30.04.18

Hämatologie Heute 2018:

Epigenetics in Hematologic Malignancies

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Division of Epigenomics and Cancer Risk Factors
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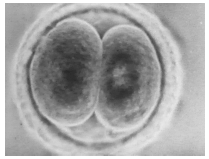
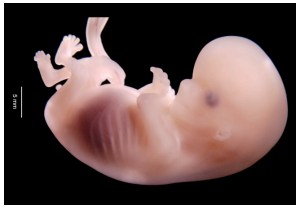
One genome, but over 200 cell and tissue types

Zygote

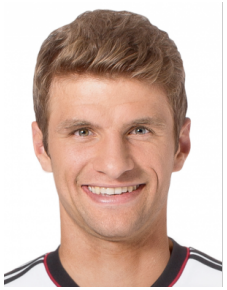


Embryo



Juvenile

Adult

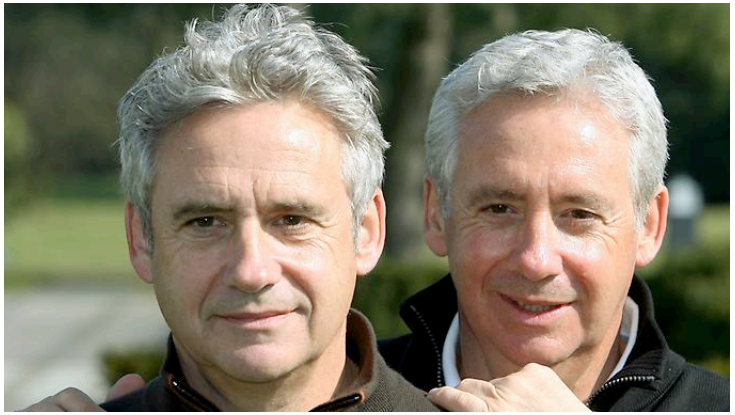


complexity

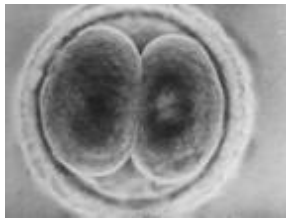
Monozygotic Twins: One genome but different fates

Different susceptibility to

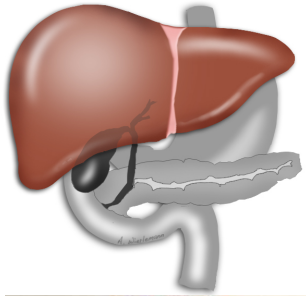
- develop cancer
- psychiatric diseases
- develop asthma/allergies

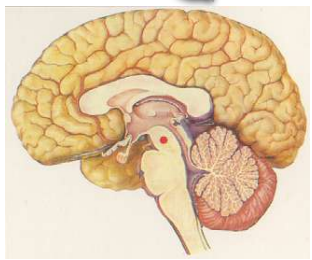


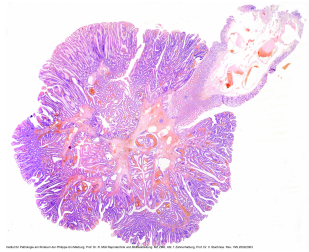
Epigenetics



- Development
- Tissue-specific gene expression
- Adopting to environmental factors
- Memory
- Aging
- Disease (diabetes, Alzheimer‘s, cancer, ...)



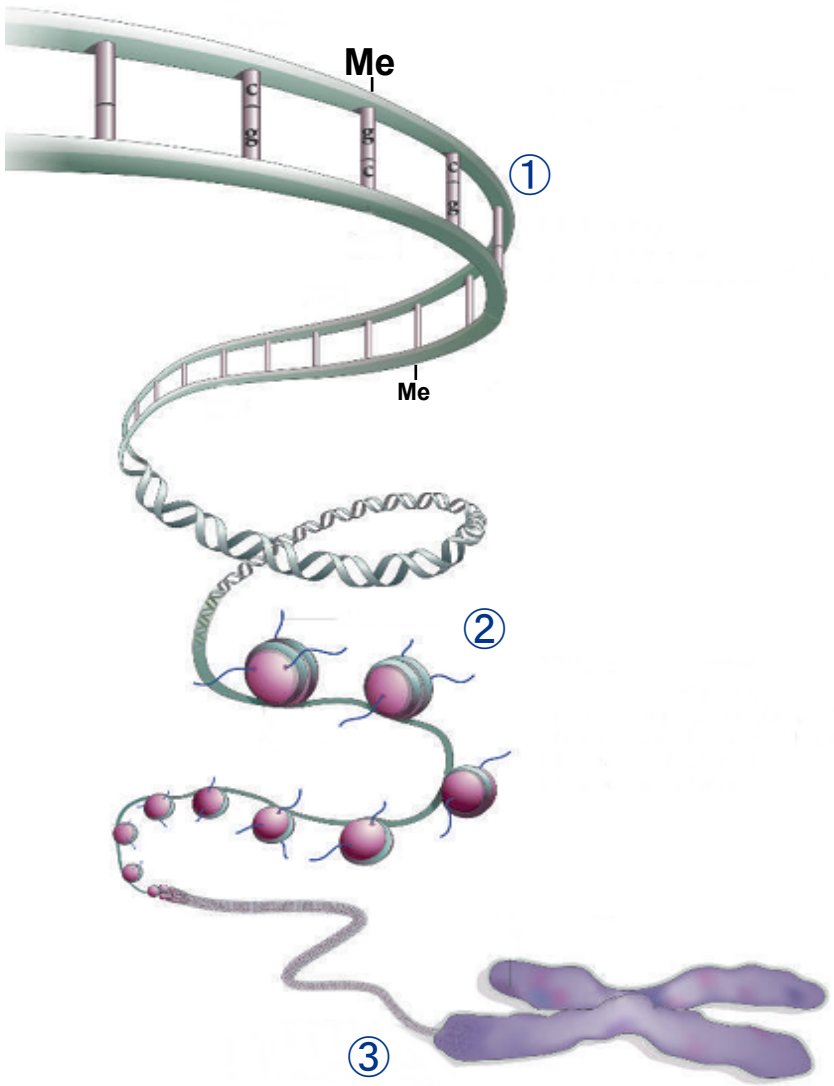




Epigenetic modifications

- **alter gene expression without affecting the DNA sequence**
- **are transmitted to daughter cells**

Layers of Epigenetic Regulation

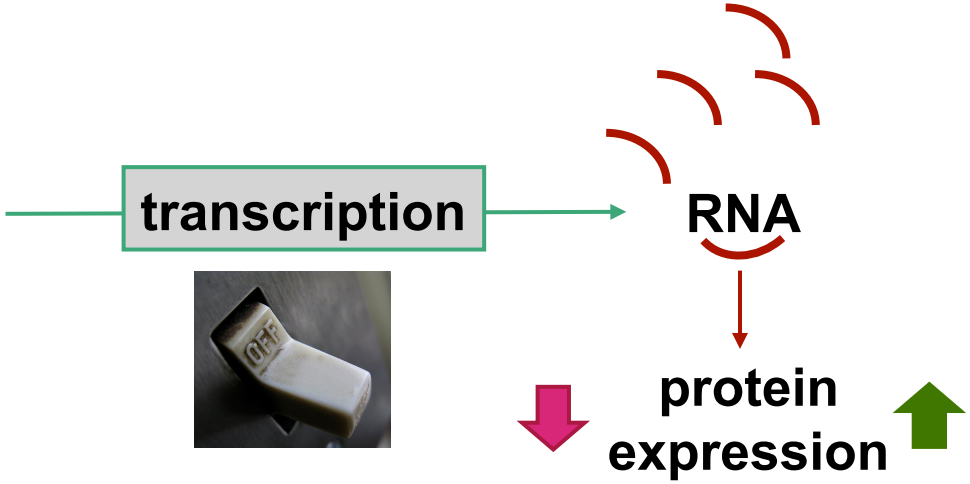


① DNA methylation

② Histone modification

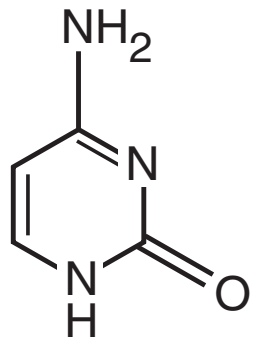
} Chromatin

③ 3D genome organization
(spatial relationship of regulatory elements)



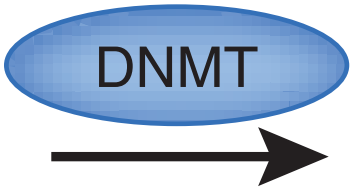
DNA methylation

Cytosine

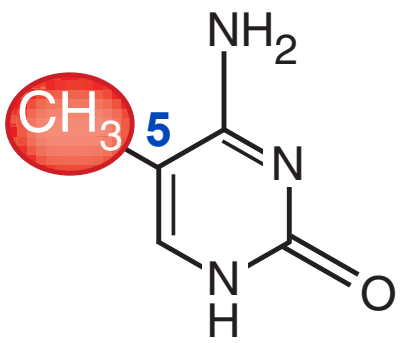


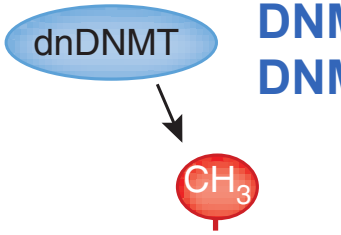
5’-CpG-3’
3’-GpC-5’

