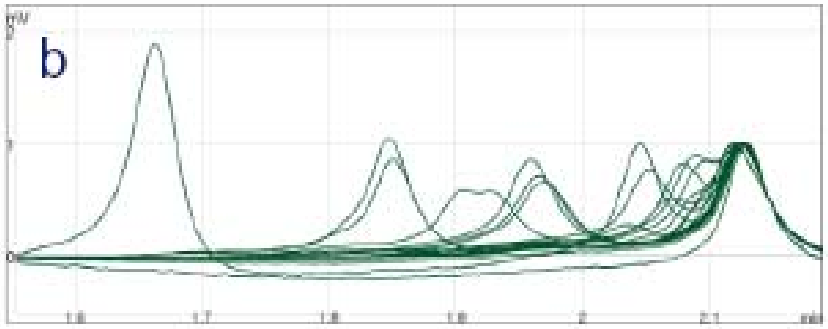
N-terminal truncation mutation

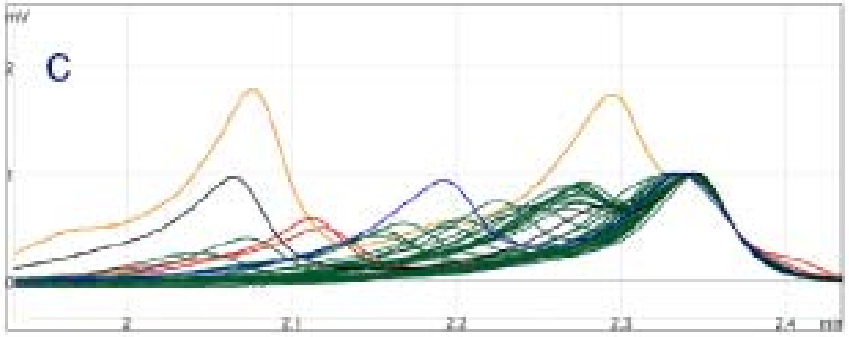


dHPLC assay to detect *CEBPA* mutations









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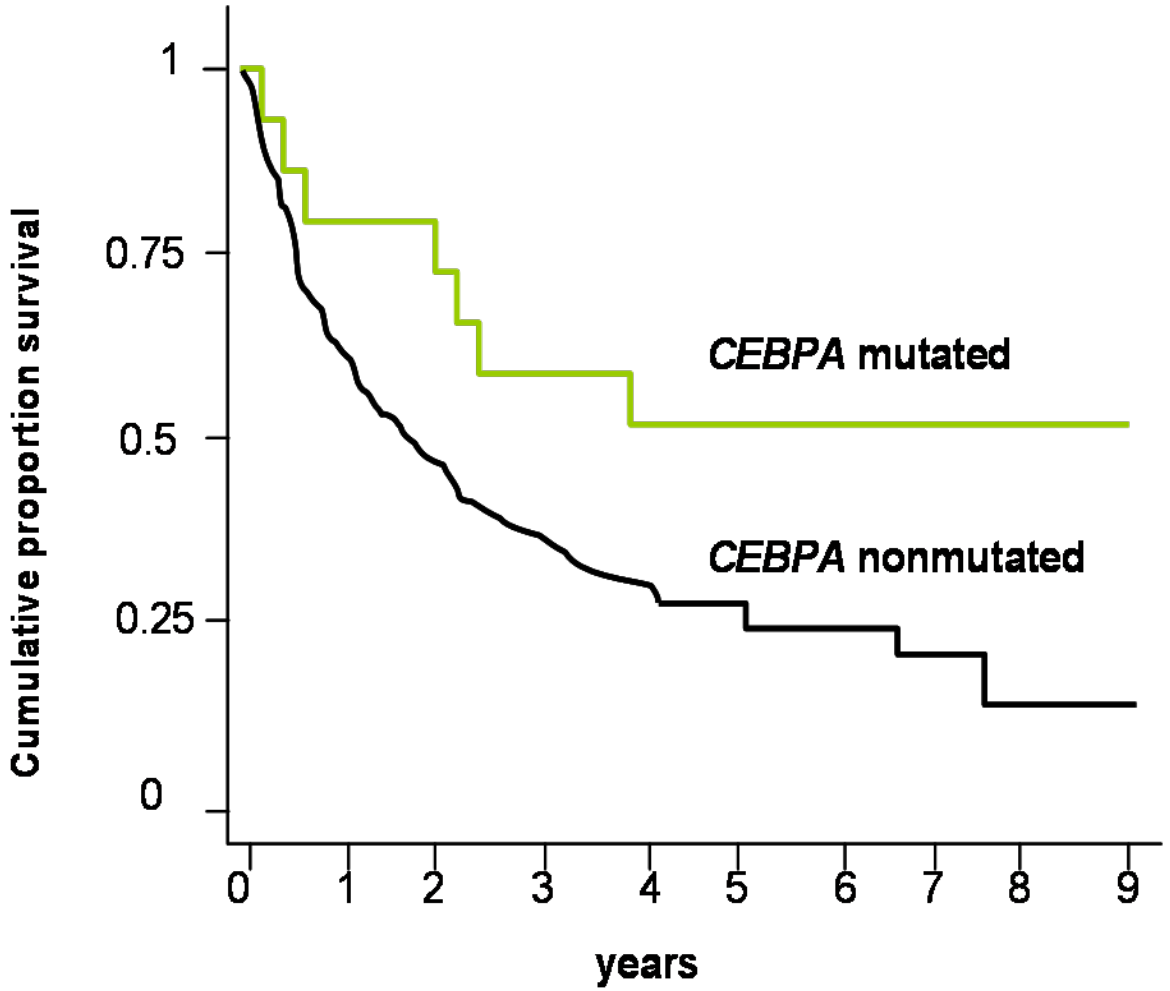
Clinical outcome of AML patients with *CEBPA* mutations



Preudhomme C. et al. (Blood 2002)

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Risk-stratification HOVON102

Risk		Definition	% pts (n=424)
Good	G1 G2 G3 G4	**t(8;21)**, WBC<=20 **inv16** MI-, **CEBPA+** MI-, **FLT3ITD-/NPM1+**, CRe	5.4 % 7.3 % 5.2 % 10.1 %
Intermediate	I1 I2	**t(8;21)**, WBC>20 CN –X –Y, CRe	2.8 % 15.8 %
Bad	B1 B2	CN –X –Y, not CRe CA, non CBF, MI-, no abn3q, **EVI1-**	22.9 % 13.0 %
Very Bad	VB1 VB2 VB3	Non CBF, MI+ or abn3q26 Non CBF, abn3q26 Non CBF, **EVI1+**	6.4 % 1.7 % 9.4 %

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AML: (cyto)genetic aberrations and prognosis

Good		Poor
	Cytogenetics	
t(8;21) inv(16) t(15;17)	normal -Y t(9;11)	-7, -5 t(3;3), inv(3) t(6;9), t(v;11) complex
	Mutations	
NPM1 (*FLT3* wild type) *CEBPA*		TET2 ASXL1 IDH1 *FLT3* ITD c-*KIT* (t(8;21)/inv(16)) *MLL* PTD
	Overexpression	
		EVI1 *BAALC*

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Microarrays and molecular diagnostics of AML

Can we use microarrays and possibly other types of genome-wide analyses to simplify AML diagnostics?

Can we use microarrays and possibly other types of genome-wide analyses to improve AML diagnostics?

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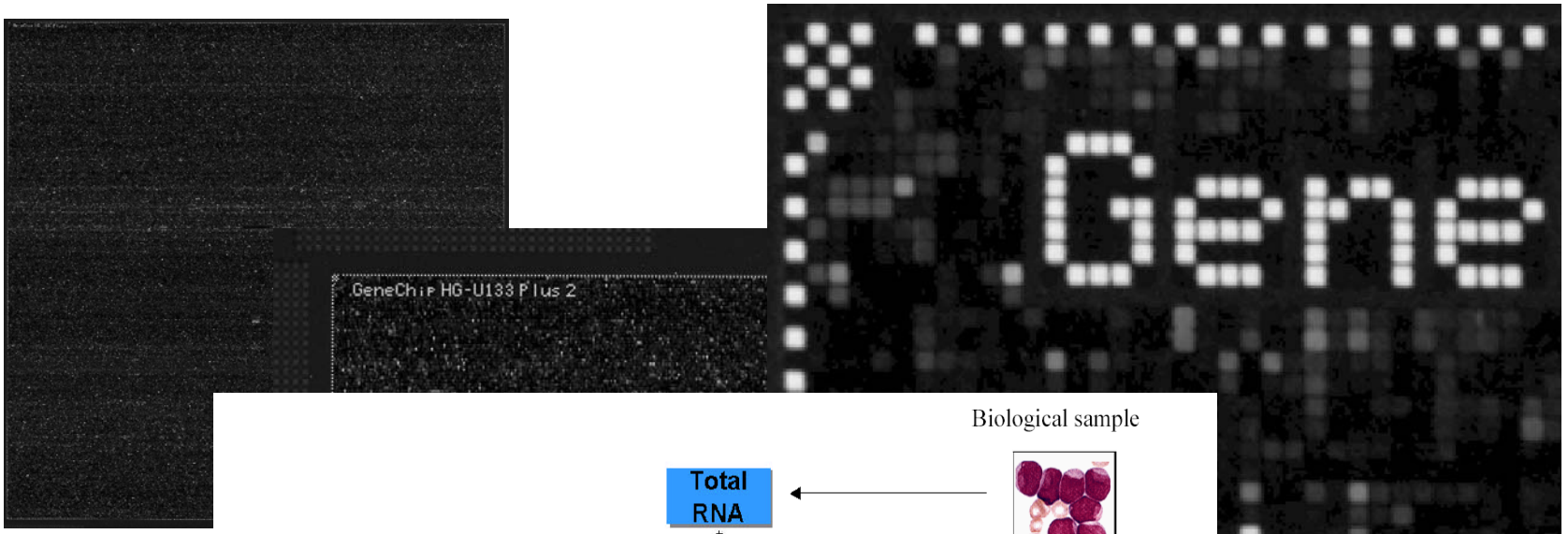
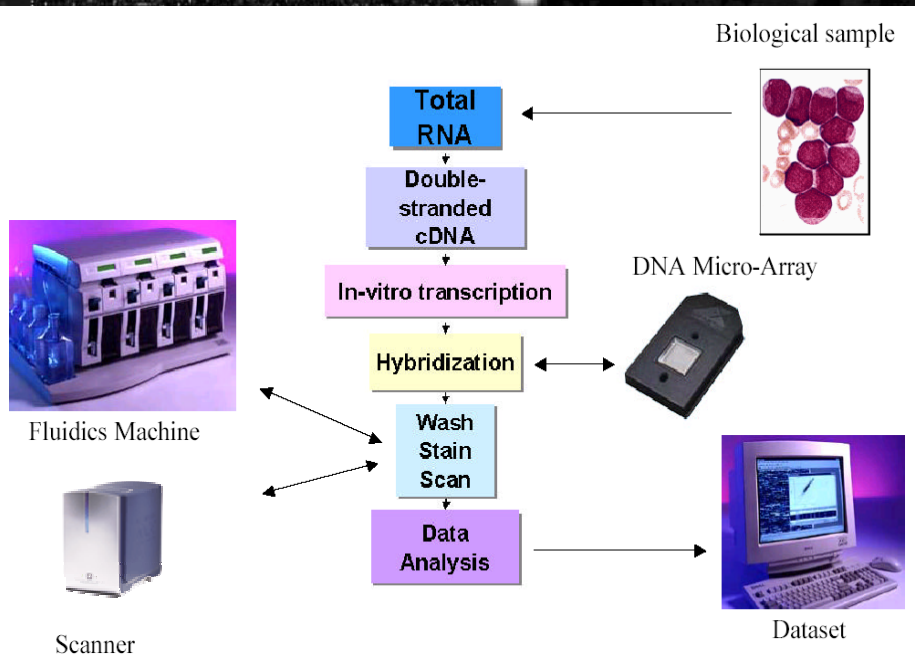
Affymetrix gene expression profiling





