ESMO Discussion

Andreas Neubauer

Wien, 1.10.2012

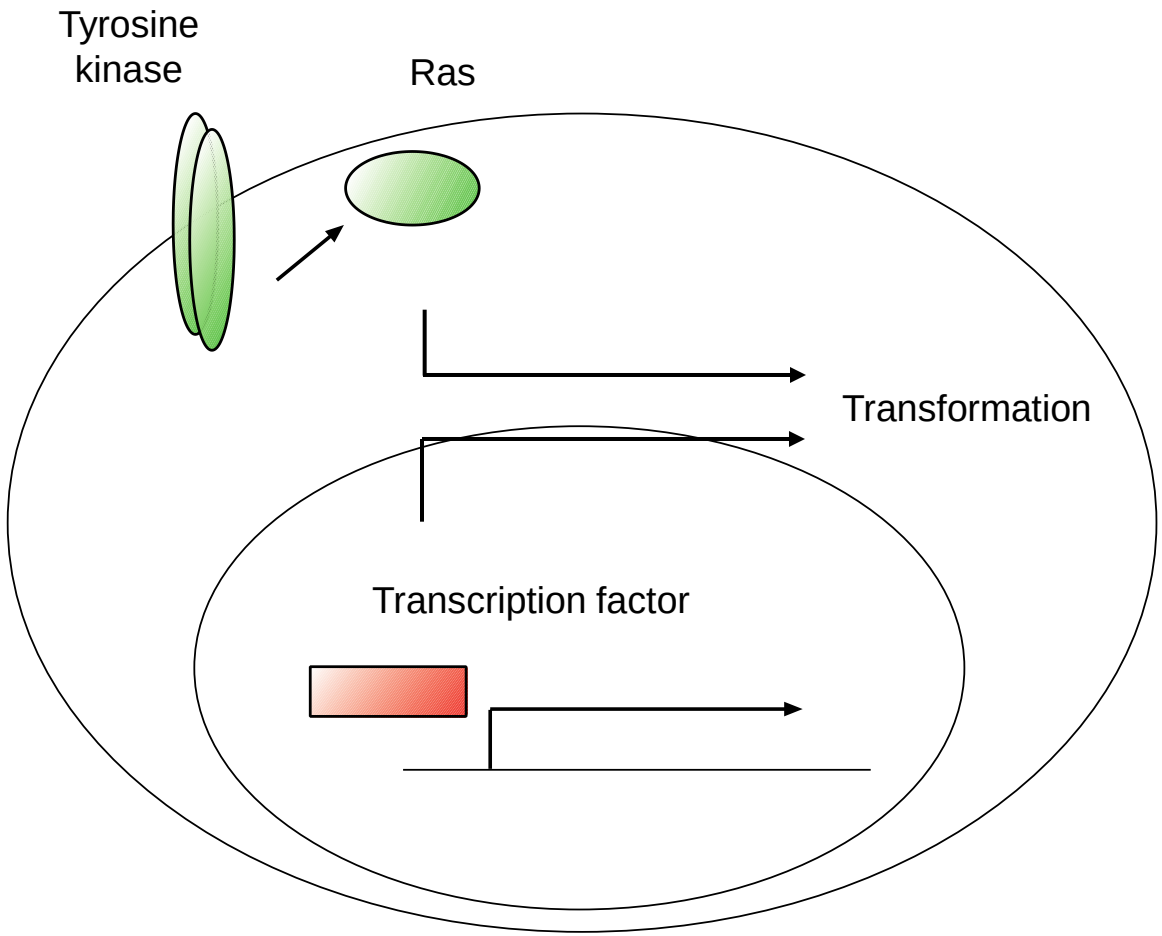
Disclosure

- ESMO policy requires that all Speakers show a Disclosure slide at the start of their presentation. Please state your disclosure, even if you have no Conflicts of Interest to declare