DNA methyltransferase



MeC **= 5mC**

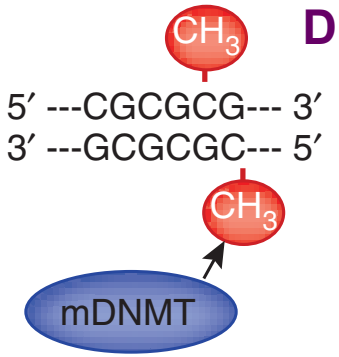




DNMT3A
DNMT3B

De novo **methylation**

e.g. early development

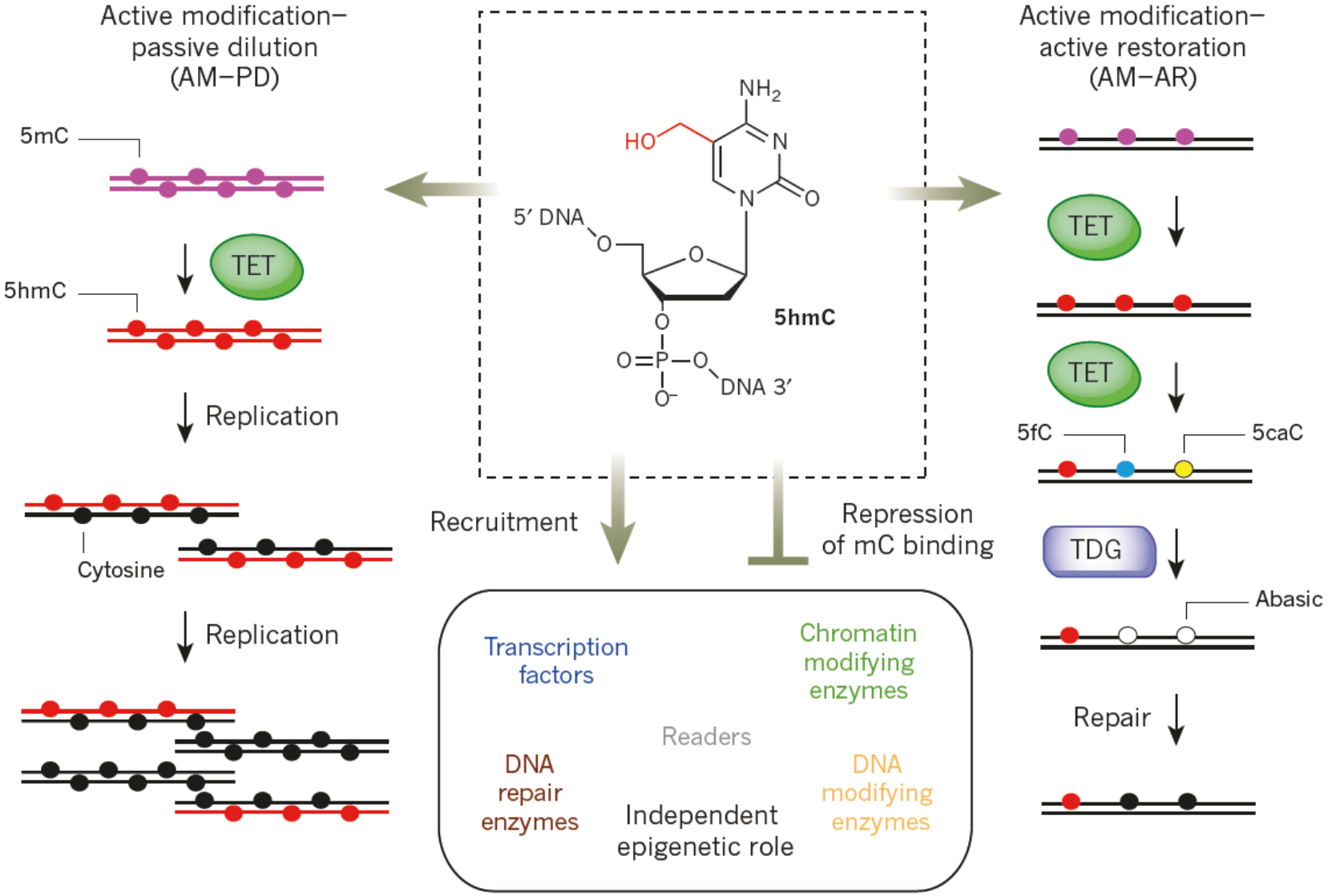


DNMT1

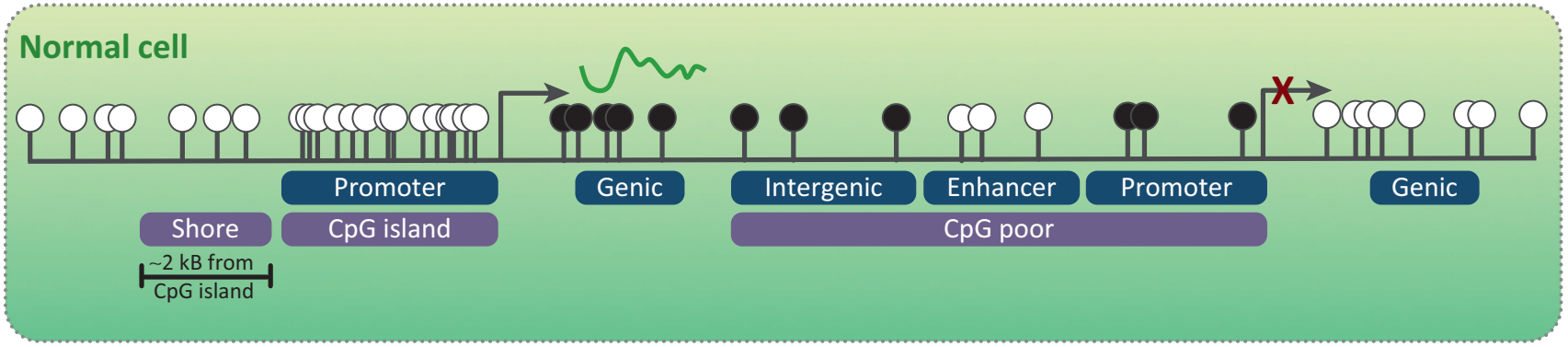
Maintenance **methylation**

e.g. replication

DNA demethylation → TET enzymes and 5hmC formation

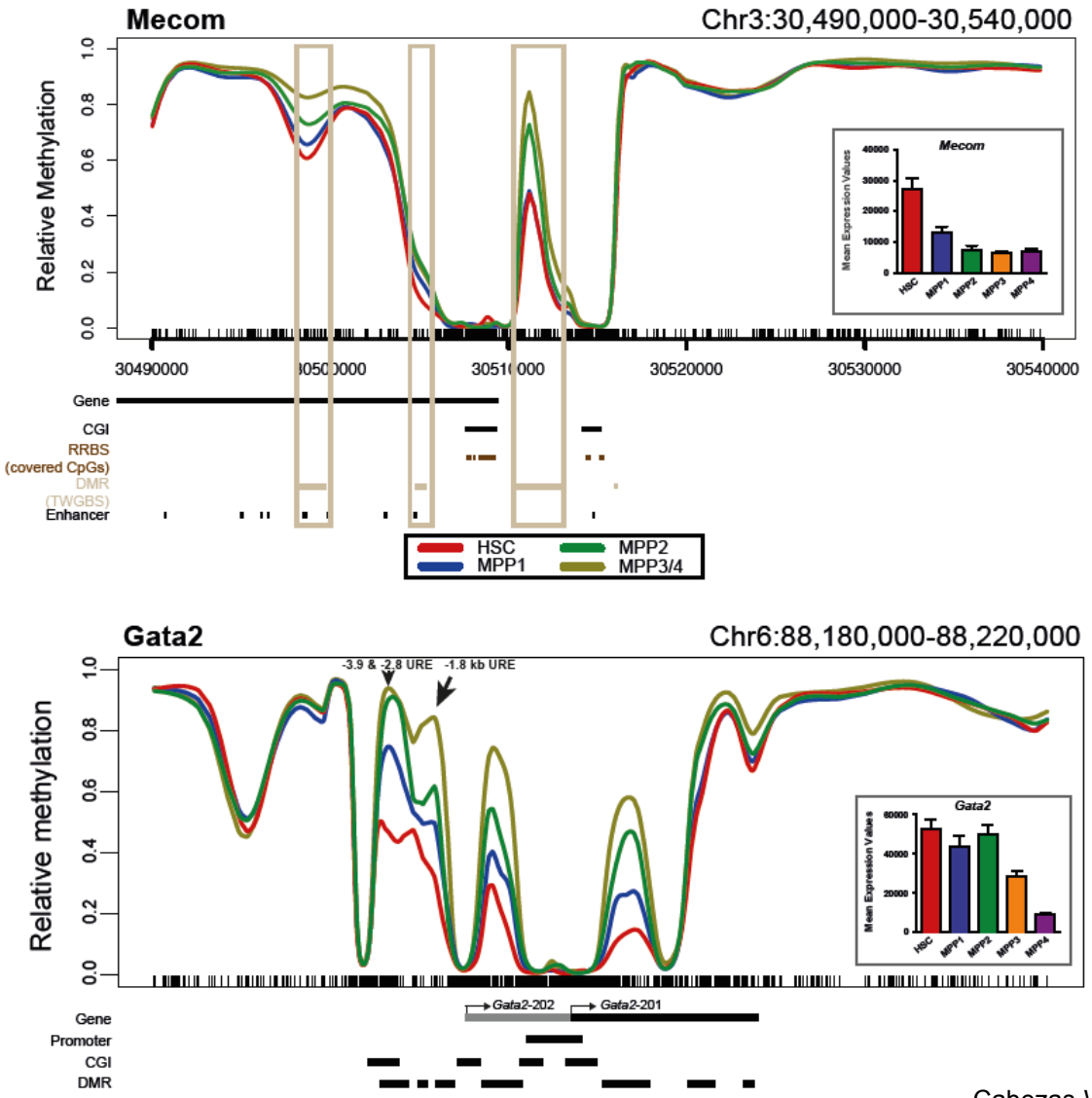


DNA methylation in normal cells



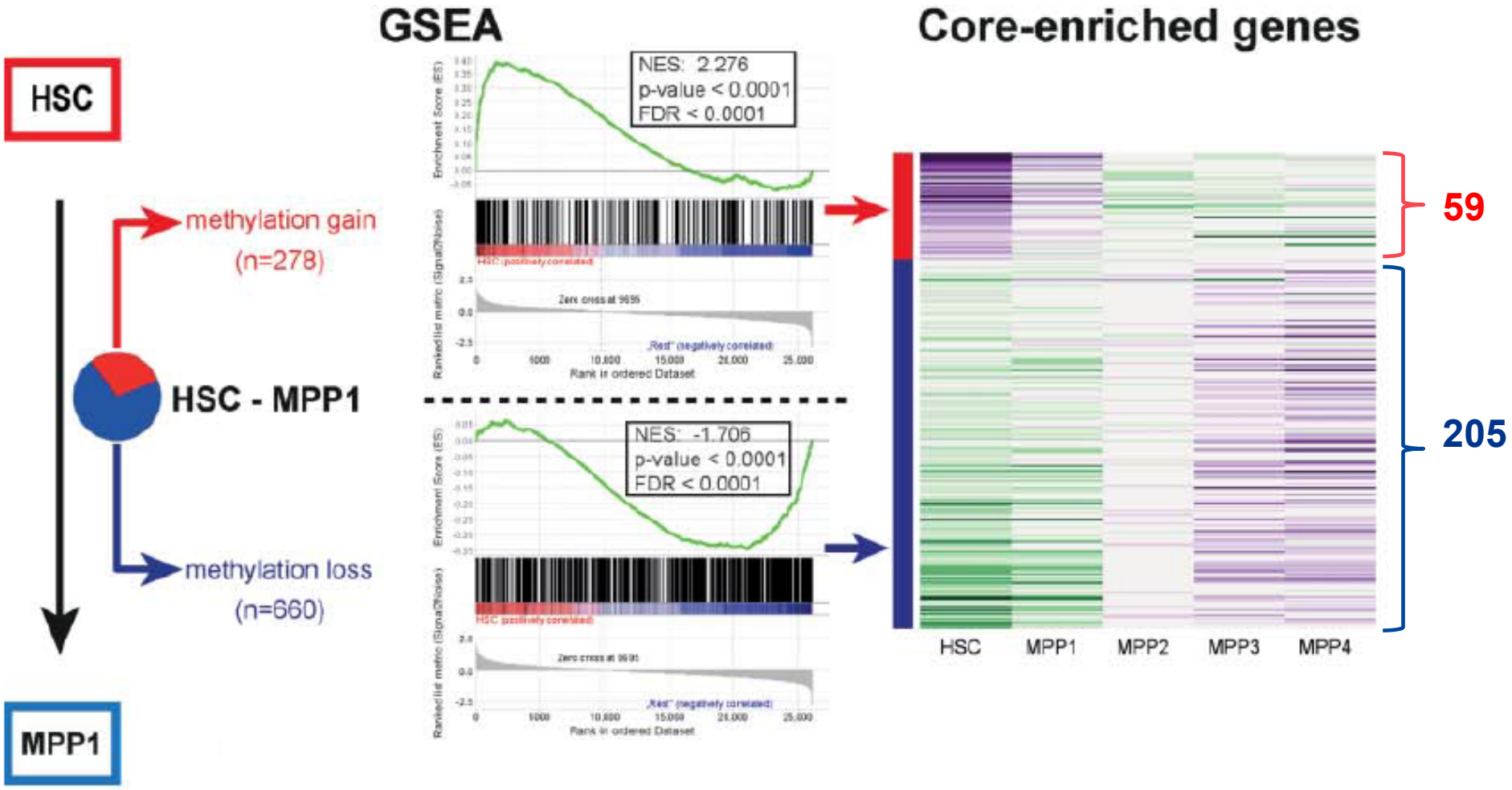
- Epigenetic patterns regulate gene expression
  - unmethylated promoter (+ intragenic methylation): gene expression ↑
  - methylated promoter (+ unmethylated gene body): gene expression ↓
- The epigenetic state determines cell fate

DNA methylation dynamics in normal hematopoiesis



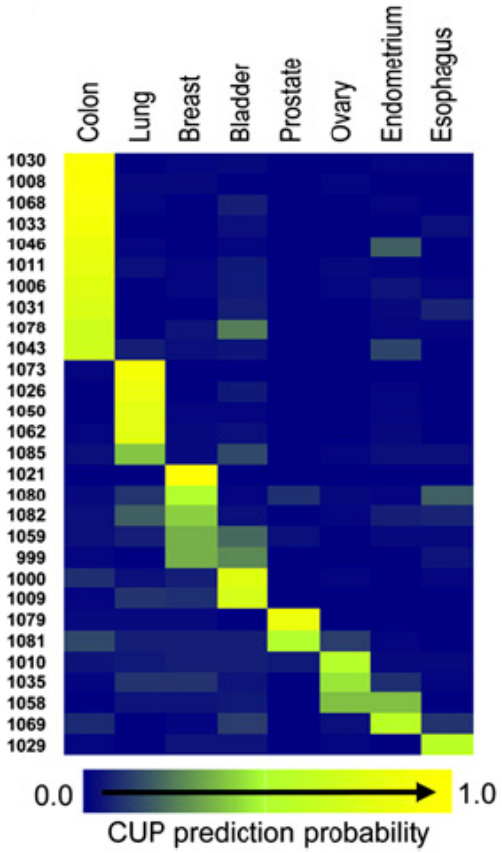
Cabezas-Wallscheid et al., Cell Stem Cell 2014

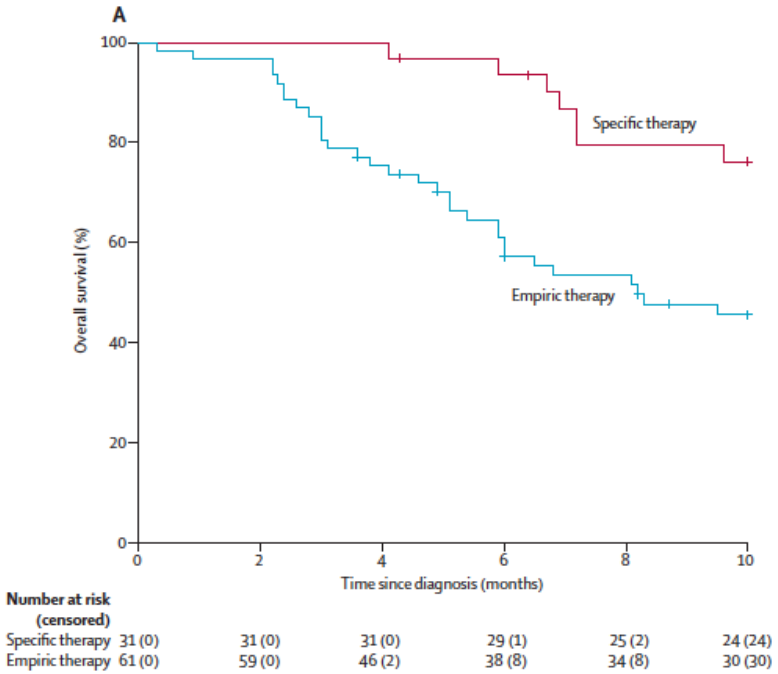
DMRs predict downstream gene-expression patterns