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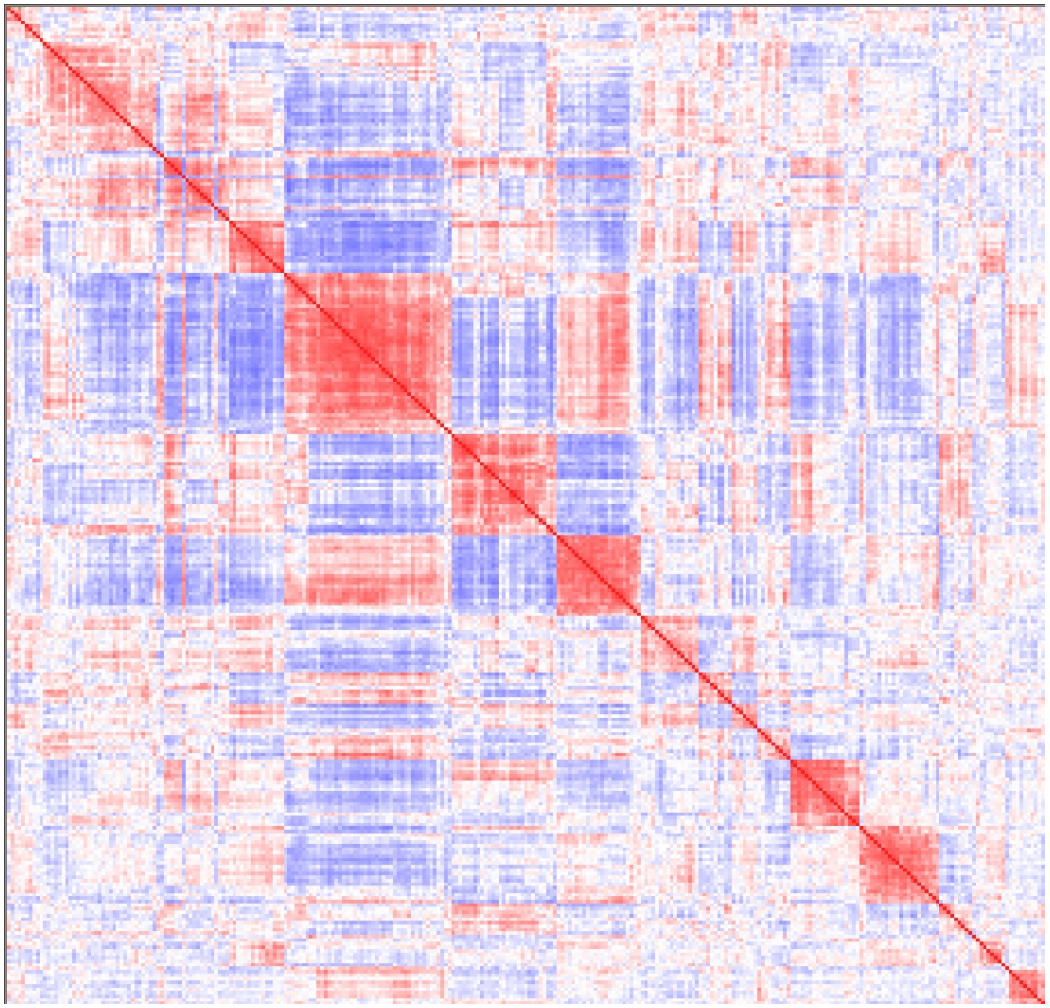
Class discovery in AML

Omniviz correlation view

Clustered order

285 AML patients



285 AML patients

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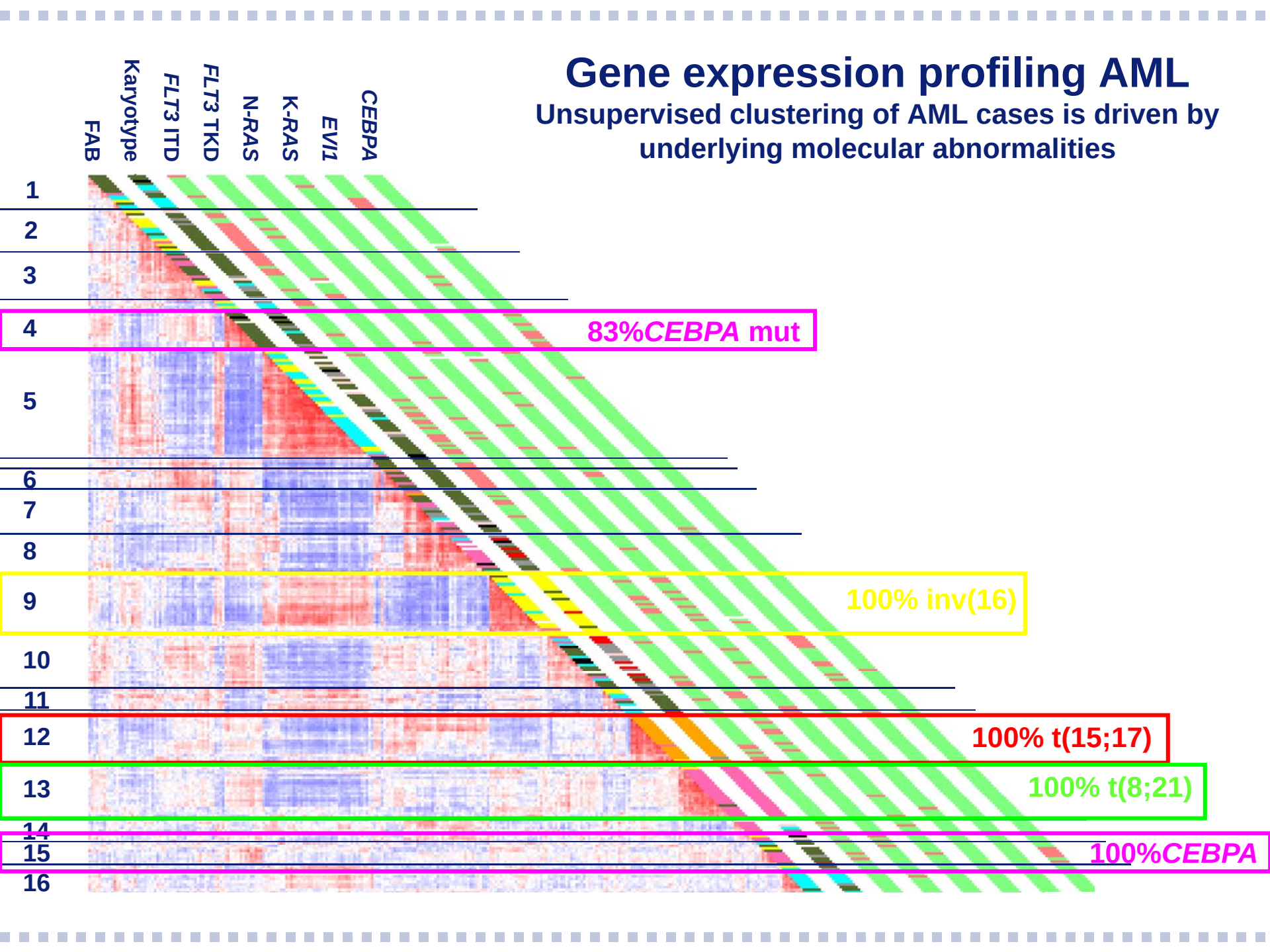
Gene expression profiling AML

Unsupervised clustering of AML cases is driven by underlying molecular abnormalities



Class prediction in AML (<60 years)

Cytogenetic and molecular abnormalities

Are we able to predict outcome or the prognostically relevant (cyto)genetic abnormalities using representative AML cohorts?

Affymetrix U133 Plus2.0 GeneChip

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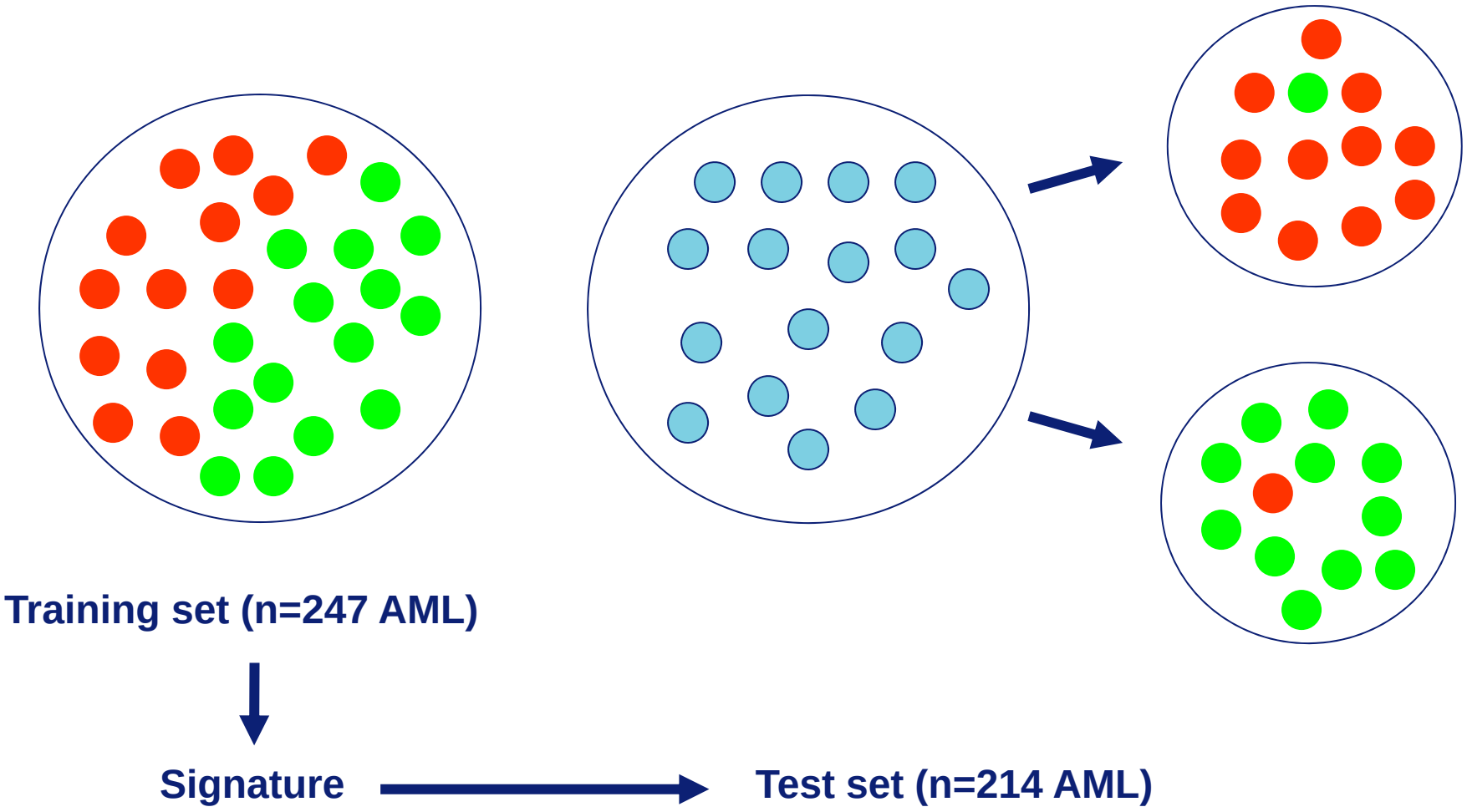
Prediction by gene expression profiling in AML



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Conclusion gene expression profiling AML

Class prediction

- Complete classification into good and poor treatment outcome possible based on gene expression profiling as single assay?