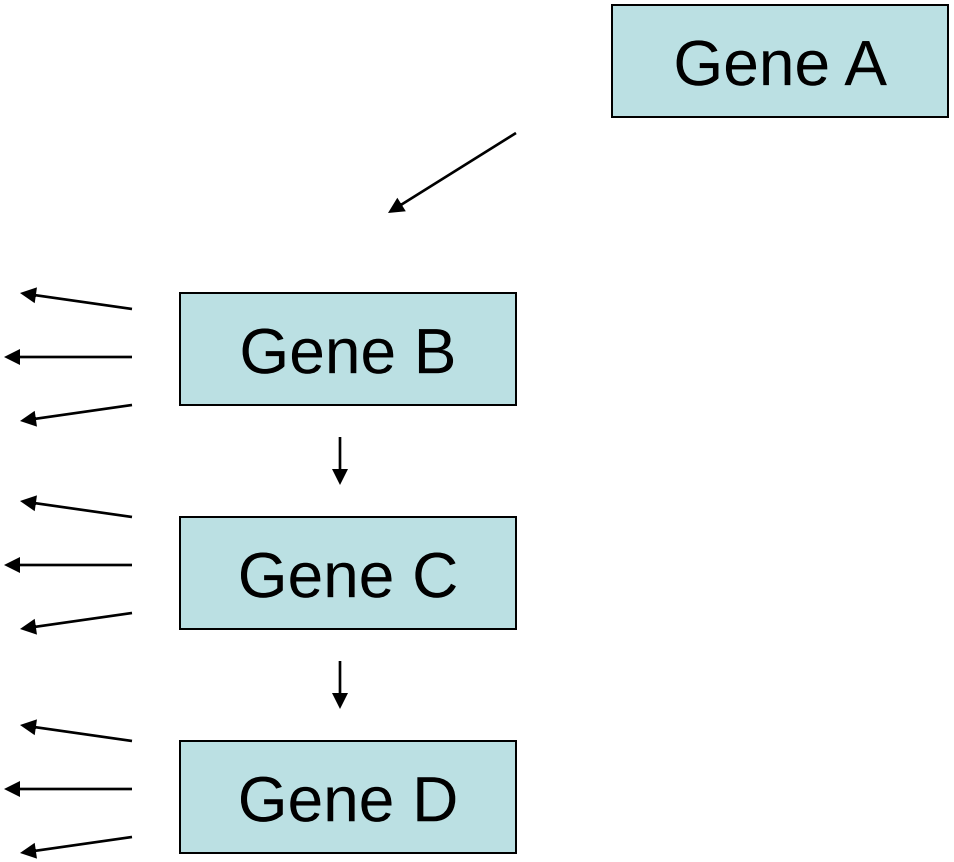
- Professor of Internal Medicine
- Director of Dept Hematology / Oncology / Immunology, University Clinic Gießen and Marburg
- Funding by DFG, BMBF, Krebshilfe, Carreras- and Behring-Foundation
- Funding for laboratory research (2003-2009) Roche
- Participation in clinical study presented by Dr. Bullinger