Lipka et al., Cell Cycle 2014

Tissue-specific DNA methylation patterns: CUP classifier



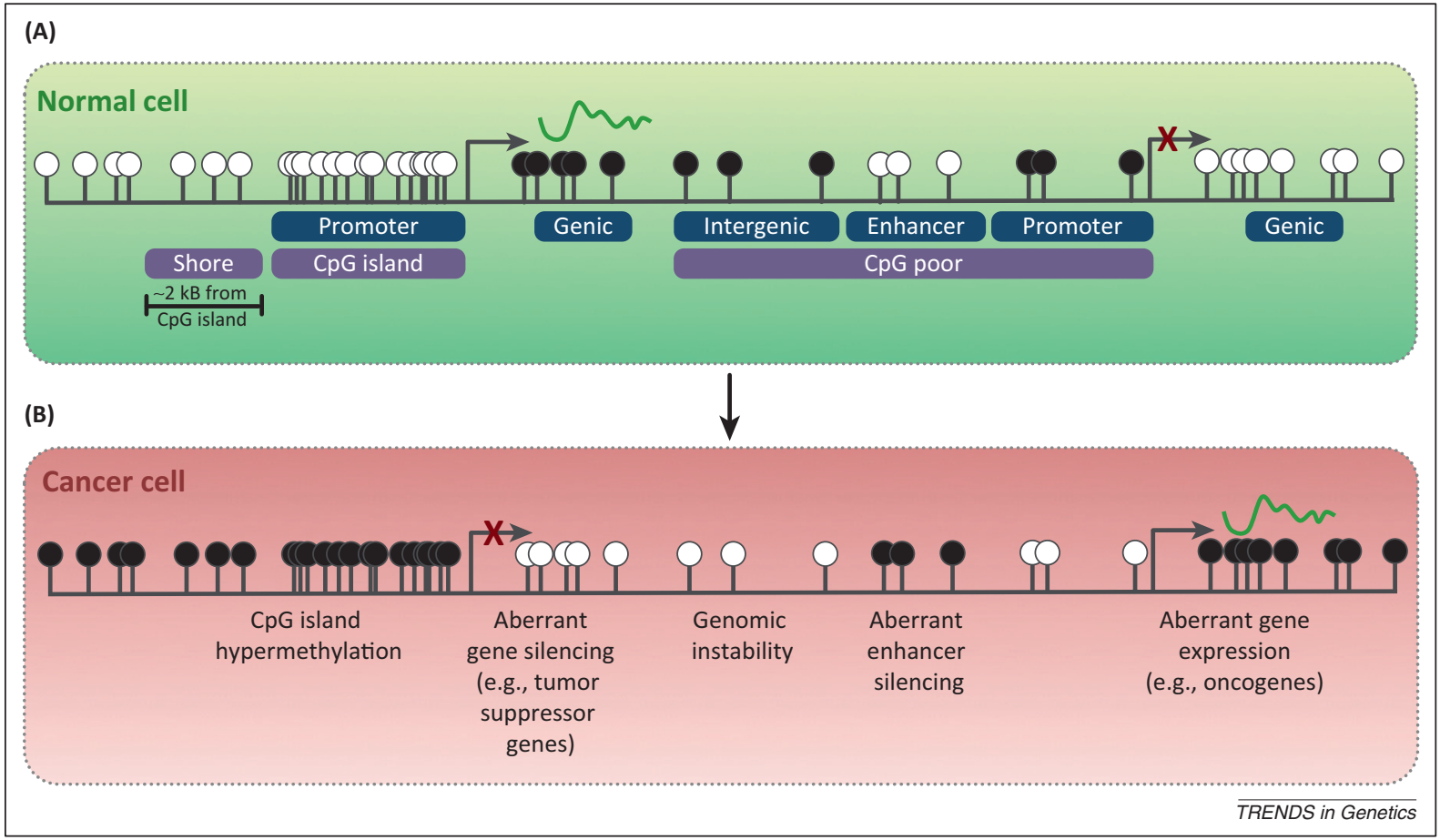


<u>Tissue of origin prediction for CUP samples:</u>
possible for 188/216 samples (87%)

Fernandez, Genome Res, 2012

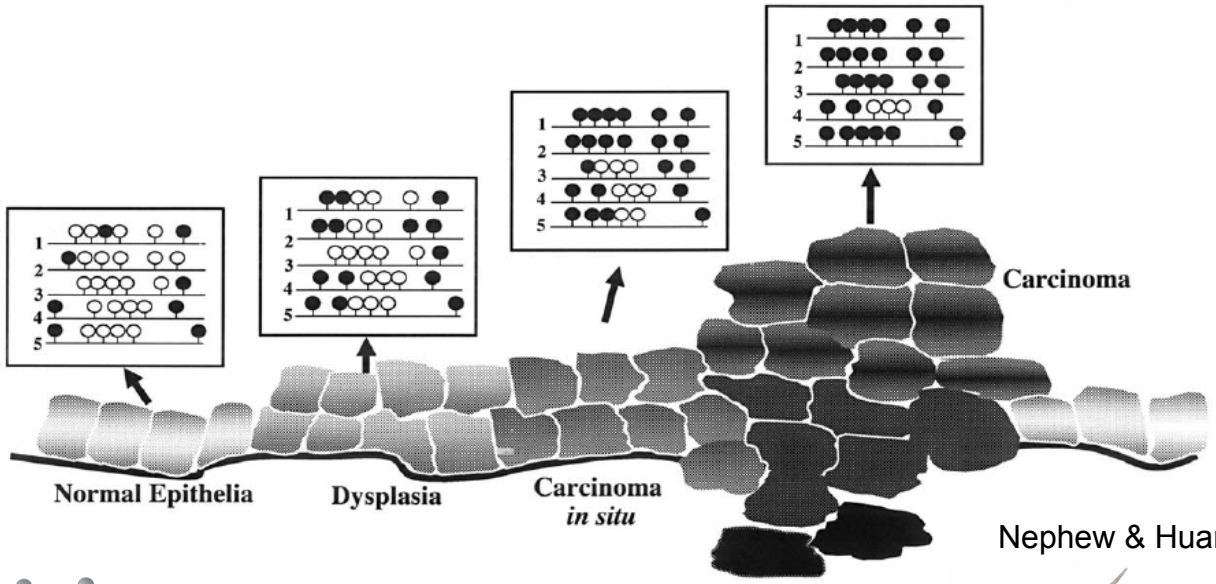
Moran et al., Lancet Oncol, 2016

DNA methylation in malignant transformation



- The epigenetic state determines cell fate
- Epigenetic deregulation can drive tumorigenesis (oncogenes ↑ & tumor-suppressors ↓)

Promoter hypermethylation in malignant transformation



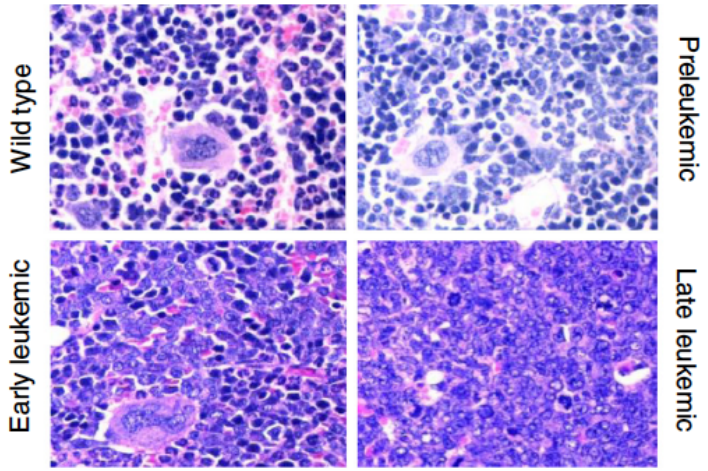
Nephew & Huang, Cancer Lett. 2003

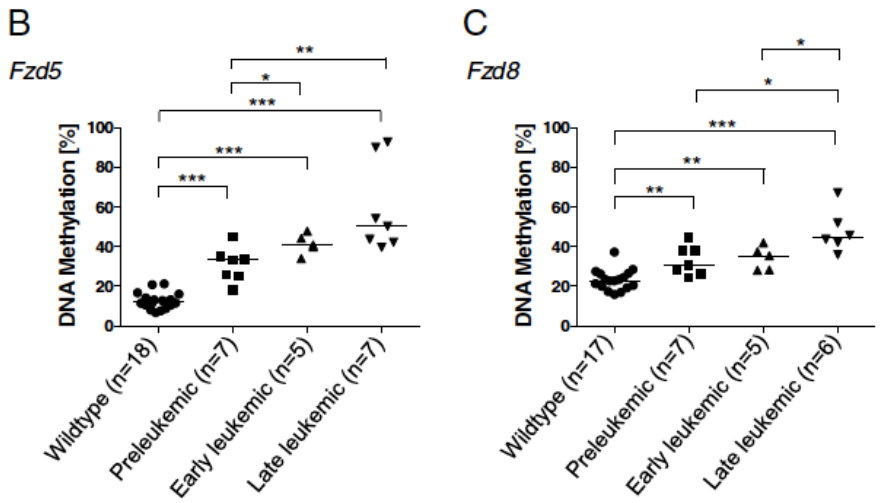
Breast **Prostate**
Colon **Lung**
MDS **Leukemia**

Fay et al., Expert Opin. Ther. Targets (2005)

Leukemia / MDS (Sonnet et al., Genome Med. 2014)
CLL (Chen et al., PNAS 2009)
Prostate (Kinney et al., MCR 2008)

Promoter hypermethylation in a murine leukemia model (PU.1 hypomorphic mice)