Classification error of 40% and higher

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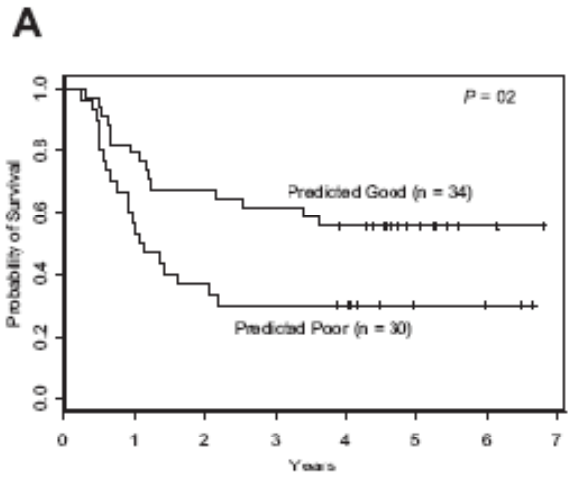
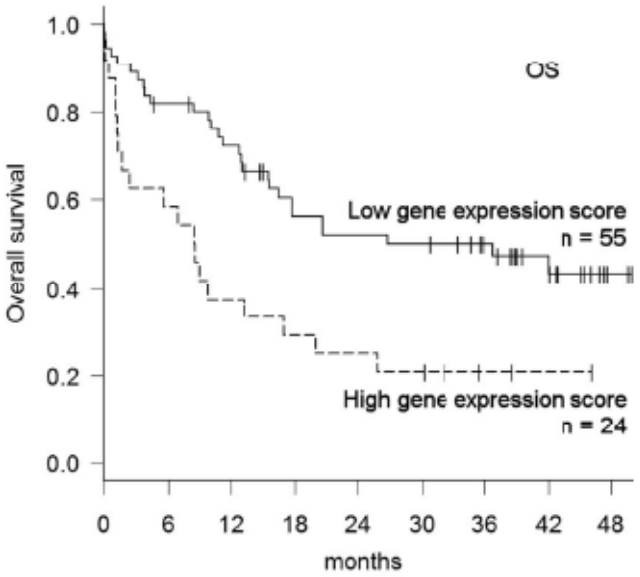
Gene expression signatures associated with OS in CN-AML





86 probe set signature

Metzeler et al 2008

C

Predicted Overall Survival Outcome Group

Actual Overall Survival Outcome	Good	Poor	
Alive	19	9	28
Dead	15	21	36
	34	30	64

Prediction Accuracy = 62.5%

101 probe set signature

Radmacher et al 2006

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Conclusion gene expression profiling AML

Class prediction

- Particular genetically defined subgroups, i.e., t(8;21), inv(16) and t(15;17) are predicted with high accuracy (positive and negative predictive value: 100%).

- *NPM1* and *CEBPA* mutations are predicted less accurate (positive predictive value: 94% and 70% and negative predictive value: 98 and 99%, respectively).

- Other recurrent molecular abnormalities are not accurately predictable using gene expression signatures.

Verhaak et al., 2009

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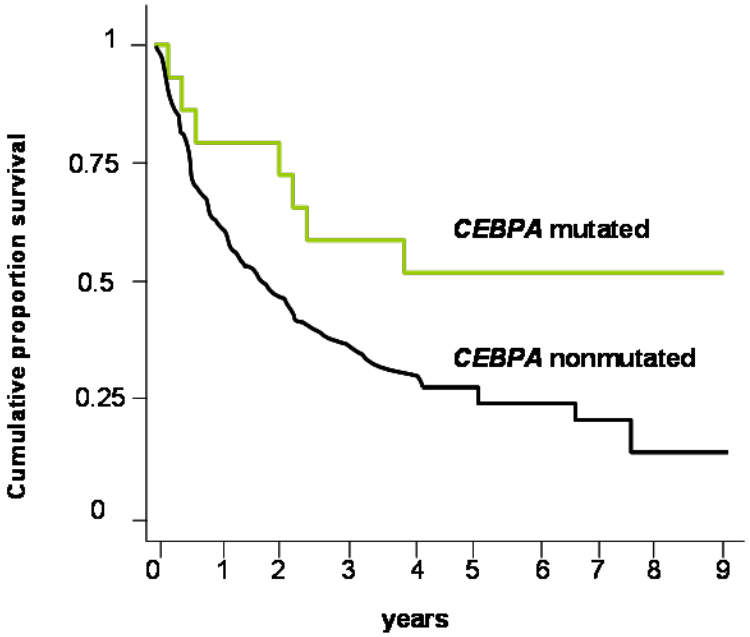
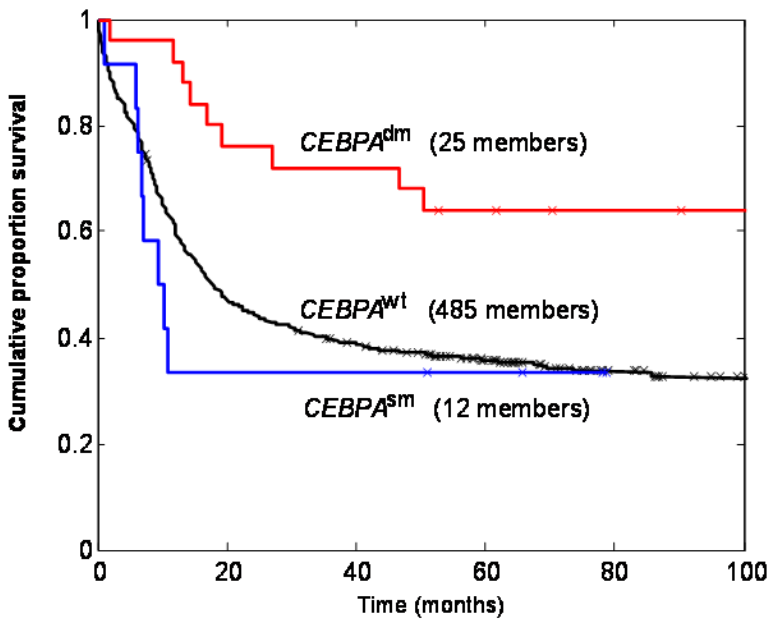
Treatment outcome of AML single and bi-allelic *CEBPA* mutations





Preudhomme C. et al. (Blood 2002)
Waalwijk van Doorn B. et al. (Hematology 2003)
Fröhling et al. (JCO 2003)
Schlenk, R.F et al. (NEJM 2008)

Wouters B. et al, (Blood 2009)
Dufour, A., et al. (J. Clin. Oncol. 2009)
Hou, H.A., et al. (Br. J. Cancer 2009)
Pabst, T., et al. (Br. J. Cancer, 2009)
Green, C.L., et al. (J. Clin. Oncol. 2010)

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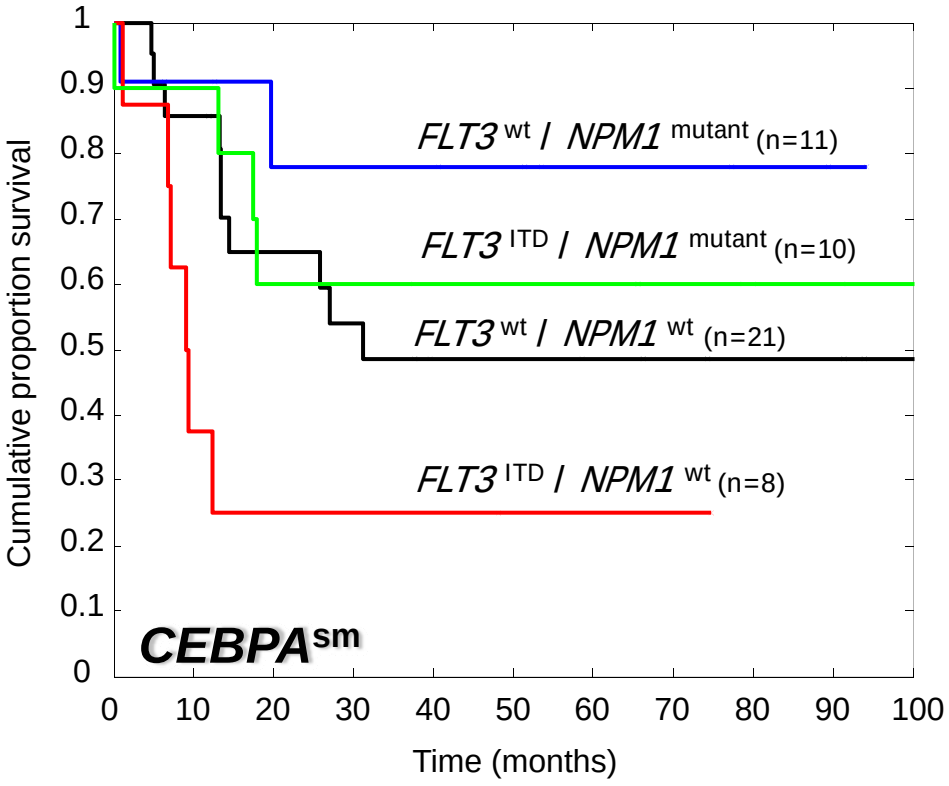
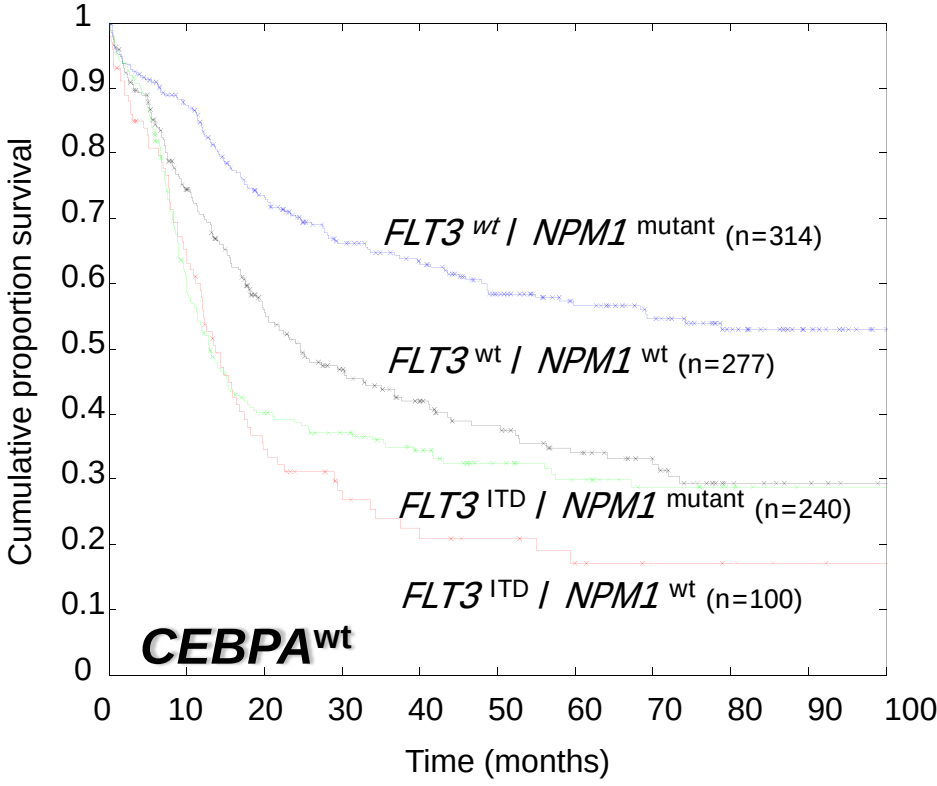
Clinical outcome of *smCEBPA* depends on concurrent mutations