- No financial / other conflicts of interests

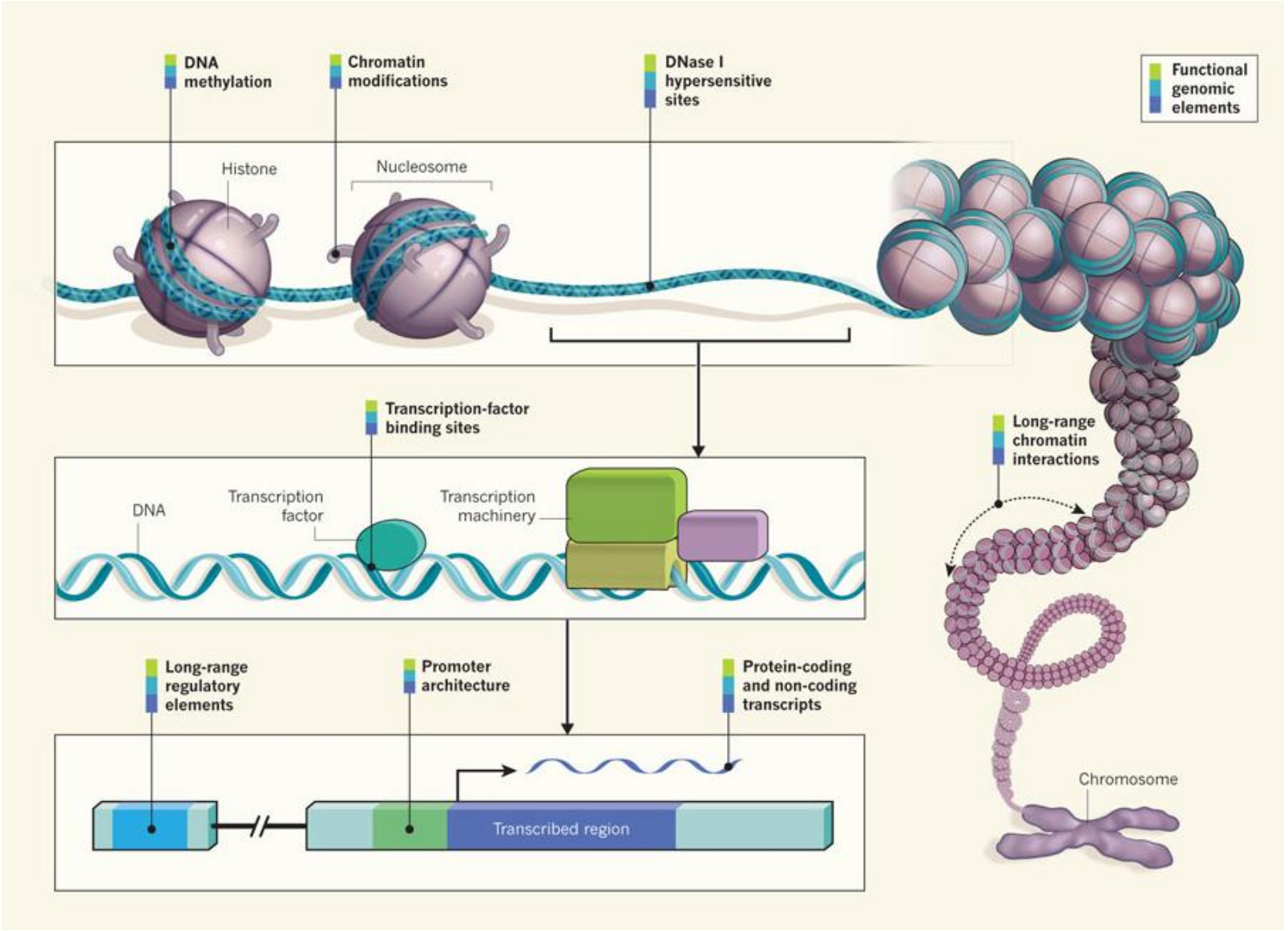
Acute myeloid leukemia



Pleiotropic actions of oncogenes



The ENCODE project: Beyond the sequence



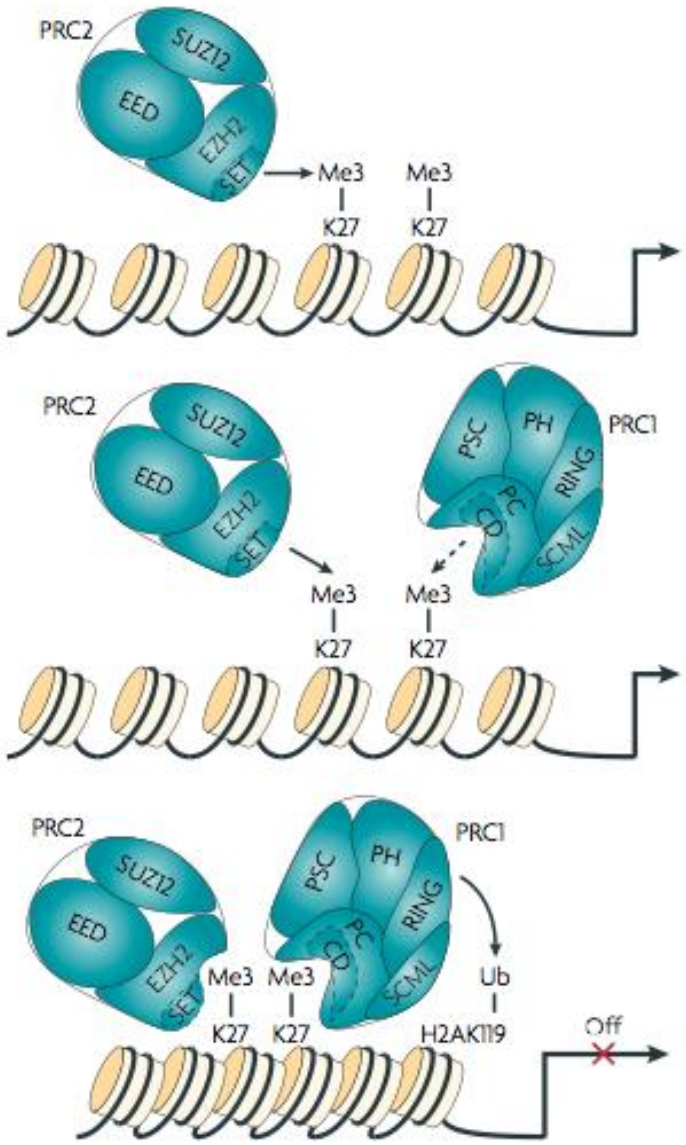
Ecker et al., Nature 2012

Overexpression of enhancer of zeste homolog 2 is associated with clinical outcome in acute myeloid leukemia patients

Po-Han Lin

Department of Medical Genetics and Internal Medicine, China Medical University Hospital, Taichung, Taiwan