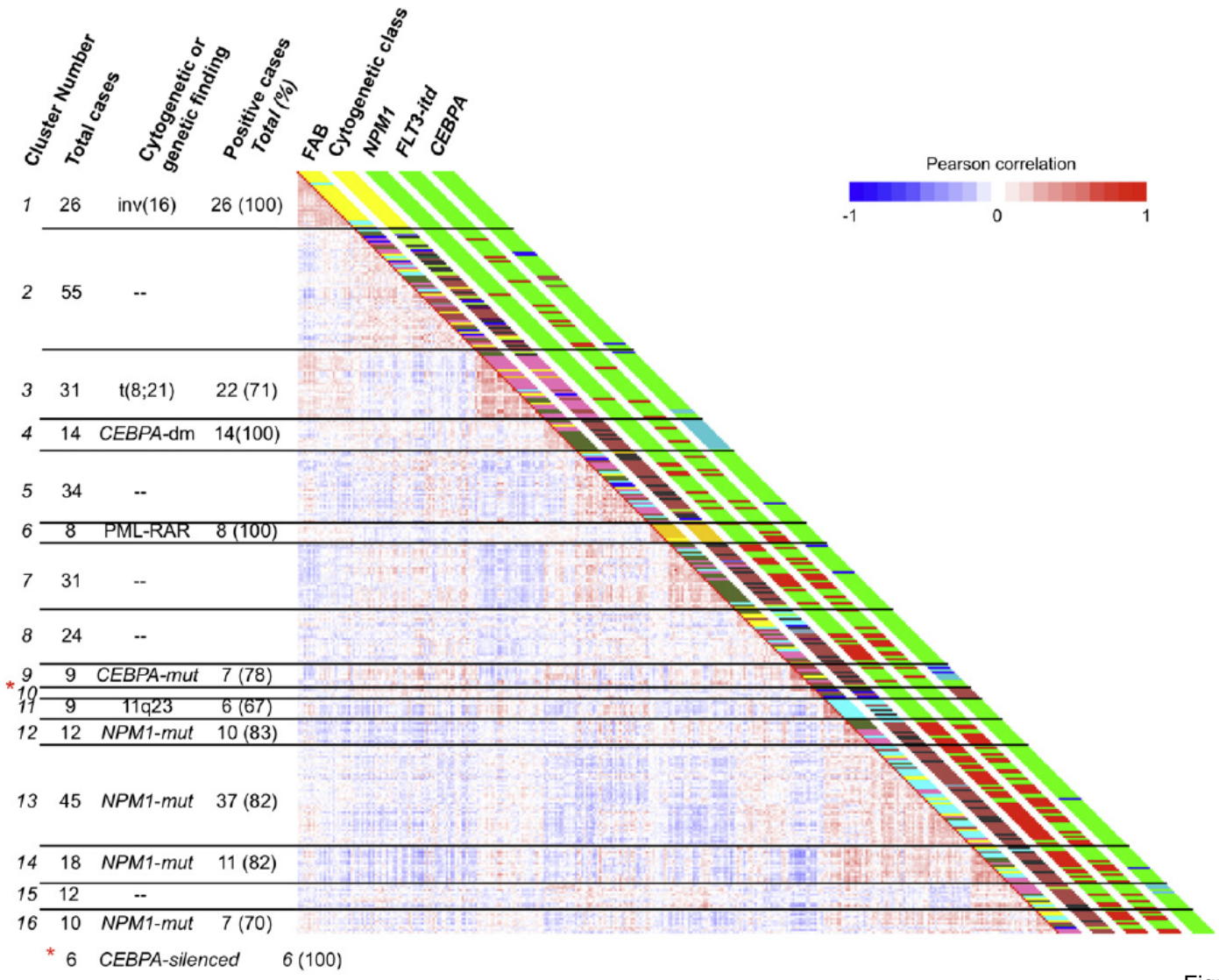




Sonnet *et al.*, Genome Med 2014

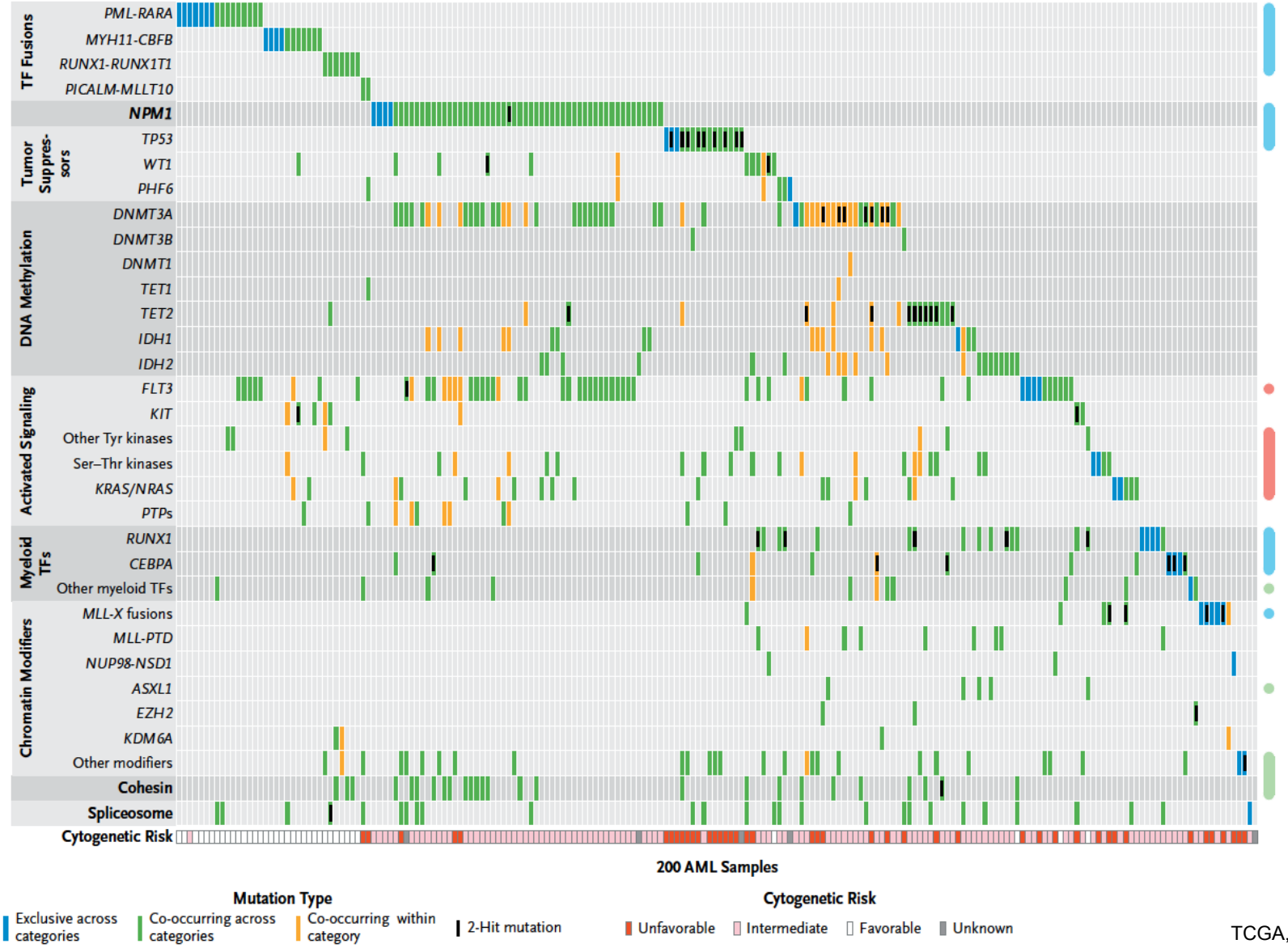
Epigenetic Alterations in Acute Myeloid Leukemia (AML)

Epigenetic signatures in AML



Figueroa et al., Cancer Cell 2010

Molecular landscape of AML



TCGA, N Engl J Med 2013

Mutation categories in AML: an epigenetic disease!?