Four composite subgroups: FLT3$^{(wt/ITD)}$ / NPM1$^{(wt/mutant)}$





Survival of *CEBPA*^{sm} follows the same trend as in *CEBPA*^{wt}

Clinical outcome in *CEBPA* subgroups

Multivariate analysis for overall survival (OS) in CN-AML

Variables	HR	95% CI		*P** value
Overall survival				
$CEBPA^{sm}$	0.73	0.48 - 1.11		.14
$CEBPA^{dm}$	0.36	0.24 - 0.56	<	.0001*
$FLT3^{ITD}$	1.75	1.44 - 2.13	<	.0001*
$FLT3^{TKD}$	0.87	0.62 - 1.20		.41
NPM1	0.57	0.47 - 0.69	<	.0001*
NRAS	1.07	0.81 - 1.42		.63
WBC count (x10^9/L)	1.27	1.05 - 1.53		.014*
Age	1.03	1.02 - 1.03	<	.0001*

The presence of a double *CEBPA* mutation is an independent prognostic factor whereas a single *CEBPA* mutation is not

AML with CEBPA^{dm} versus CEBPA^{sm} mutations

Is AML with CEBPA^{dm} different to AML with CEBPA^{sm}?

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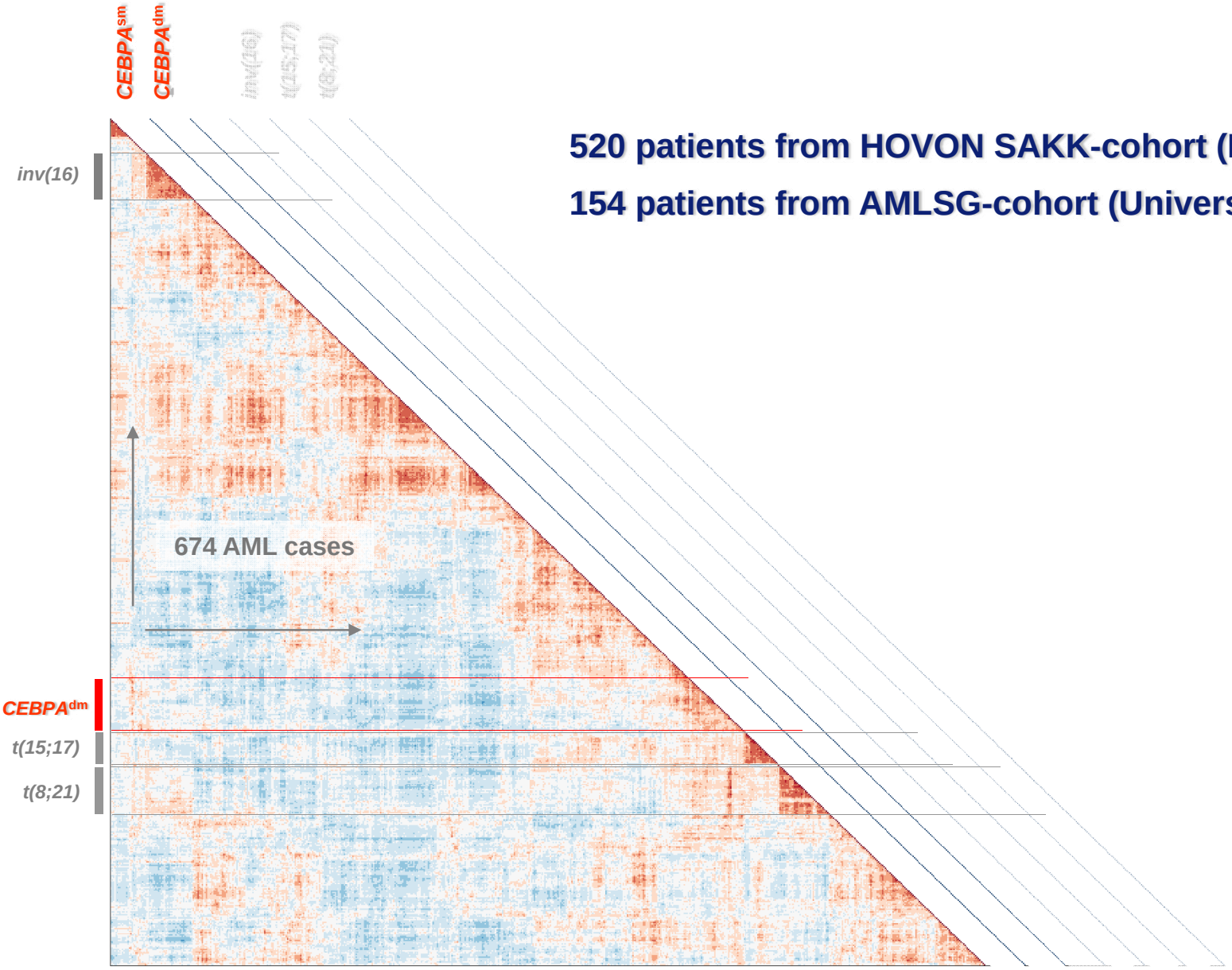
Unsupervised gene expression analyses

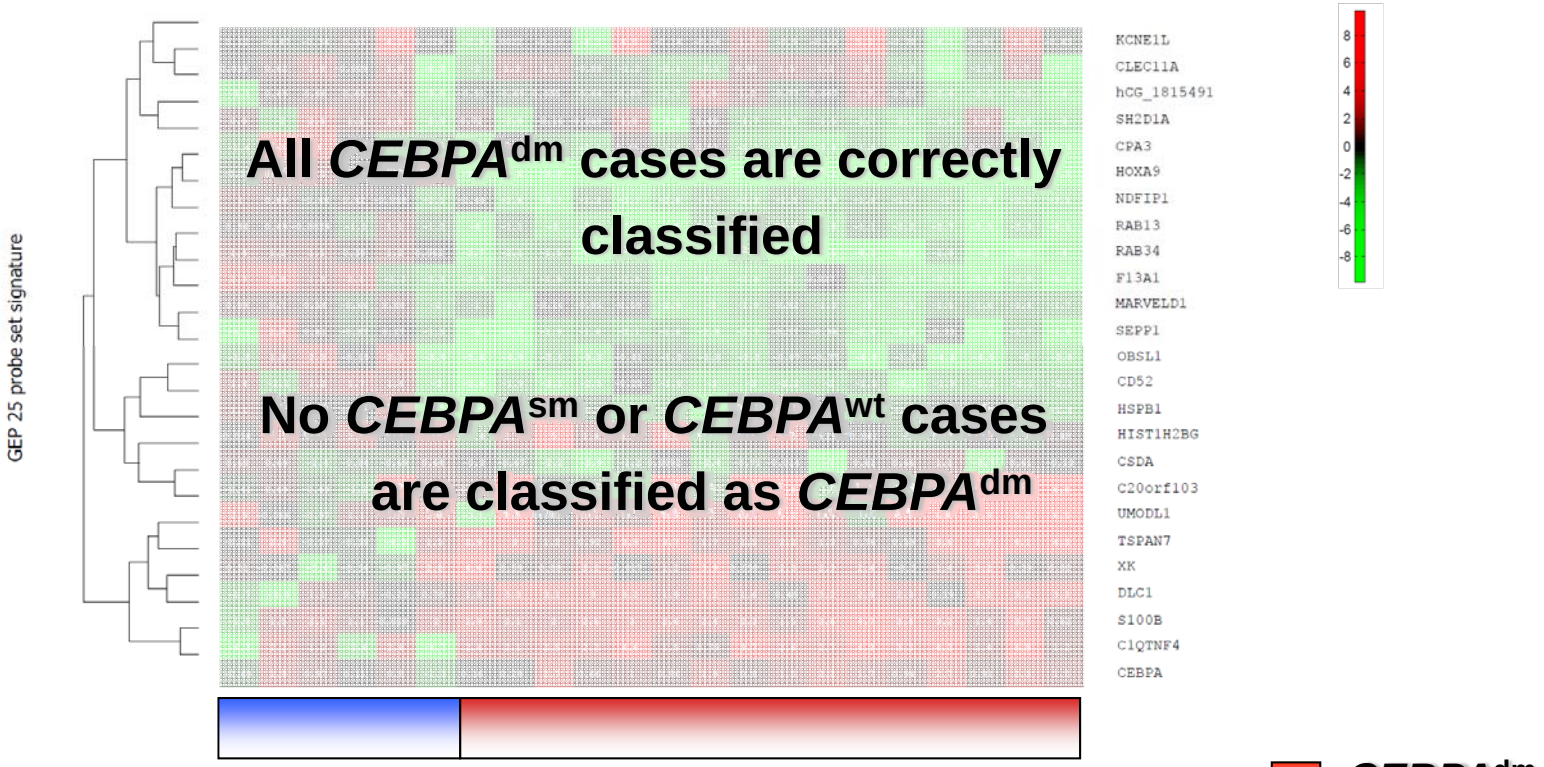


520 patients from HOVON SAKK-cohort (EMC)

154 patients from AMLSG-cohort (University of ULM)

Supervised analyses: prediction of *CEBPA*^{dm}

- Independent datasets (train: HOVON SAKK-, test: AMLSG-cohort)
- Logistic regression model with lasso regularization
- 25 probe set predictive signature



100% sensitivity and specificity

Conclusions

- ***CEBPA*^{dm}**
  - Is a unique subtype within AML
  - Is a prognostic factor which is associated with favorable clinical outcome
  - Significant lower incidence of concurrent mutations than wild-type *CEBPA*
  - Strong homogeneity in gene expression profile between patients
  - Classified with maximum specificity and sensitivity using GEP

AML: (cyto)genetic aberrations and prognosis

Good		Poor
	Cytogenetics	
t(8;21) inv(16) t(15;17)	normal -Y t(9;11)	-7, -5 t(3;3), inv(3) t(6;9), t(v;11) complex
	Mutations	
NPM1 (*FLT3* wild type) *CEBPA*		*FLT3* ITD c-*KIT* (t(8;21)/inv(16)) *MLL* PTD
	Overexpression	
		EVI1 *BAALC* ……

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Gene expression markers in AML

EVI1

BAALC

ERG

CD34

INDO1

FLT3

BCL2

MN1

WT1

ABCB1

Affymetrix U133Plus2.0

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Prognostic gene expression markers in AML

Mutation and expression markers in intermediate risk AML

- 442 patients under age 60 newly diagnosed with AML
- AML-specific mutations considered for the analysis
  - ITD at fms-like tyrosine kinase-3 gene : **FLT3ITD**
  - TKD at fms-like tyrosine kinase-3 gene : **FLT3TKD**
  - Abberations of CCAAT/enhancer binding protein alpha: **CEBP double mutation**
  - Insertion in the nucleophosmin : **NPM1**
  - Mutation at GTP-ase **NRAS**
- Selected expression markers considered for the analysis
  - **BAALC, CD34, MN1, ERG, ABCB1, BCL2, WT1, EVI1, FLT3, INDO1**