The polycomb repressor complex



Bracken and Helin, Nat Rev Cancer 2009

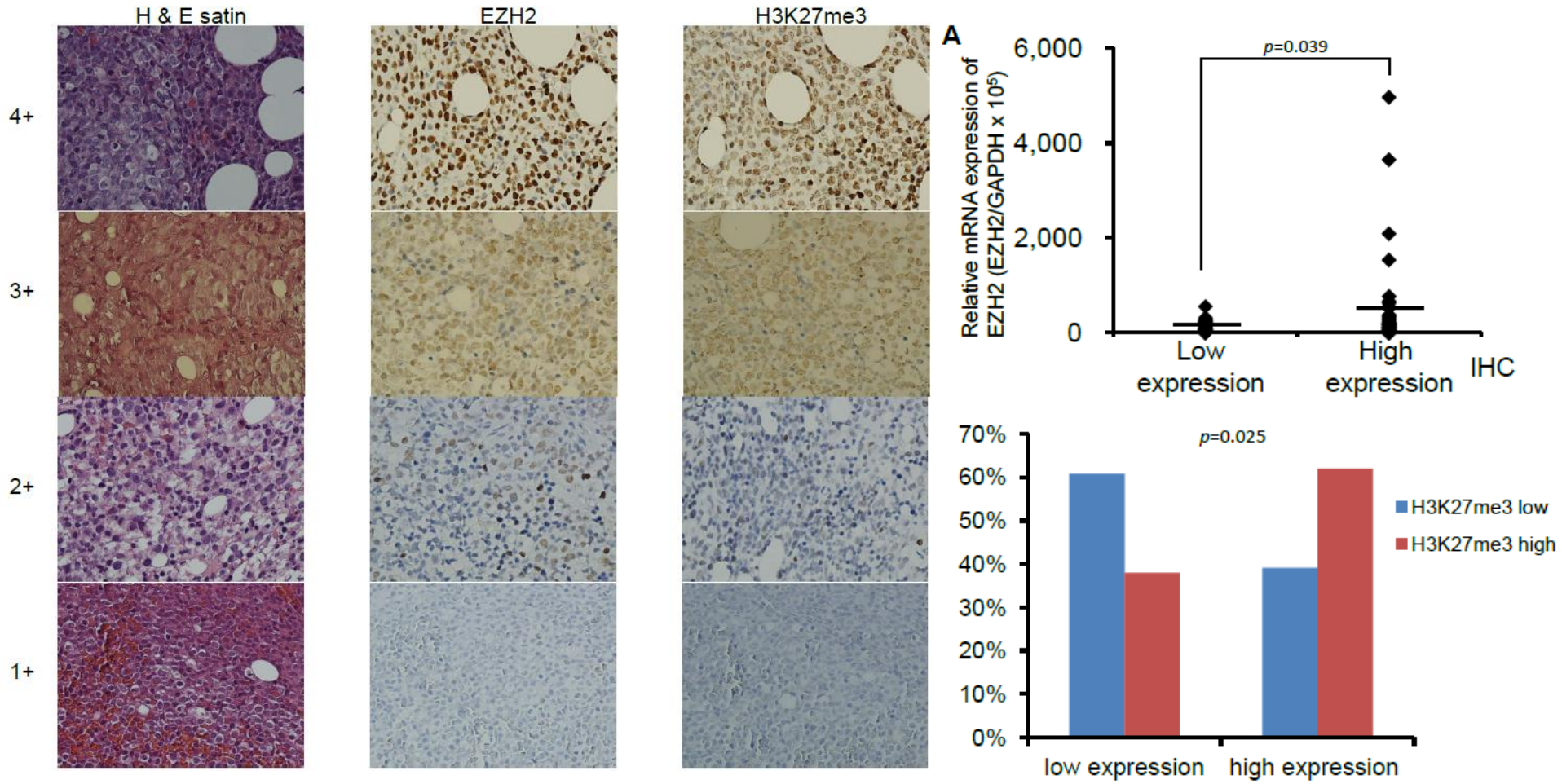
Study objective and design

- To determine the role of EZH2 expression and mutation in AML

- N=105 AML („with intensive treatment“)
- Immunohistochemistry: EZH2 and H3K27me3
- RQ PCR: EZH2
- Sequencing EZH2 exons 2-20
- Overall survival