Transcription factor fusions **18%**

PML-RARA, CBFB-MYH11, RUNX1-RUNX1T1, PICALM-MLLT10

NPM1 mutations **27%**

Tumor suppressor genes **16%**

TP53, WT1, PHF6

DNA methylation **44%**

- *DNMT3A, DNMT3B, DNMT1, TET1/2, IDH1/2*

Activated signaling **59%**

FLT3, KIT, other TK, other Ser-Thr kinases, PTPs

Myeloid transcription factors **22%**

RUNX1, CEBPA, others

Chromatin modifiers **30%**

- *MLL* fusions, *MLL-PTD, NUP98-NSD1, ASXL1, EZH2, KDM6A*, others

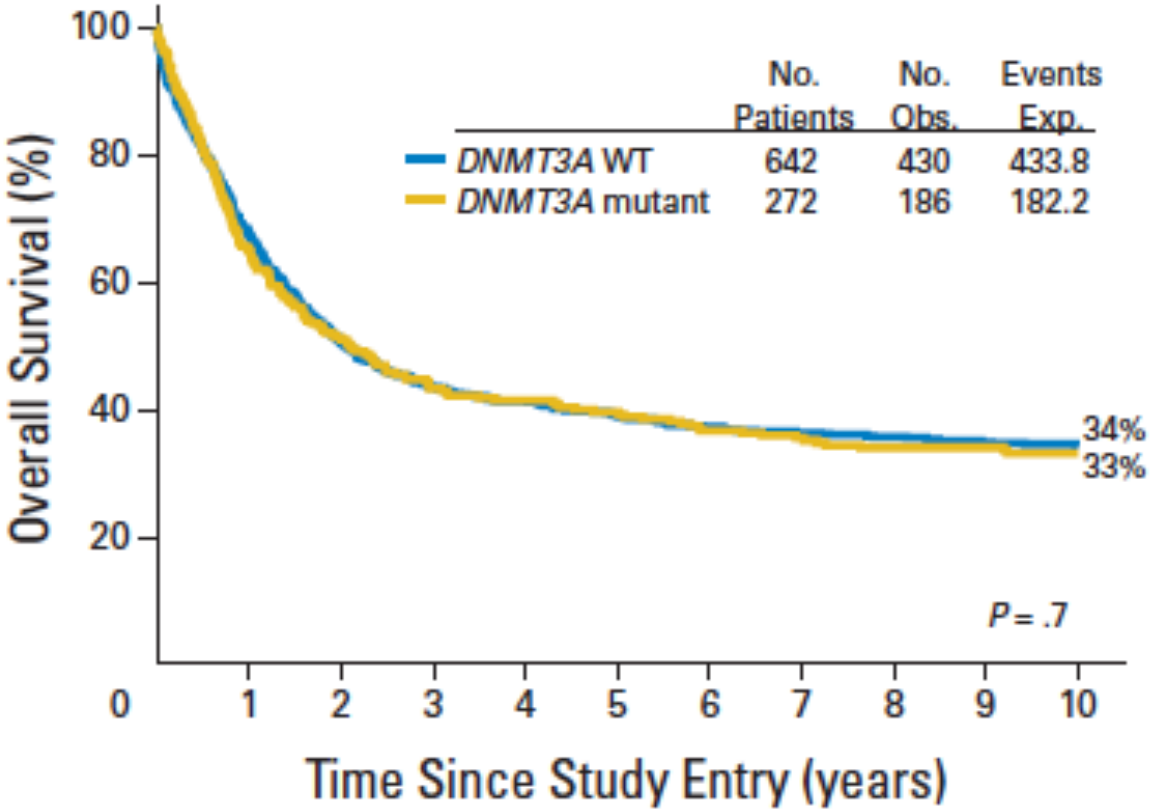
Cohesin complex **13%**

Spliceosome complex **14%**

TCGA, N Engl J Med 2013

Interactions between mutations

All patients

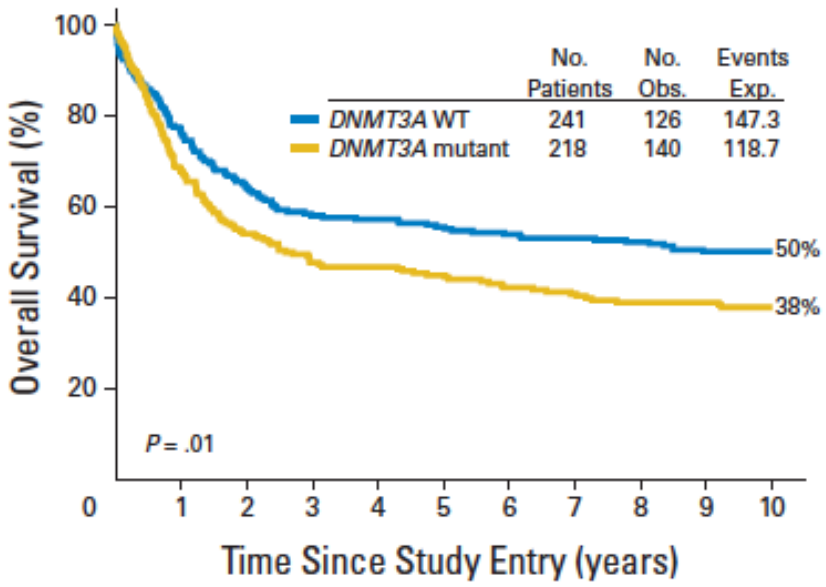


Gale et al., J Clin Oncol 2015

Interactions between mutations

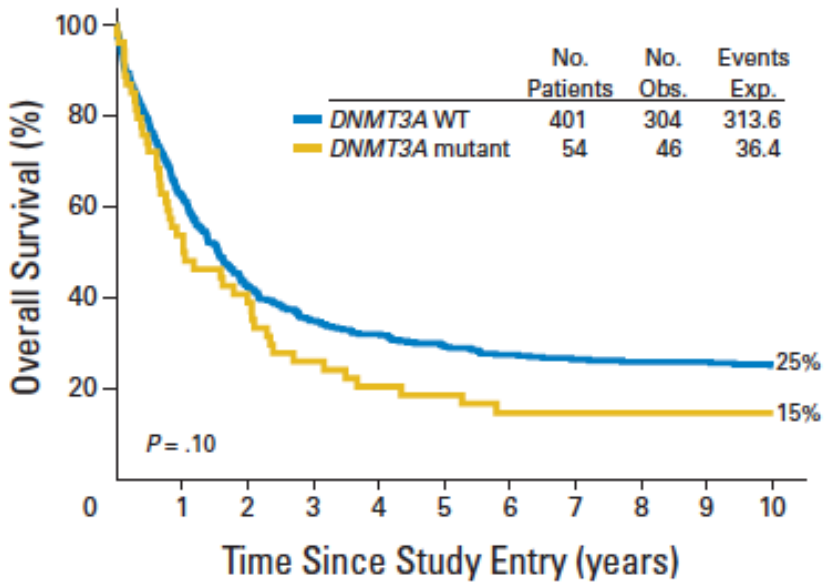
NPM1-mutant

E



NPM1-wildtype

F



Gale et al., J Clin Oncol 2015

Clonal evolution of AML

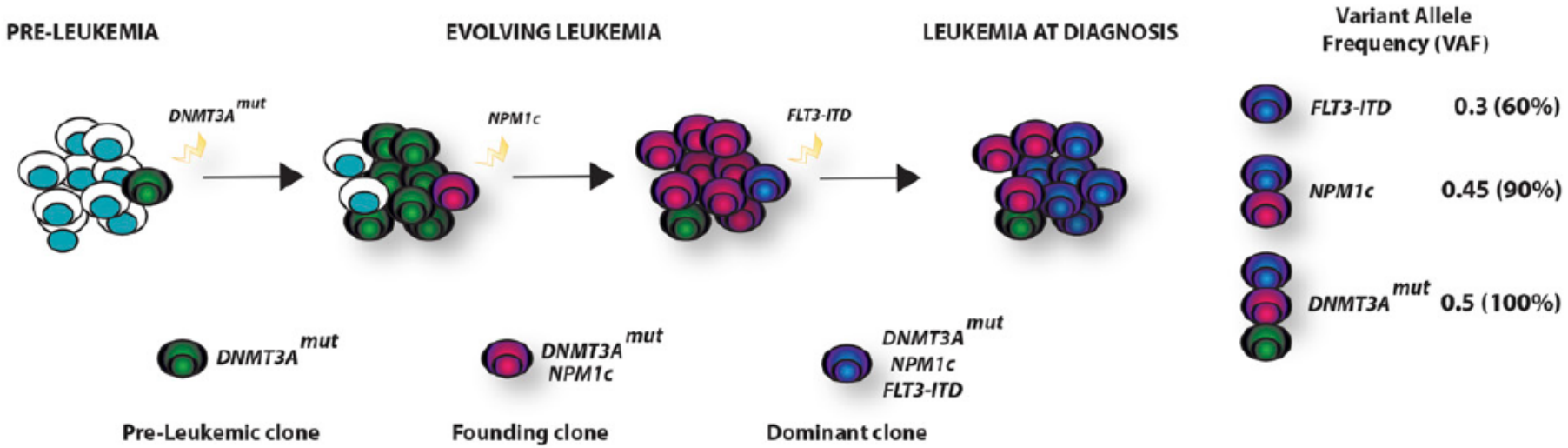
ARTICLE

doi:10.1038/nature13038

Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia

Nature 2014

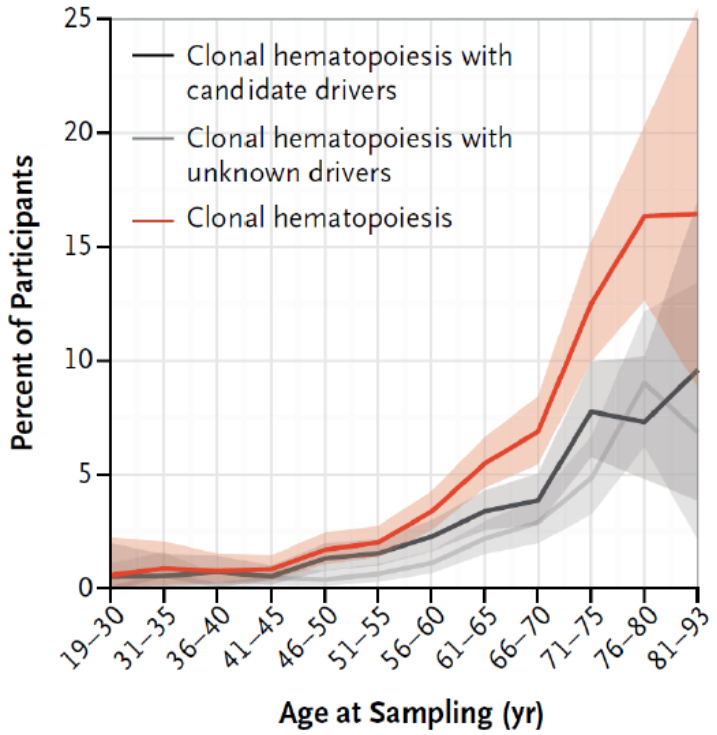
Liran I. Shlush[1]*, Sasan Zandi[1]*, Amanda Mitchell[1], Weihsu Claire Chen[1], Joseph M. Brandwein[1,2,3], Vikas Gupta[1,2,3], James A. Kennedy[1], Aaron D. Schimmer[1,2,3,4], Andre C. Schuh[1,2,3], Karen W. Yee[1,2,3], Jessica L. McLeod[1], Monica Doedens[1], Jessie J. F. Medeiros[1], Rene Marke[1,5], Hyeoung Joon Kim[6], Kwon Lee[6], John D. McPherson[4,7], Thomas J. Hudson[4,7,8], The HALT Pan-Leukemia Gene Panel Consortium†, Andrew M. K. Brown[7], Fouad Yousif[7], Quang M. Trinh[7], Lincoln D. Stein[7,8], Mark D. Minden[1,2,3,4], Jean C. Y. Wang[1,2,3] & John E. Dick[1,8]