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Prognostic gene expression markers in AML

Mutation and expression markers in intermediate risk AML

	Cytogenetical subgroup						P-value
Mutation	t(8;21)	inv(16)	t(15;17)	CN	CA	MK	
FLT3 ITD							< 0.0001
No	32	37	17	110	101	27	
Yes	3	0	8	82	25	0	
FLT3 TKD							0.048*
Neg	34	30	19	172	114	26	
Pos	1	7	6	20	12	1	
N-RAS							< 0.0001*
Neg	32	25	25	174	119	24	
Pos	3	12	0	18	7	3	
NPM1							< 0.0001
Neg	35	37	25	79	110	26	
Pos	0	0	0	113	16	1	
CEBP DM							0.034*
Neg	35	37	25	174	121	27	
Pos	0	0	0	18	5	0	
FLT3 ITD×NPM1							⋆
Neg Neg	32	37	17	58	91	26	
Neg Pos	0	0	0	52	10	1	
Pos Neg	3	0	8	21	19	0	
Pos Pos	0	0	0	61	6	0	

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Prognostic gene expression markers in AML

Association between mutation and expression markers in intermediate risk AML

		BAALC	CD34	MN1	ERG	ABCB	BCL	WT	EVI	FLT3	INDO1
CEBP DM	p-value	0.062	**0.003**	**0.006**	**0.001**	**<0.001**	0.258	**0.008**	0.518	**0.002**	0.963
	Median difference	0.39	1.11	0.98	0.36	1.7	0.21	-0.52	-0.08	-0.62	0.05
FLT3ITD	p-value	**<0.001**	**0.005**	**<0.001**	0.832	**<0.001**	0.205	**<0.001**	**0.039**	**<0.001**	0.572
	Median difference	-0.51	-0.68	-0.92	0.01	-0.63	-0.03	0.44	-0.13	0.22	-0.06
FLT3TKD	p-value	**0.002**	**0.005**	0.058	0.188	**0.001**	0.126	0.352	0.525	0.152	**0.021**
	Median difference	-0.75	-1.10	-0.80	-0.06	-0.41	-0.24	0.19	-0.06	0.03	-0.40
NRAS	p-value	0.118	0.176	**0.001**	**0.034**	0.492	0.312	0.154	0.823	**0.047**	0.952
	Median difference	0.47	0.40	1.06	0.25	0.19	0.24	-0.12	0.07	-0.14	0.03
NPM1	p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.137	**0.004**	0.101
	Median difference	-1.22	-2.07	-2.07	-0.46	-0.83	-0.33	0.42	0.04	0.17	-0.15

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Prognostic gene expression markers in AML

Association between expression markers in intermediate risk AML

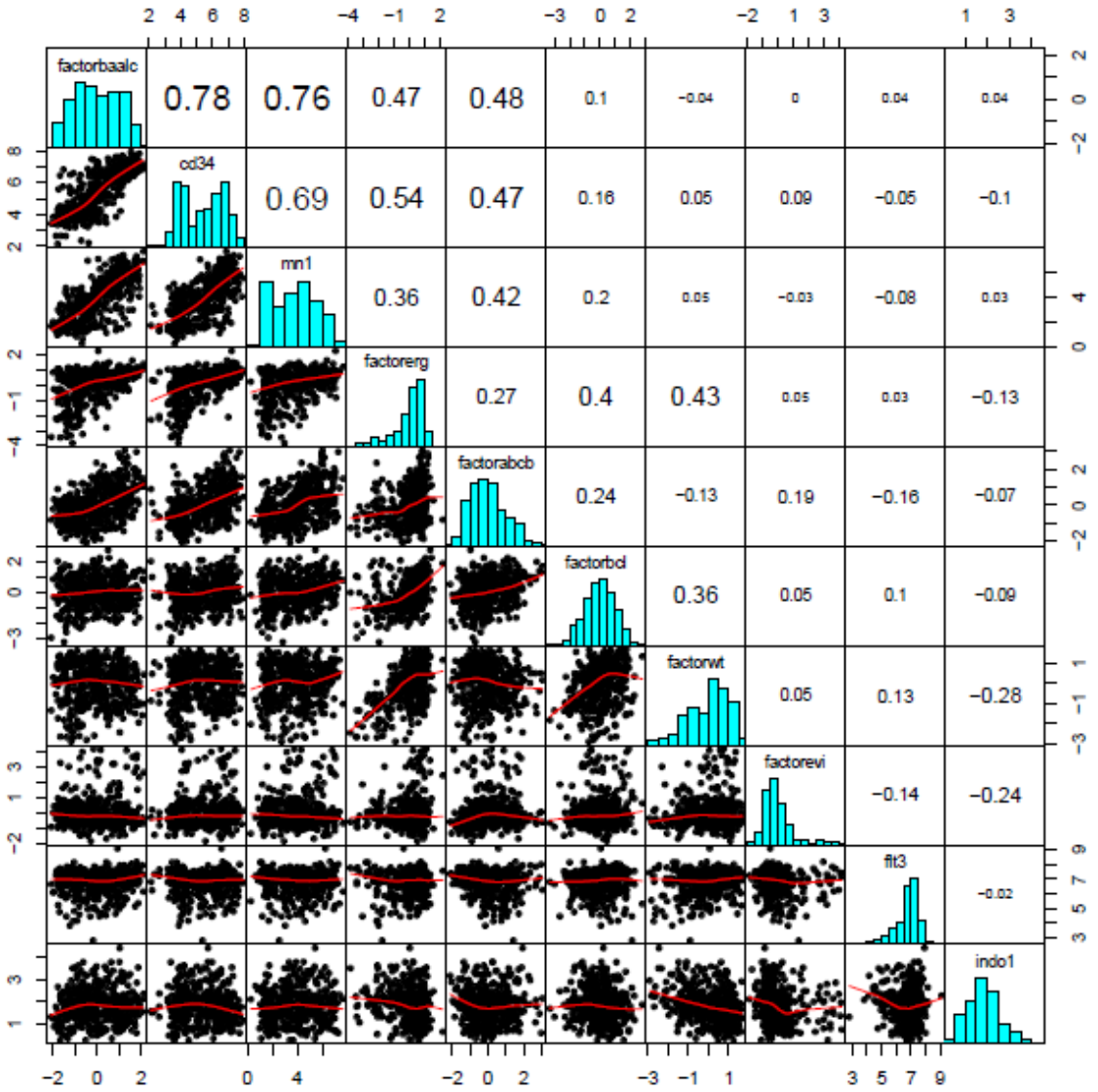
BAALC

CD34

MN1

ERG

FLT3



Erasmus MC

Prognostic gene expression markers in AML

Univariate survival analysis

Variable		Overall Survival					Event Free Survival				
		Hazard Ratio	Lower	Upper	p-value	Survival	Hazard Ratio	Lower	Upper	p-value	Survival
CEBP DM	-	0.38	0.19	0.74	**0.004**	0.328	0.45	0.25	0.81	**0.007**	0.244
	+					0.652					0.522
FLT3ITD	-	1.41	1.06	1.86	**0.017**	0.384	1.3	0.99	1.7	**0.059**	0.275
	+					0.287					0.242
NPM1	-	0.73	0.55	0.97	**0.03**	0.296	0.69	0.53	0.9	**0.006**	0.207
	+					0.432					0.347
FLT3TKD	-	0.82	0.51	1.32	0.418	0.341	0.74	0.47	1.16	0.192	0.255
	+					0.438					0.344
NRAS	-	0.94	0.57	1.54	0.798	0.349	1.23	0.77	1.94	0.386	0.268
	+					0.378					0.215
FLT3ITD× NPM1	+ +	1.03	0.72	1.47	0.875	0.355	0.9	0.64	1.27	0.549	0.312
	- +	0.63	0.42	0.94	**0.022**	0.515	0.64	0.45	0.93	**0.018**	0.386
	+ -	1.67	1.13	2.46	**0.01**	0.171	1.76	1.21	2.58	**0.003**	0.125
	- -					0.329					0.229
BAALC		1.32	1.14	1.52	**<0.001**	0.358	1.29	1.13	1.48	**<0.001**	0.267
CD34		1.28	1.15	1.41	**<0.001**	0.373	1.26	1.14	1.39	**<0.001**	0.278
MN1		1.13	1.05	1.23	**0.002**	0.358	1.14	1.05	1.23	**<0.001**	0.269
ERG		1.24	1.09	1.42	**0.001**	0.324	1.23	1.08	1.4	**0.001**	0.238
ABCB		1	0.88	1.14	0.983	0.352	0.98	0.87	1.11	0.793	0.264
BCL		1.01	0.89	1.15	0.861	0.352	1.03	0.91	1.16	0.644	0.265
WT		1.12	0.98	1.28	0.092	0.344	1.11	0.98	1.26	0.106	0.258
EVI		1.1	0.96	1.26	0.168	0.361	1.16	1.02	1.33	0.028	0.276
FLT3		0.97	0.83	1.14	0.746	0.354	0.93	0.8	1.08	0.348	0.27
INDO		0.9	0.76	1.08	0.254	0.353	0.92	0.78	1.08	0.3	0.265