Lin et al., ESMO 2012

IHC analysis of EZH2 and H3k27me3; QPCR for EZH2 expression level

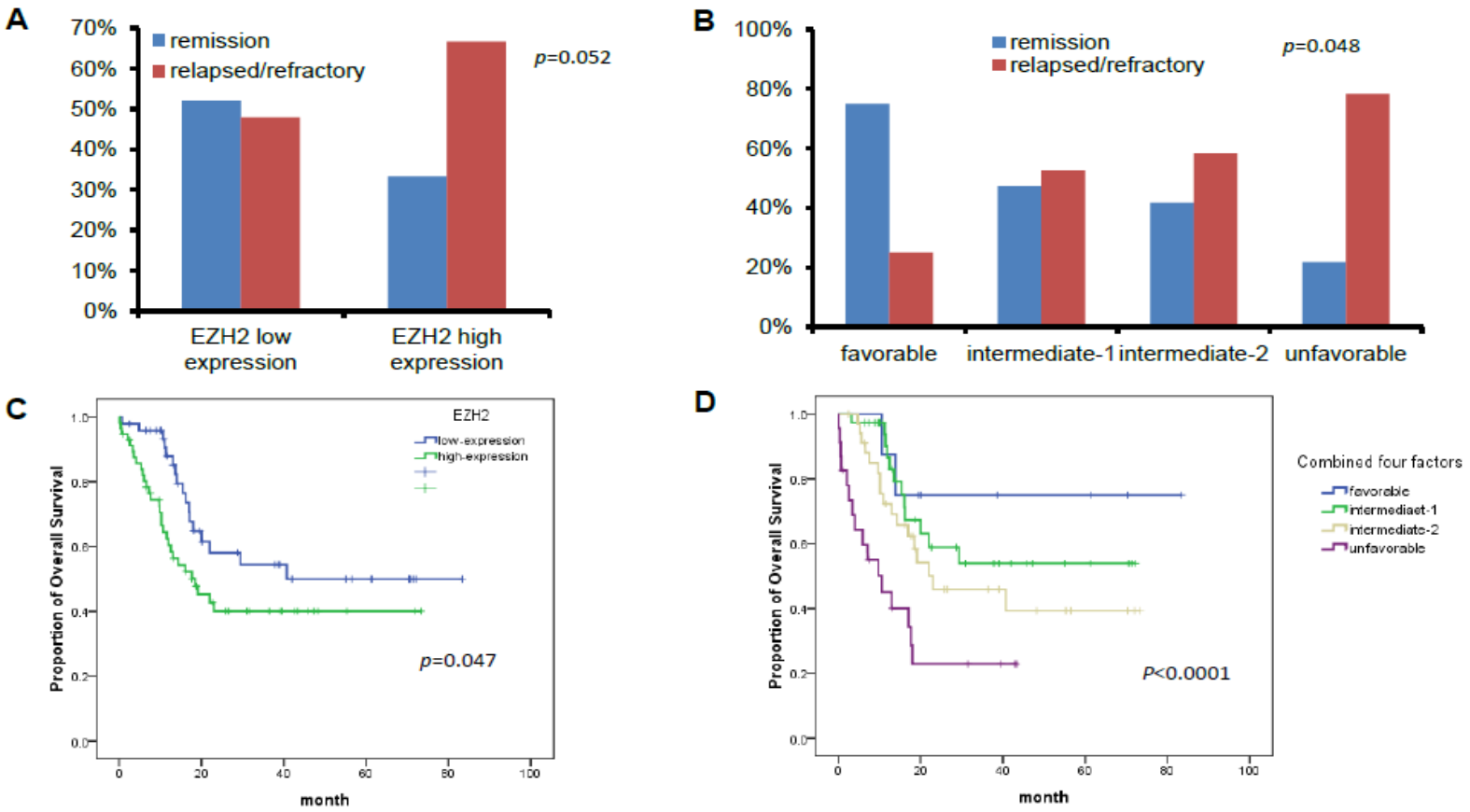


The expression level of IHC result is correlated with QPCR mRNA expression in EZH2 gene. The nuclear staining of H3K27me3 is positively associated with expression level of EZH2.

Proposed scoring classification

Prognostic variances					
Chromosome	Favorable	0	*FLT3*	ITD	+1
	Intermediate	+1		Wild type	0
	Unfavorable	+2	EZH2	High expression	+1
NPM1	Mutation	-1		Low expression	0
	Wild type	0			

Proposed scoring classification	
Favorable	0
Intermediate-1	1
Intermediate-2	2
Unfavorable	3-4