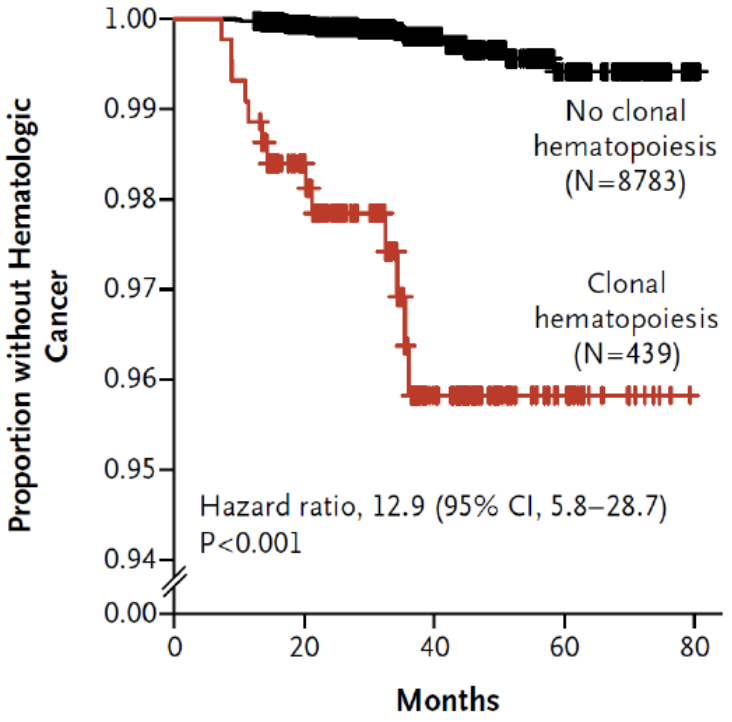
Grimwade et al., Blood 2016

Clonal hematopoiesis & pre-leukemia

CHIP – incidence with age

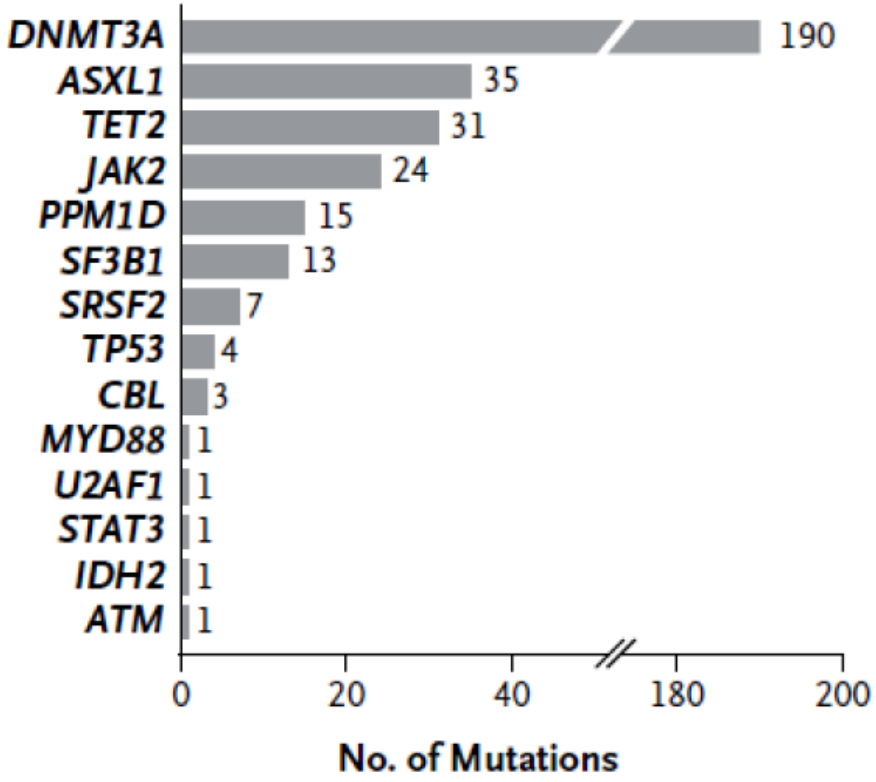


CHIP is associated with hematologic malignancies



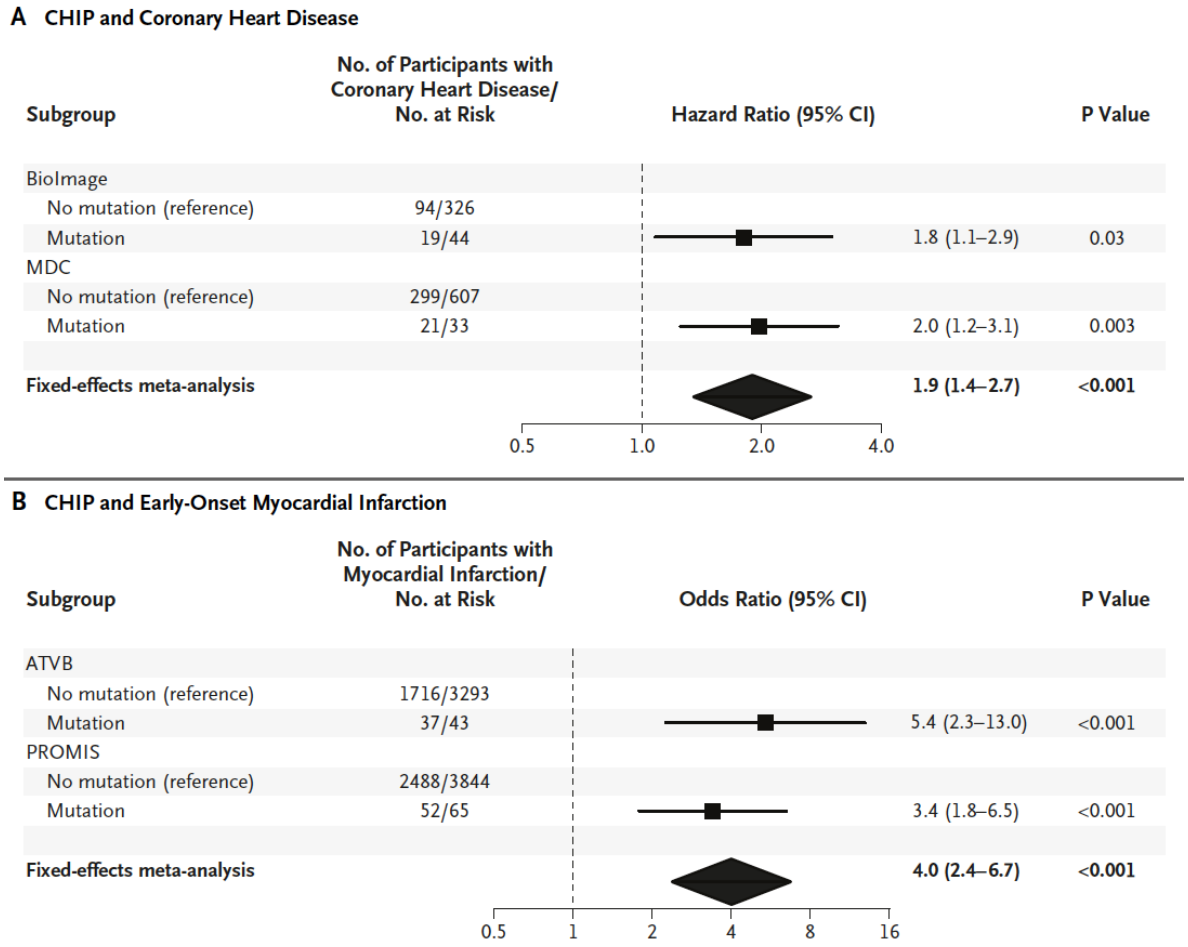
Genovese et al., NEJM 2015

CHIP – underlying mutations



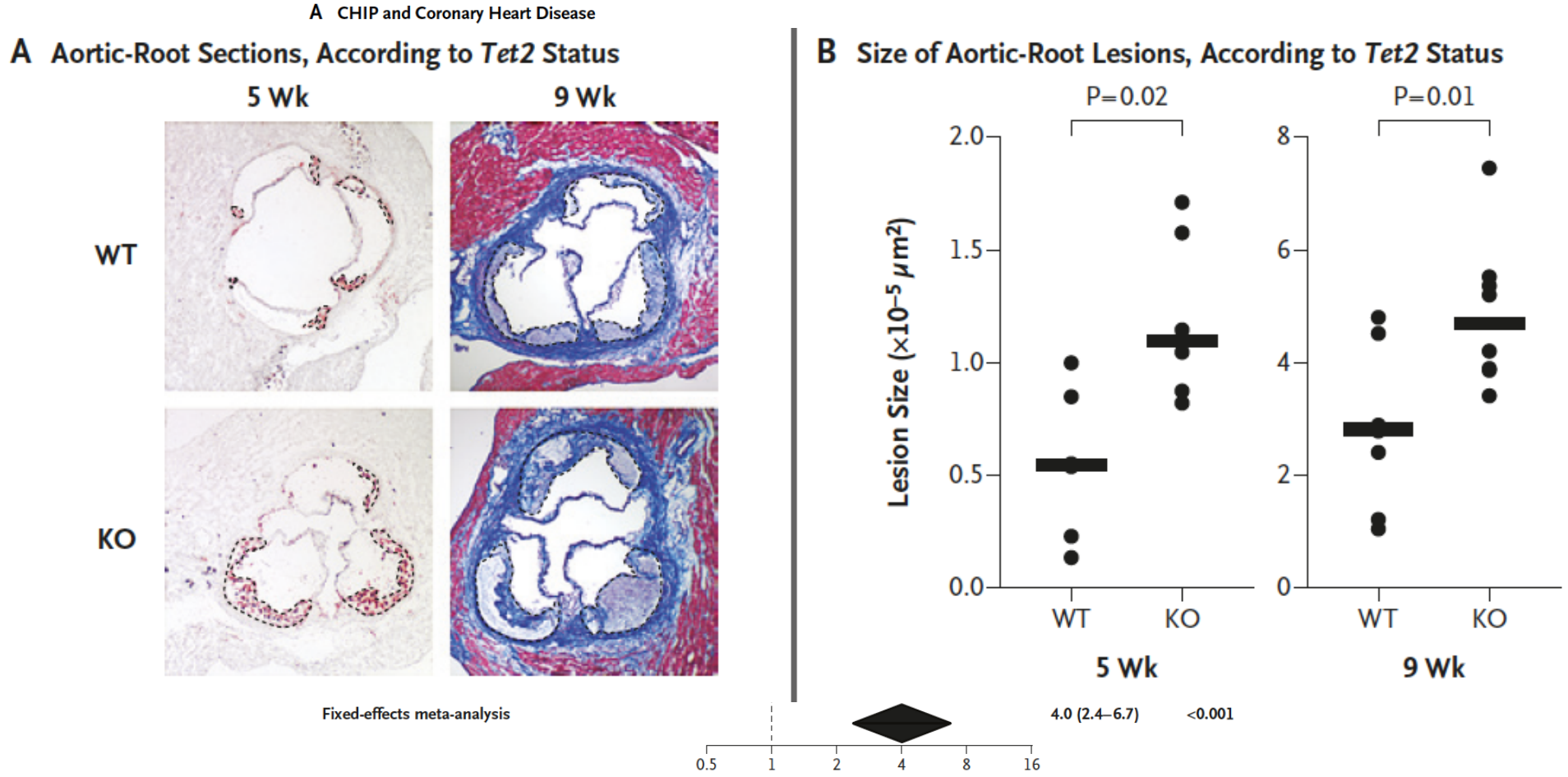
Genovese et al., NEJM 2015

Clonal hematopoiesis & cardiovascular risk



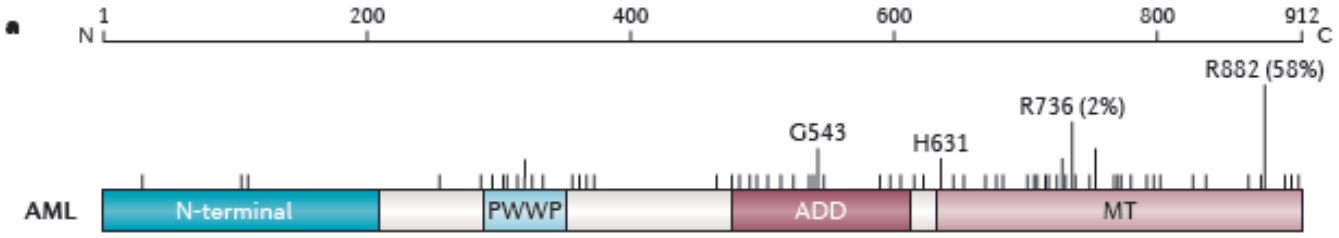
Jaiswal et al., NEJM 2017

Clonal hematopoiesis & cardiovascular risk



Jaiswal et al., NEJM 2017

DNMT3A-R882H mutations



Yan et al., Nat Genet. 2011

The R882H DNMT3A Mutation Associated with AML Dominantly Inhibits Wild-Type DNMT3A by Blocking Its Ability to Form Active Tetramers

David A. Russler-Germain,[1] David H. Spencer,[2] Margaret A. Young,[1] Tamara L. Lamprecht,[1] Christopher A. Miller,[3] Robert Fulton,[3] Matthew R. Meyer,[4] Petra Erdmann-Gilmore,[4,6] R. Reid Townsend,[4,6] Richard K. Wilson,[3,5,6] and Timothy J. Ley[1,3,5,6,*]

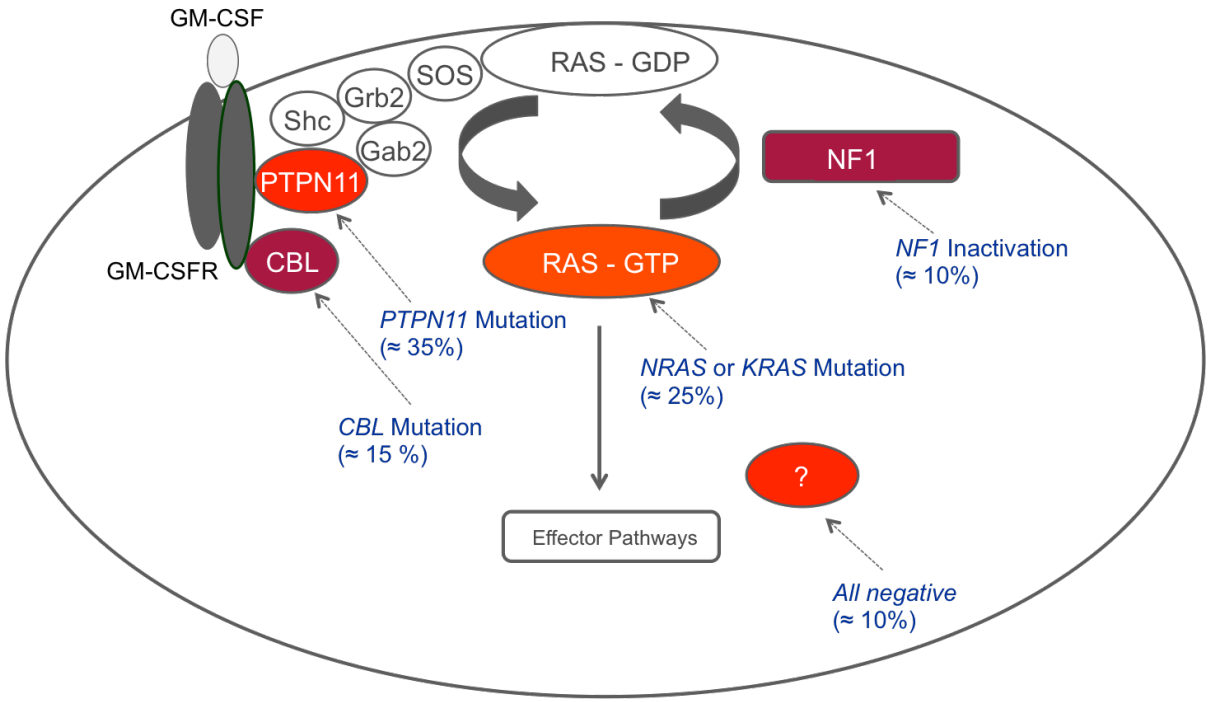
DNMT3A-R882 mutations

- affect the methyltransferase domain of DNMT3A in
- present in **~60% AML** cases and in pre-leukemia
- exhibits **80% reduction in methyltransferase activity**
- **'Dominant Negative' mutation** by inhibiting oligomerisation with wild-type DNMT3A

Juvenile Myelomonocytic Leukemia: an epigenetic disease?

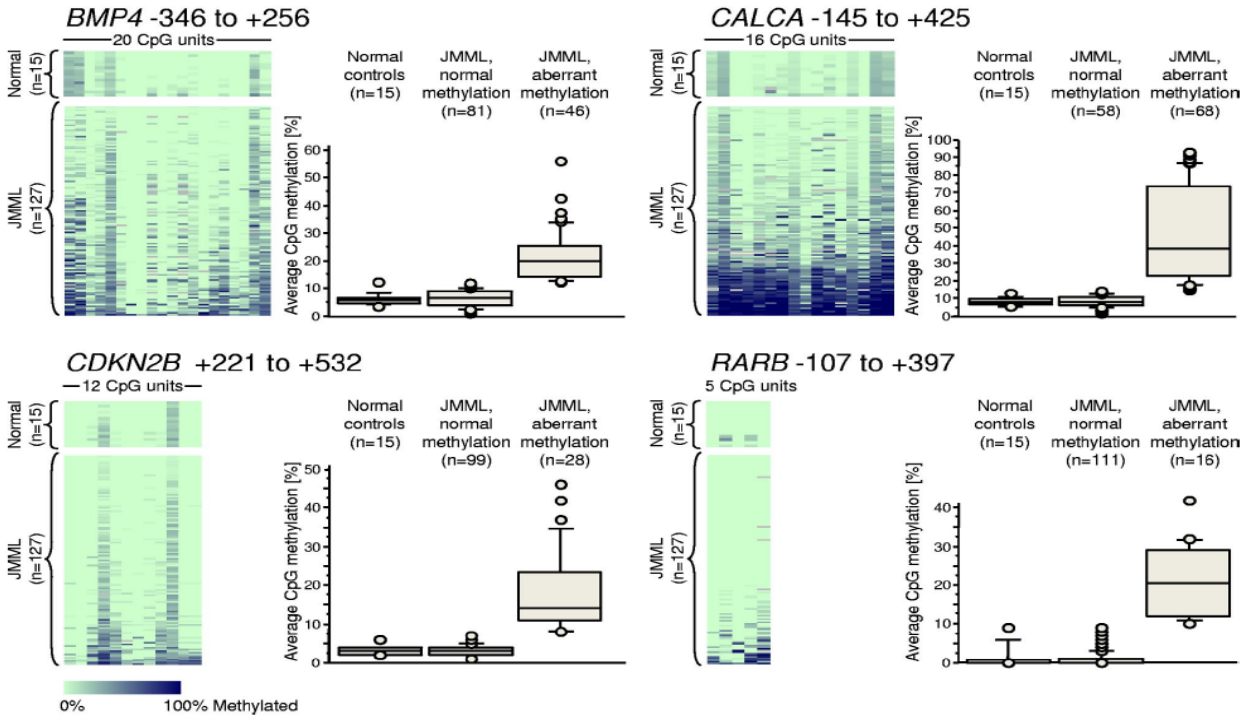
Juvenile myelomonocytic leukemia (JMML): Background

- Aggressive myeloid malignancy of early childhood
- Only allo-HSCT is potentially curative
- 5-year EFS is 60%
- Hyperactivation of the Ras signaling pathway



JMML: Background

- DNA methylation so far only studied in few candidate gene loci
  - DNA methylation status of promoter CGI from 14 genes associated with cancer or Ras signaling were studied in a large JMML cohort (n=127)
  - Hypermethylated genes: *BMP4*, *CALCA*,*CDKN2B*, *RARB* (+ *RASA4*)



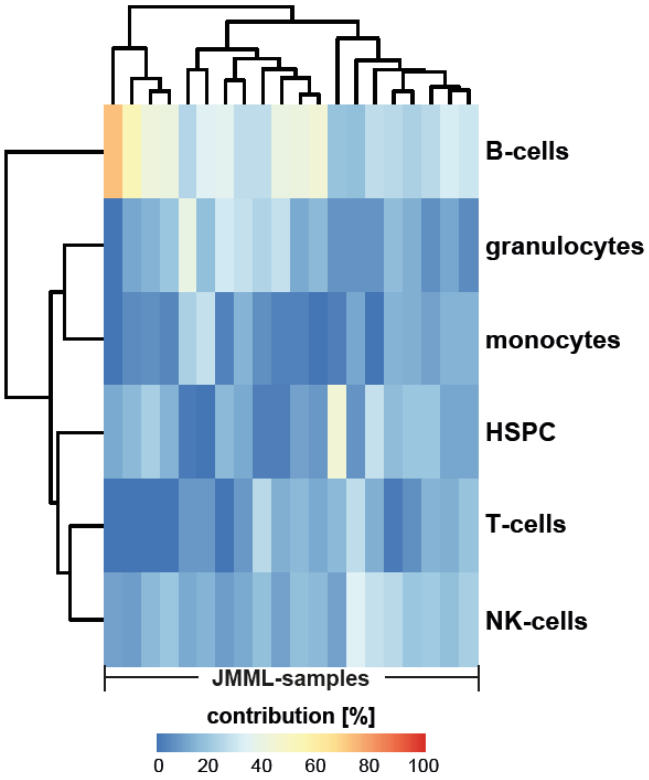
Olk-Batz et al., Blood 2011; Poetsch et al., Epigenetics 2014