Erasmus MC

Prognostic gene expression markers in AML

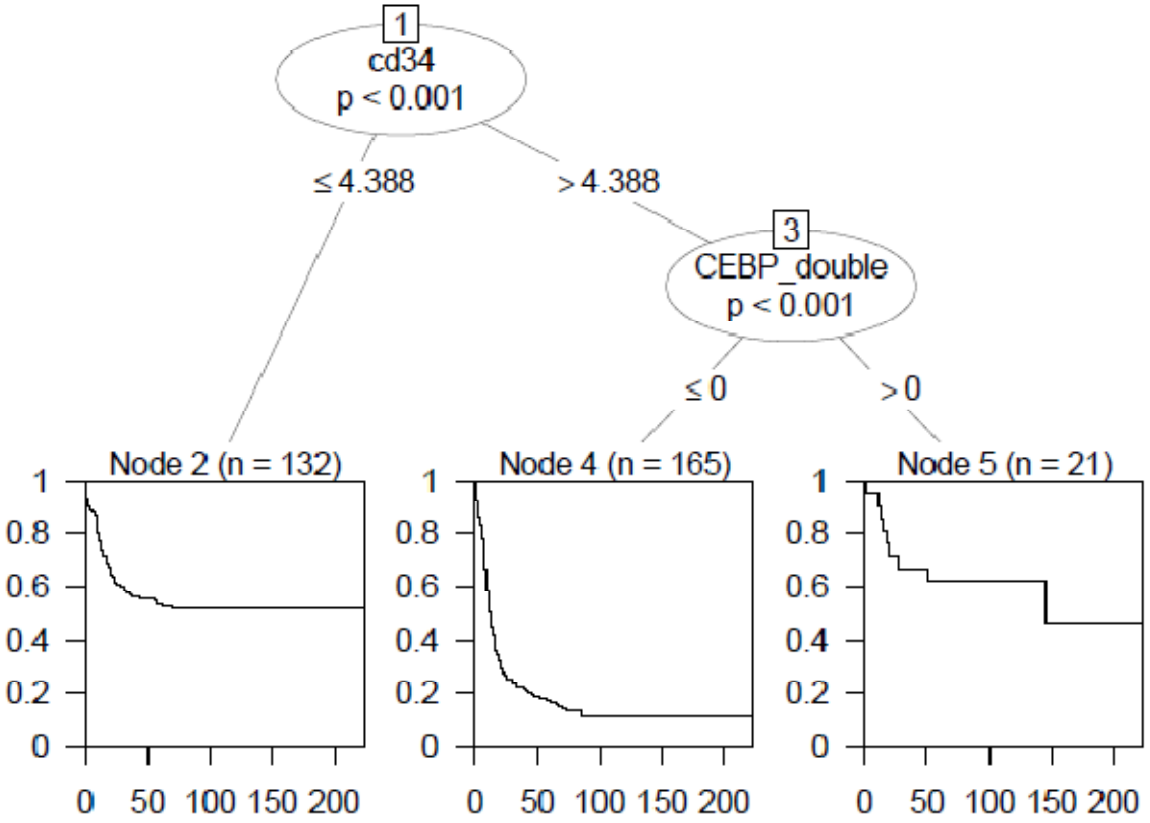
Multivariate survival analysis

	Overall survival				Event free survival			
Variable	Hazard Ratio	Lower	Upper	p-value	Hazard Ratio	Lower	Upper	p-value
BAALC	1.099	0.826	1.462	0.518	1.052	0.804	1.376	0.711
CD34	1.333	1.099	1.618	**0.004**	1.292	1.077	1.548	**0.006**
MN1	0.942	0.812	1.091	0.424	0.960	0.828	1.112	0.586
ERG	1.236	0.981	1.558	0.073	1.228	0.990	1.523	0.061
ABCB	0.925	0.768	1.113	0.409	0.890	0.748	1.060	0.192
BCL	0.828	0.699	0.982	**0.030**	0.862	0.732	1.014	0.072
WT	0.941	0.777	1.140	0.536	0.930	0.774	1.116	0.434
EVI	1.011	0.876	1.168	0.879	1.057	0.916	1.220	0.449
FLT3	0.919	0.766	1.102	0.363	0.914	0.766	1.092	0.322
INDO1	0.921	0.750	1.131	0.434	0.928	0.766	1.125	0.449
CEBP	0.299	0.161	0.557	**0.0001**	0.330	0.187	0.582	**0.0001**
FLT3ITD	1.265	0.813	1.970	0.298	1.536	1.001	2.357	**0.050**
FLT3TKD	1.200	0.714	2.017	0.491	1.007	0.616	1.645	0.979
NRAS	1.039	0.619	1.742	0.886	1.328	0.820	2.149	0.249
NPM1	0.737	0.434	1.251	0.259	0.741	0.450	1.221	0.239
FLT3ITD:NPM1	1.214	0.646	2.281	0.546	0.855	0.470	1.557	0.609

Erasmus MC

Prognostic gene expression markers in AML

Risk-stratification modeling



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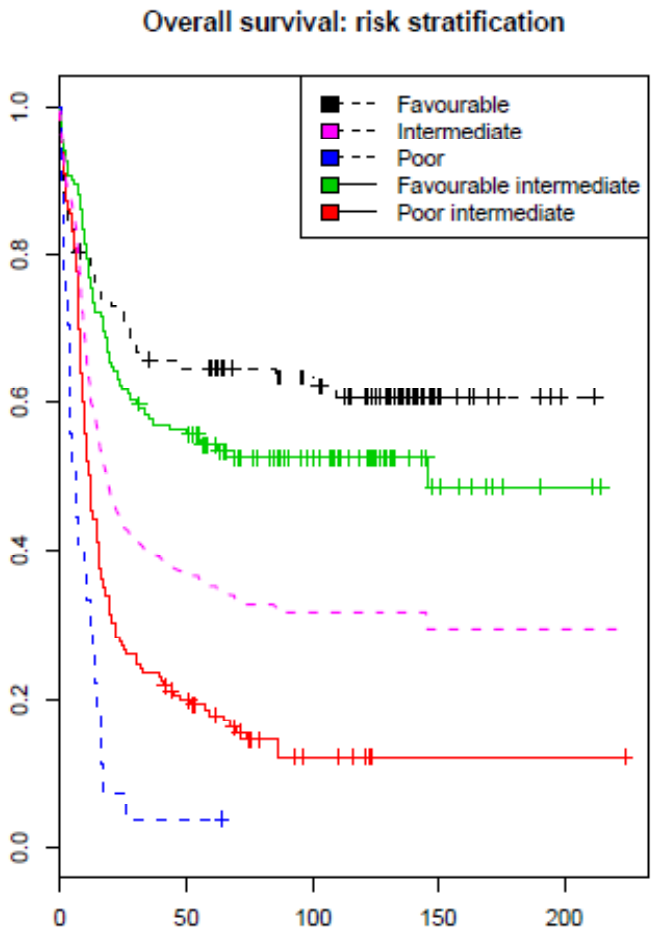
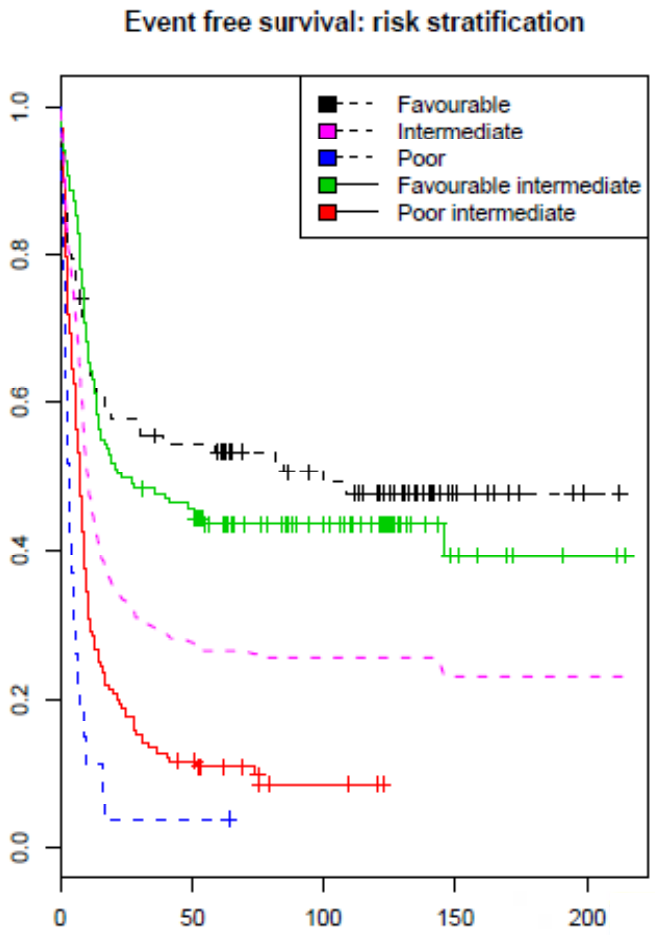
Prognostic gene expression markers in AML

Risk-stratification in intermediate-risk AML





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Conclusions

- We have confirmed prognostic ability of some established markers in AML
- We have demonstrated that **CD34 has dominant predictive effect**
- In the hierarchy of importance, **CEBPDM** is the second most **important marker**
- The combination of CD34 and CEBPDM can contribute in risk stratification of the intermediate group

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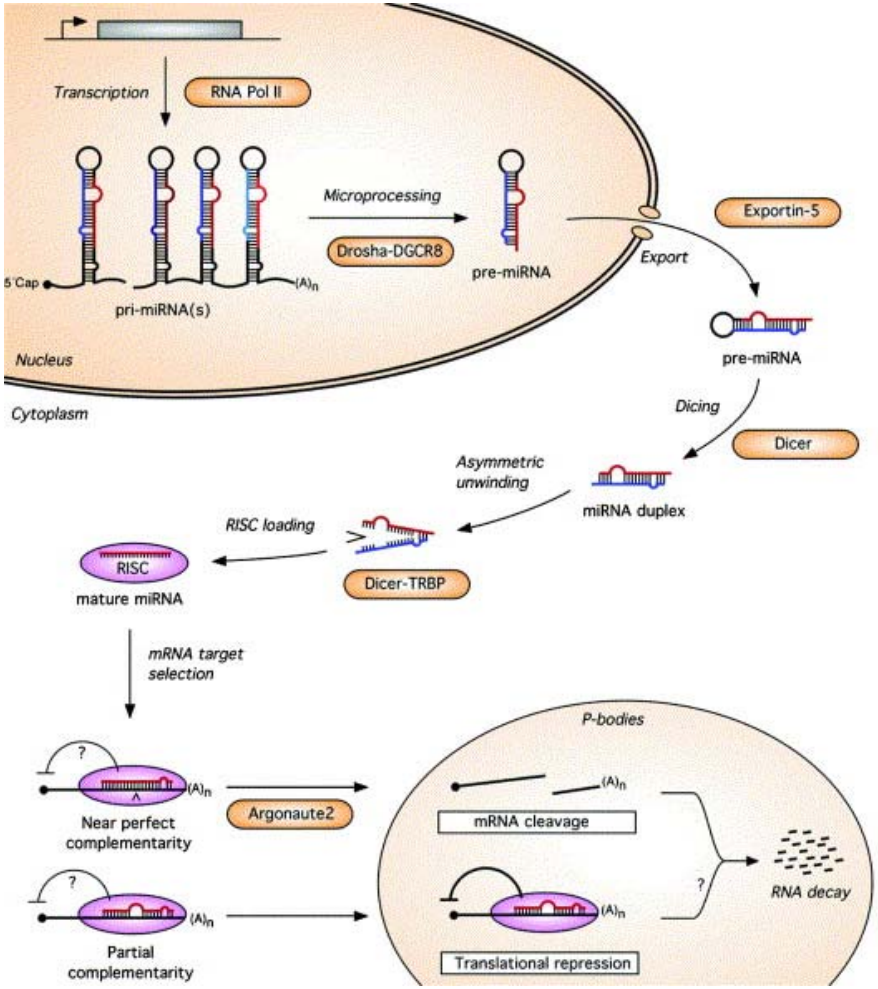
microRNAs in AML

Small conserved RNAs (20-23 nt)

Non-protein coding

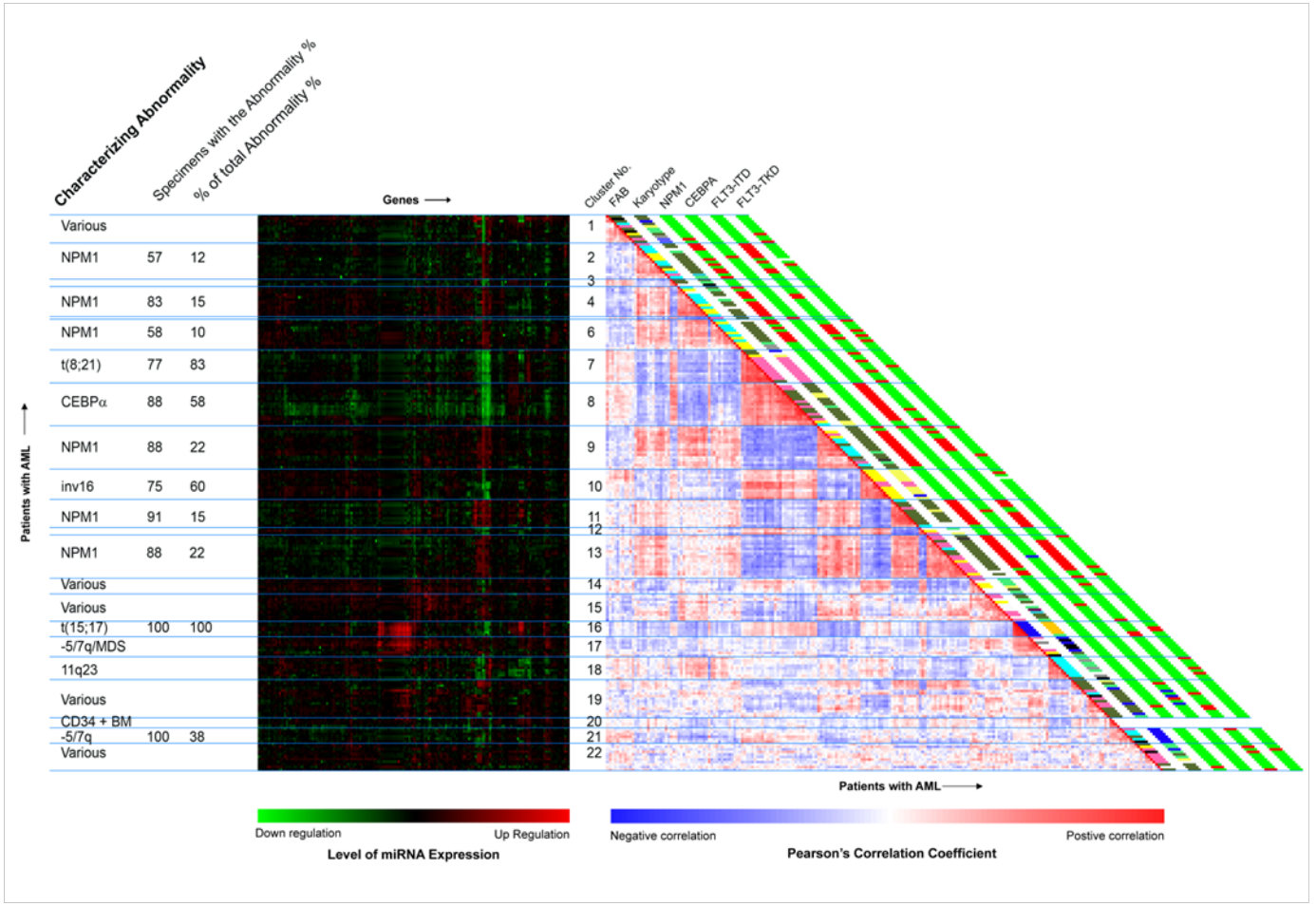
Regulate translation

Bind 3’ UTR mRNA



MicroRNA Expression Profiling (GEP) in AML

Unsupervised clustering and class comparison



Jongen et al., 2008

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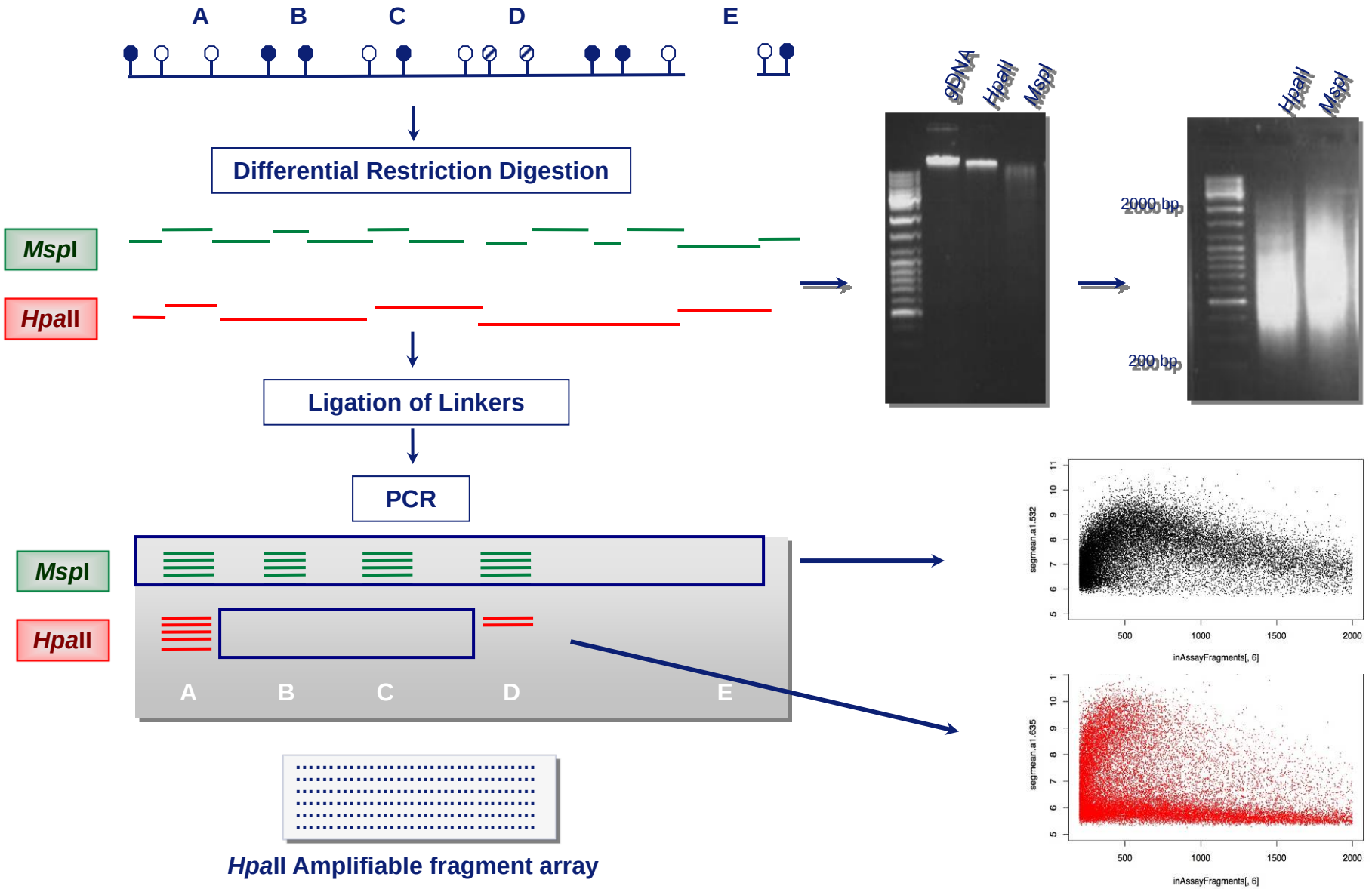
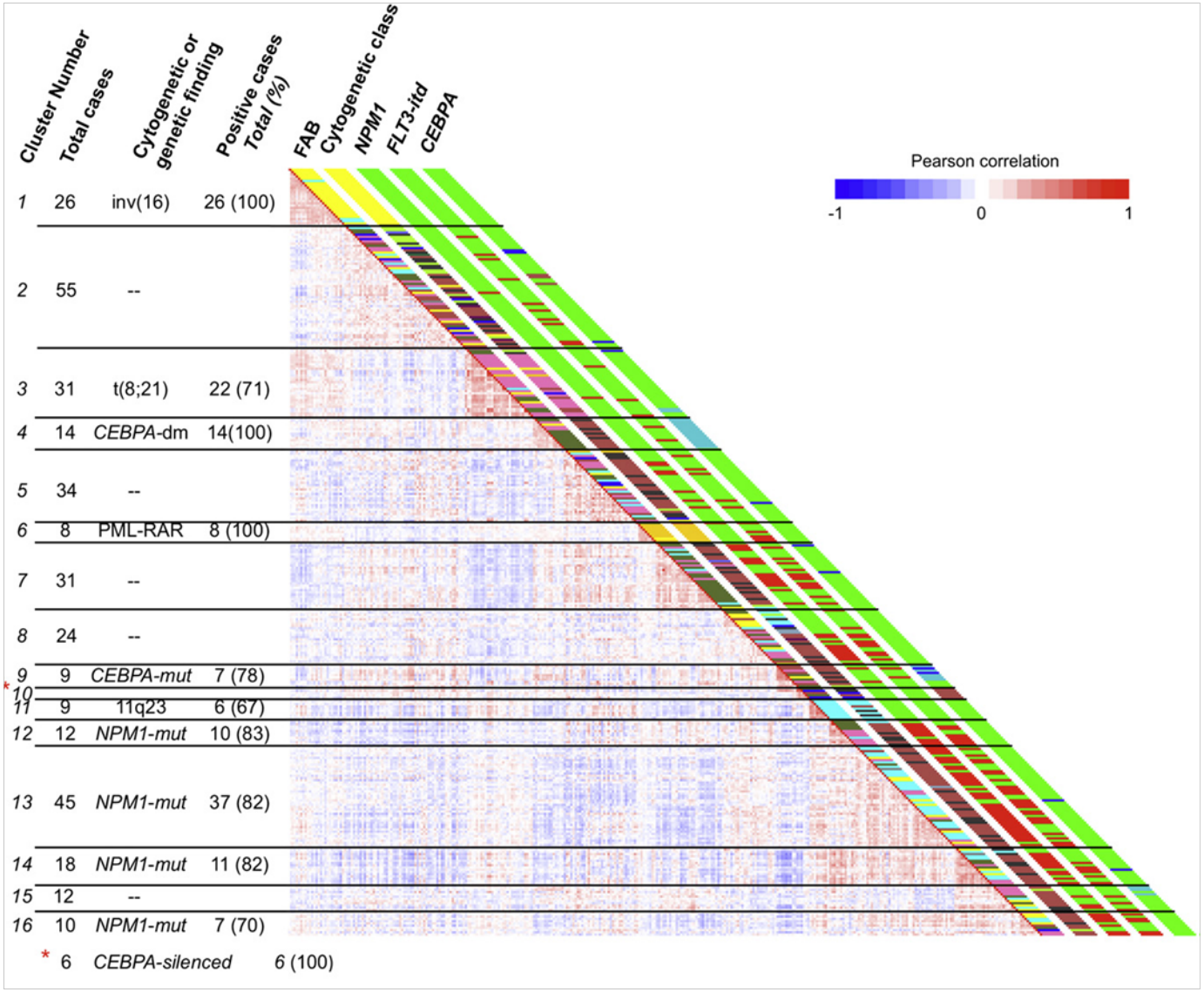
Methylation profiling of AML