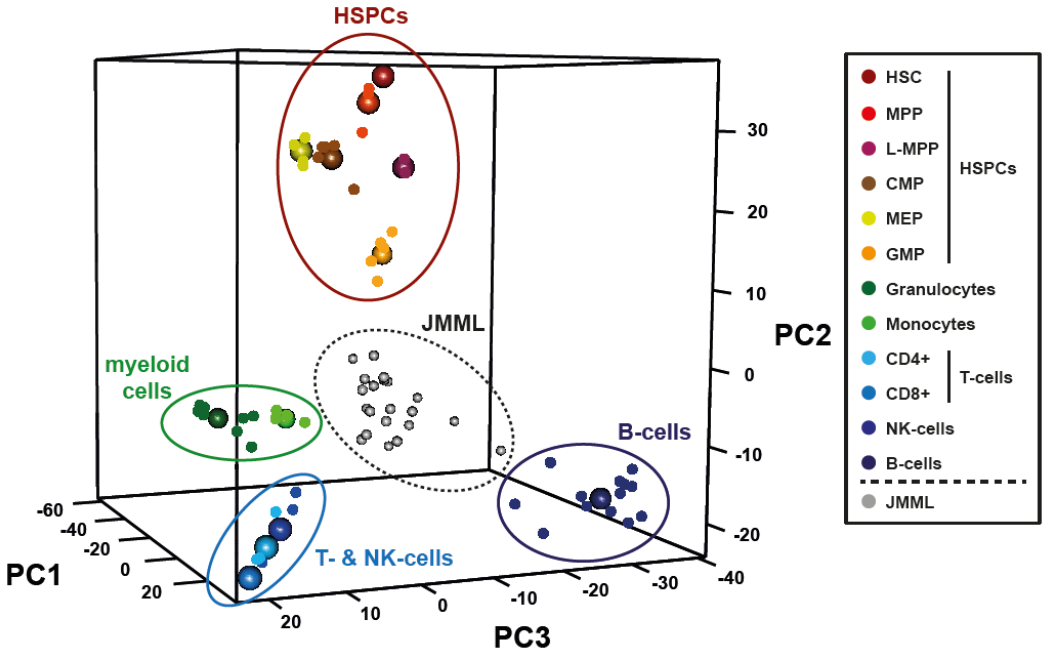
Hypothesis

JMML shows aberrant DNA methylation patterns which might serve to discriminate groups with different biologic behavior and provide insights into the pathogenesis and progression of the disease

Cell type composition is heterogeneous

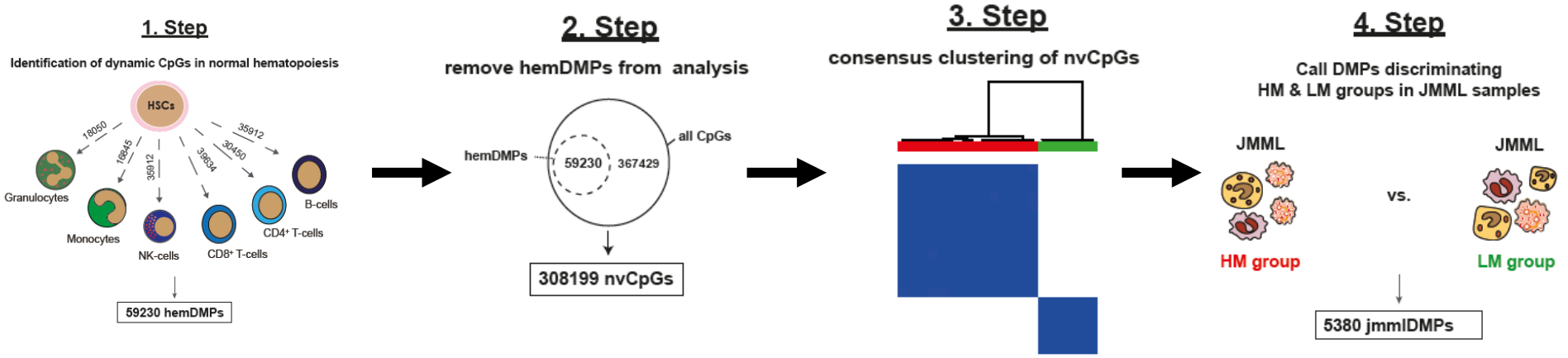
- JMML discovery cohort (n=20)
- Methylome analysis: 450k Illumina Bead Chip Array





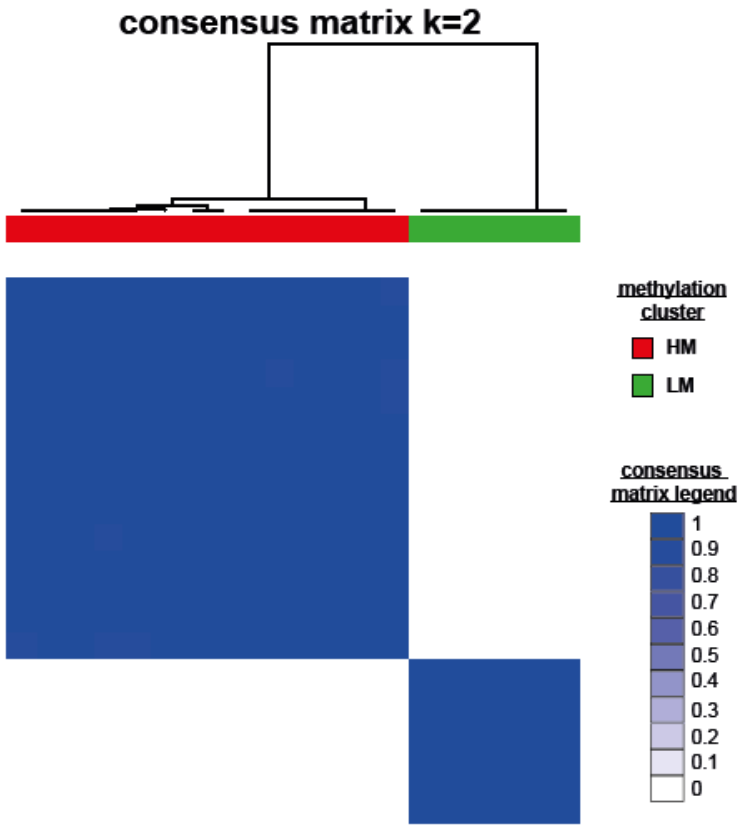
Lipka et al., Nat. Commun. 2017

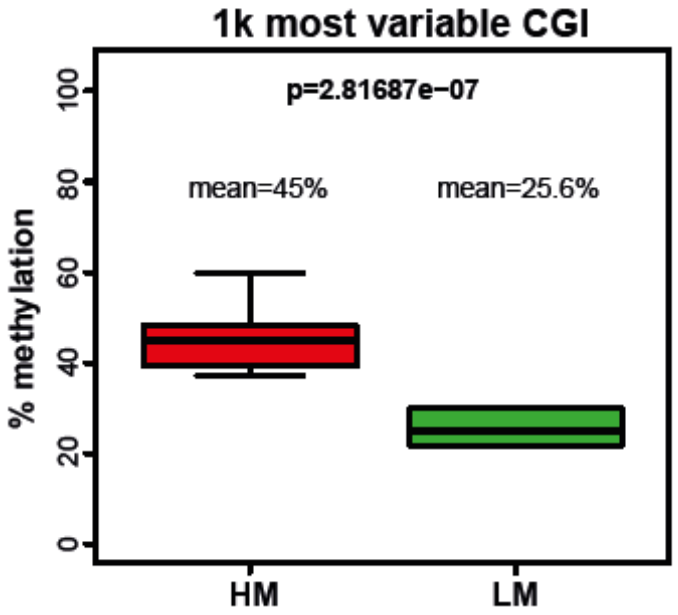
Strategy to identify disease-specific aberrant methylation events



Lipka et al., Nat. Commun. 2017

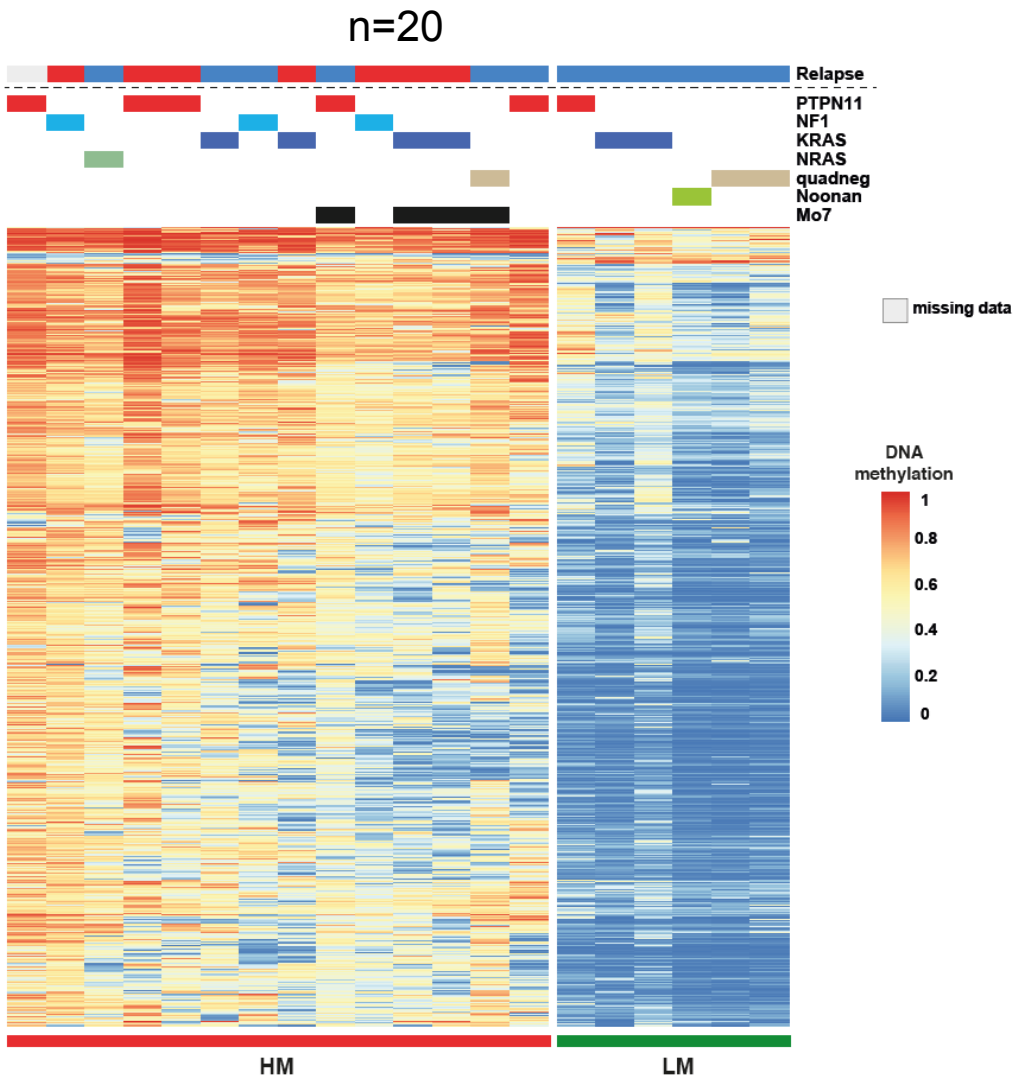
Consensus clustering (k=2; discovery cohort)