HELP Assay voor genoom-brede 5me-Cy detectie



Methylation profiling AML



Figueroa ME et al. 2010

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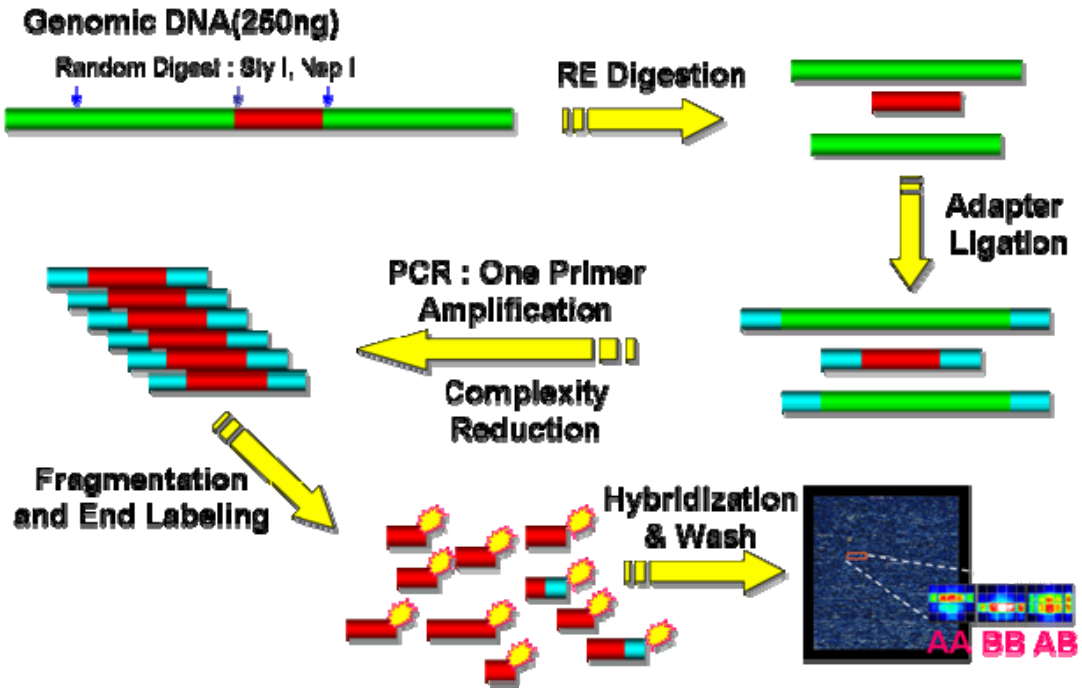
Genome wide genotyping of AML

Identification of novel (recurrent) abnormalities

Affymetrix 500K Mapping SNP GeneChips

*Nsp*I (250K)

*Sty*I (250K)



Genotype

Copy number (normal diploid genome as reference)

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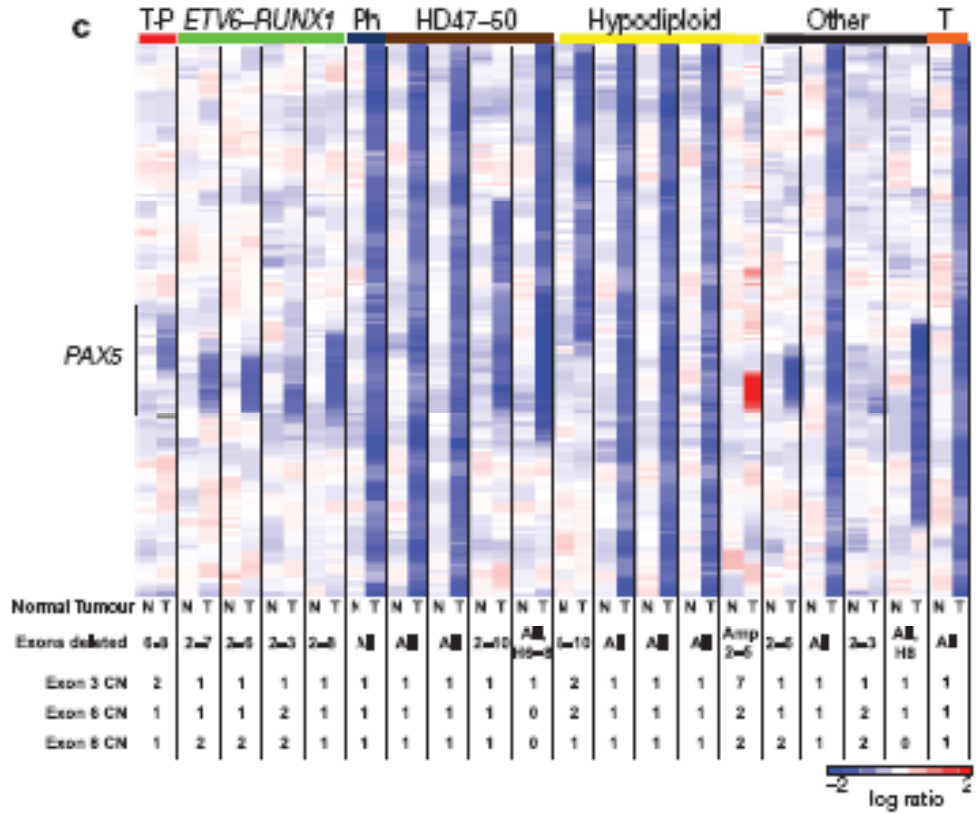
Genome-wide genotyping of ALL



Mullighan et al., 2007

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Genome-wide genotyping and gene expression

SNPExpress

Sanders et al., BMC Genomics 2008

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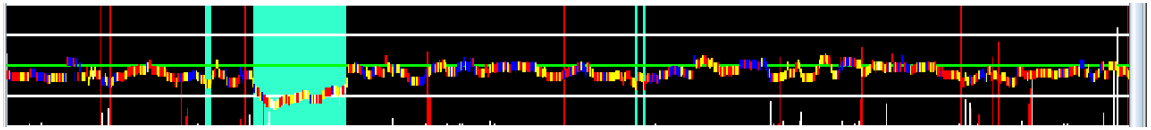
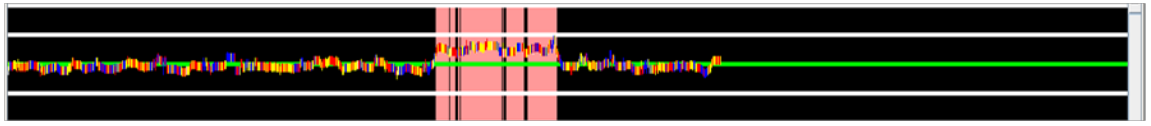
Genome-wide genotyping and gene expression of AML

SNPExpress

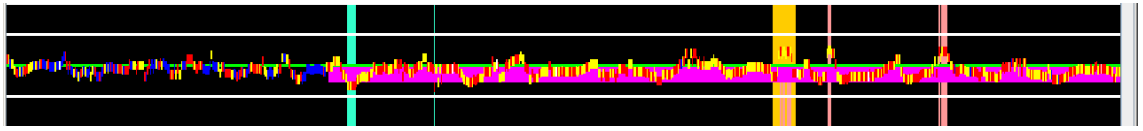
Deletion : tumor suppressor gene



Amplification : oncogene



Uni parental dysomy : recessive mutations



‘Cryptic’ translocations?

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Genome-wide genotyping
AML versus ALL

ALL many recurrent aberrations present

(PAX5 and IKAROS)

AML few (recurrent) aberrations present

(Downing/Young/others)

→ RAG-mediated rearrangements in ALL

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Whole genome sequencing AML

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

ARTICLES

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]

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Recurring Mutations Found by Sequencing an Acute Myeloid Leukemia Genome

Elaine R. Mardis, Ph.D., Li Ding, Ph.D., David J. Dooling, Ph.D., David E. Larson, Ph.D., Michael D. McLellan, B.S., Ken Chen, Ph.D., Daniel C. Koboldt, M.S., Robert S. Fulton, M.S., Kim D. Delehaunty, B.A., Sean D. McGrath, M.S., Lucinda A. Fulton, M.S., Devin P. Locke, Ph.D.,

1000 mutations per AML

'driver' versus 'passenger' mutations

IDH1 mutations

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General conclusions

All genome-wide approaches are strongly associated with the know (cyto)genetic subgroups (genetics and epi-genetics)

A number of novel subtypes of AML have been identified using the novel technologies

Validation of these novel subtypes in independent studies is essential, but difficult

Integrated analyses of the various genome-wide data sets

AML is not a single disease, one should study AML within relatively homogeneous subsets, such as t(8;21) inv(16) or mutant *CEBPA*

Next generation sequencing may replace microarray analyses

Gene/microRNA expression
Methylation profiling
Novel markers

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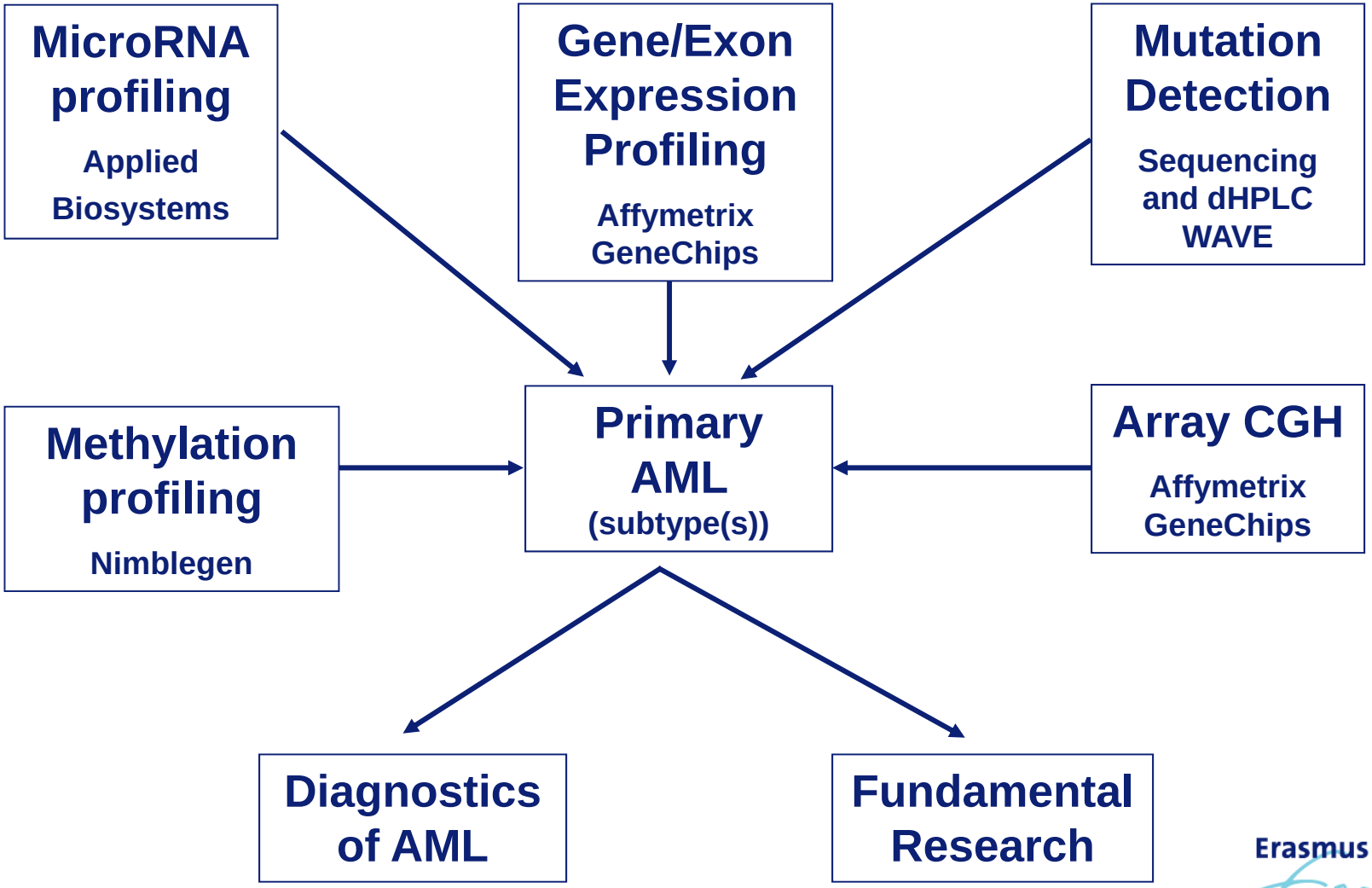
Genome-wide Approaches to Identify New Subtypes of AML



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Contribution of microarrays in Acute Myeloid Leukemia diagnostics

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Antoinette Beijen
Saman Abbas
Claudia Erpelinck
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