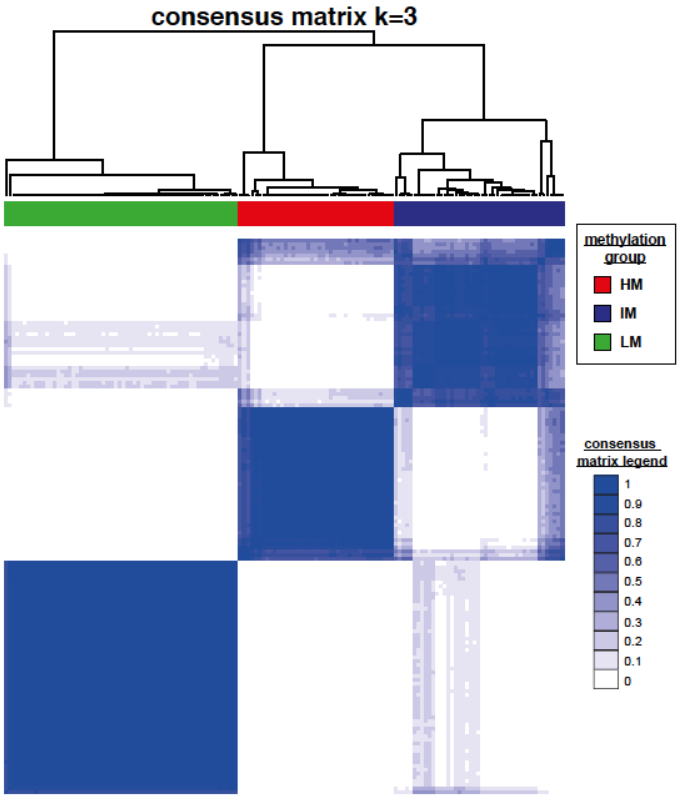
Lipka et al., Nat. Commun. 2017

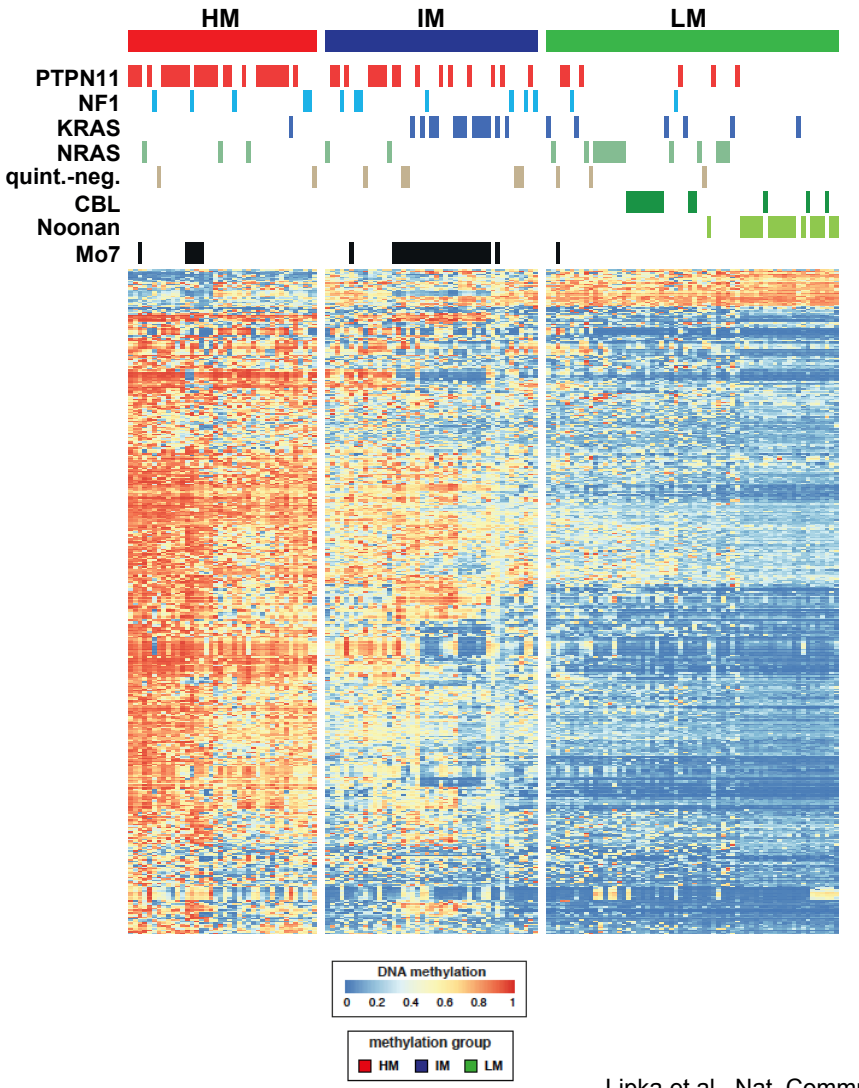
Clustering of the JMML discovery cohort



Lipka et al., Nat. Commun. 2017

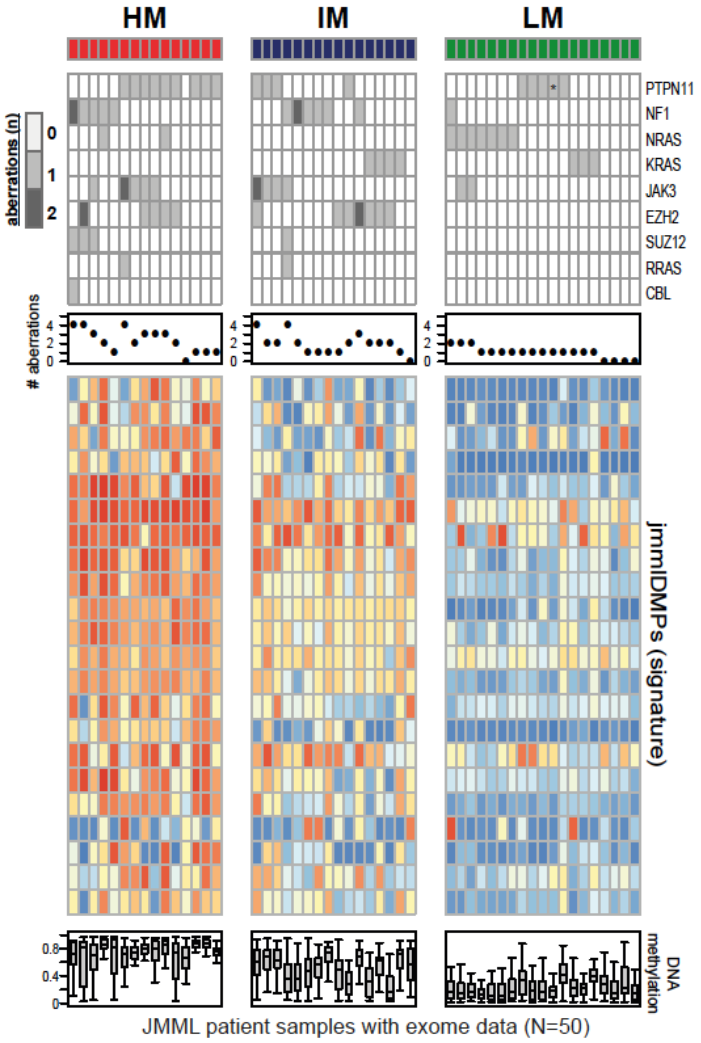
Validation cohort: three distinct molecular subtypes (n=147)



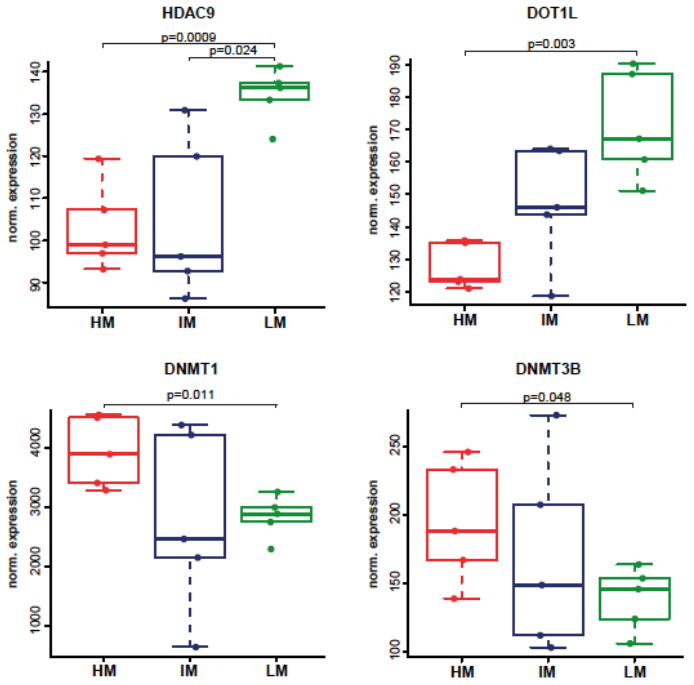


Lipka et al., Nat. Commun. 2017

RAS-mediated deregulation of the epigenetic machinery in JMML?

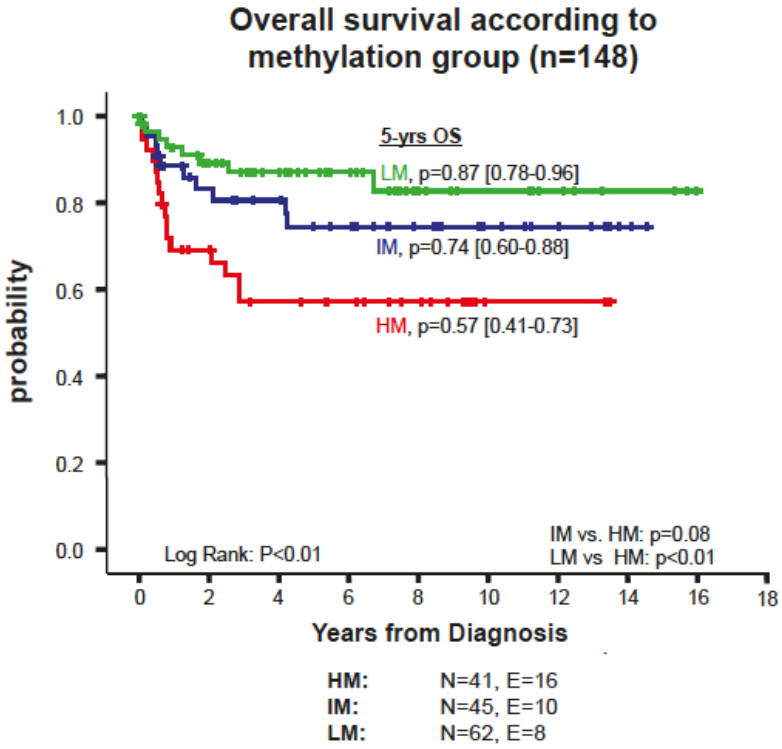


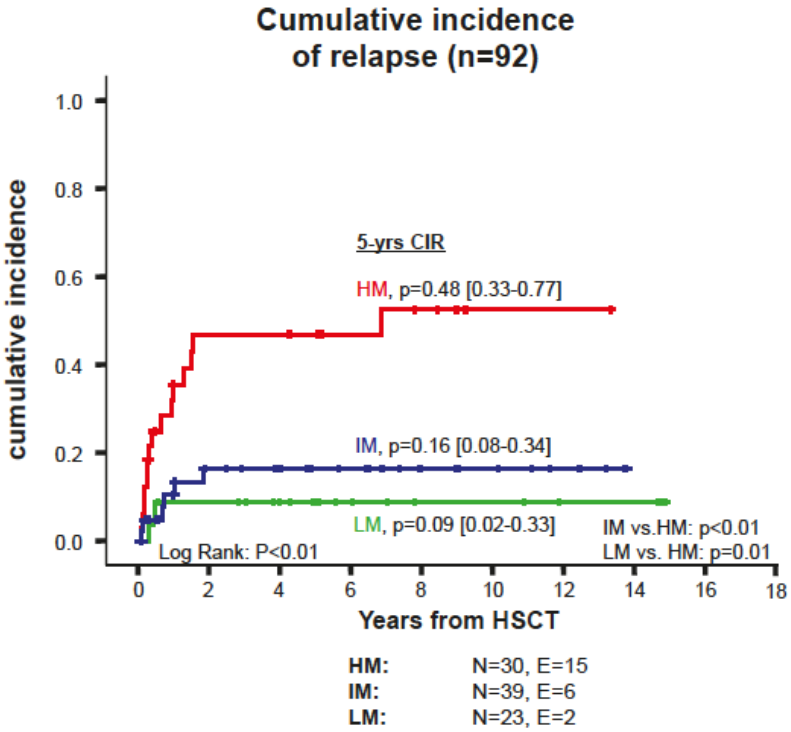
HM: 6
LM: 1



Lipka, Witte et al., Nat. Commun. 2017

High risk of relapse in the HM group





Lipka et al., Nat. Commun. 2017

Univariate analysis

		Total	HM-group	IM-group	LM-group	p
	n	147	40	45	62	
Age at diagnosis [years]	***mean (range)***	1.4 (0.1-12.3)	3.1 (1.0-12.3)	1.4 (0.1-6.0)	0.4 (0.1-3.6)	*<0.01*
	< 2 yrs.	95 (65%)	5 (13%)	32 (71%)	58 (94%)	*<0.01*
	>= 2 yrs.	52 (35%)	*35 (88%)*	13 (29%)	4 (6%)	
Platelets [10^9/l]	***mean (range)***	79 (5-548)	*38 (5-234)*	99 (12-442)	110 (9-548)	*<0.01*
	< 70	62 (47%)	*28 (78%)*	18 (43%)	16 (29%)	*<0.01*
	>= 70	71 (53%)	8 (22%)	24 (57%)	39 (71%)	
	missing	14	4	3	7	
Monocytes (PB) [%]	***mean (range)***	19 (0-55)	15 (5-31)	26 (5-55)	20 (0-38)	*<0.01*
	<10%	20 (14%)	10 (25%)	5 (11%)	5 (8%)	*<0.01*
	10-19%	55 (37%)	19 (48%)	12 (27%)	24 (39%)	
	>=20%	72 (49%)	11 (28%)	*28 (62%)*	33 (53%)	
Hemoglobin F (age-adjusted)	***normal***	43 (41%)	0 (0%)	13 (41%)	30 (71%)	*<0.01*
	elevated	63 (59%)	*32 (100%)*	19 (59%)	12 (29%)	
	missing	41	8	13	20	
Karyotype	***normal***	93 (72%)	28 (76%)	20 (47%)	45 (90%)	*<0.01*
	aberrant	37 (29%)	9 (24%)	*23 (54%)*	5 (10%)	
	missing	17	3	2	12	
Mutation	***NF1***	14 (11%)	5 (14%)	7 (16%)	2 (5%)	*<0.01*
	PTPN11 som	48 (39%)	*26 (70%)*	16 (37%)	*6 (14%)*	
	KRAS som	20 (16%)	1 (3%)	*13 (30%)*	6 (14%)	
	NRAS som	19 (15%)	3 (8%)	2 (5%)	*14 (32%)*	
	CBL	13 (11%)	0 (0%)	*0 (0%)*	*13 (30%)*	
	No mutation	10 (8%)	2 (5%)	5 (12%)	3 (7%)	
	Noonan	18	0	0	*18*	
	incomplete	5	3	2	0	

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Multivariate analysis

- **Cox model for relapse with TRM as competing event**
  - methylation group
  - age at Dx
  - sex
  - *PTPN11* mutation status (somatic only)
  - platelet count

- **<u>Results:</u>**
  - **Methylation group**
    - HM vs. LM: **RR 10.9** [1.8-66.2]
    - HM vs IM: RR 4.8 [1.4-17.2]
    - IM vs. LM: RR 2.2 [0.4-11.2]
  - **PTPN11 mutation status**
    - *PTPN11*-mut vs. all other: **RR 3.3** [1.2-8.9]

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Take-home Message

Complex epigenetic alterations contribute to tumor initiation and progression and are as important for the understanding of tumor biology as genetic alterations.

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