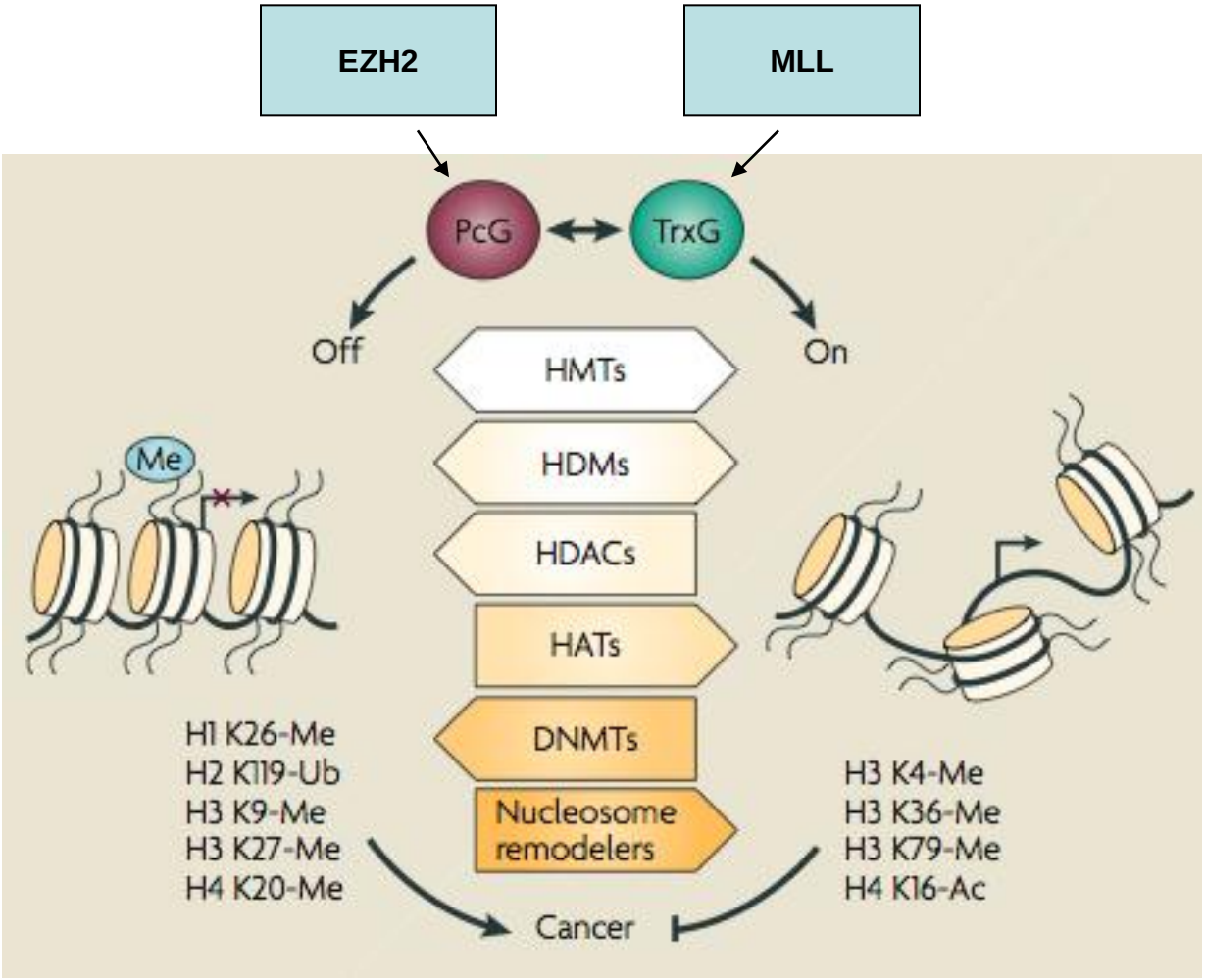


Patients with EZH2 overexpression had a significant shorter median overall survival (OS; 18.5 vs 40.7 months, p=0.047) as well as a higher trend of relapsed/refractory disease (47.9% vs 66.7%, p=0.052). We defined a prognostic classification based on karyotype, *NPM*, *FLT3*-ITD and EZH2 expression level that was able to classify patients into four risk groups showing significant different survivals (median OS: not reached, not reached, 22.0 and 10.6 months, respectively; p< .0001) and probabilities of disease relapsed/refractory (p=0.048).

Discussion Lin et al.

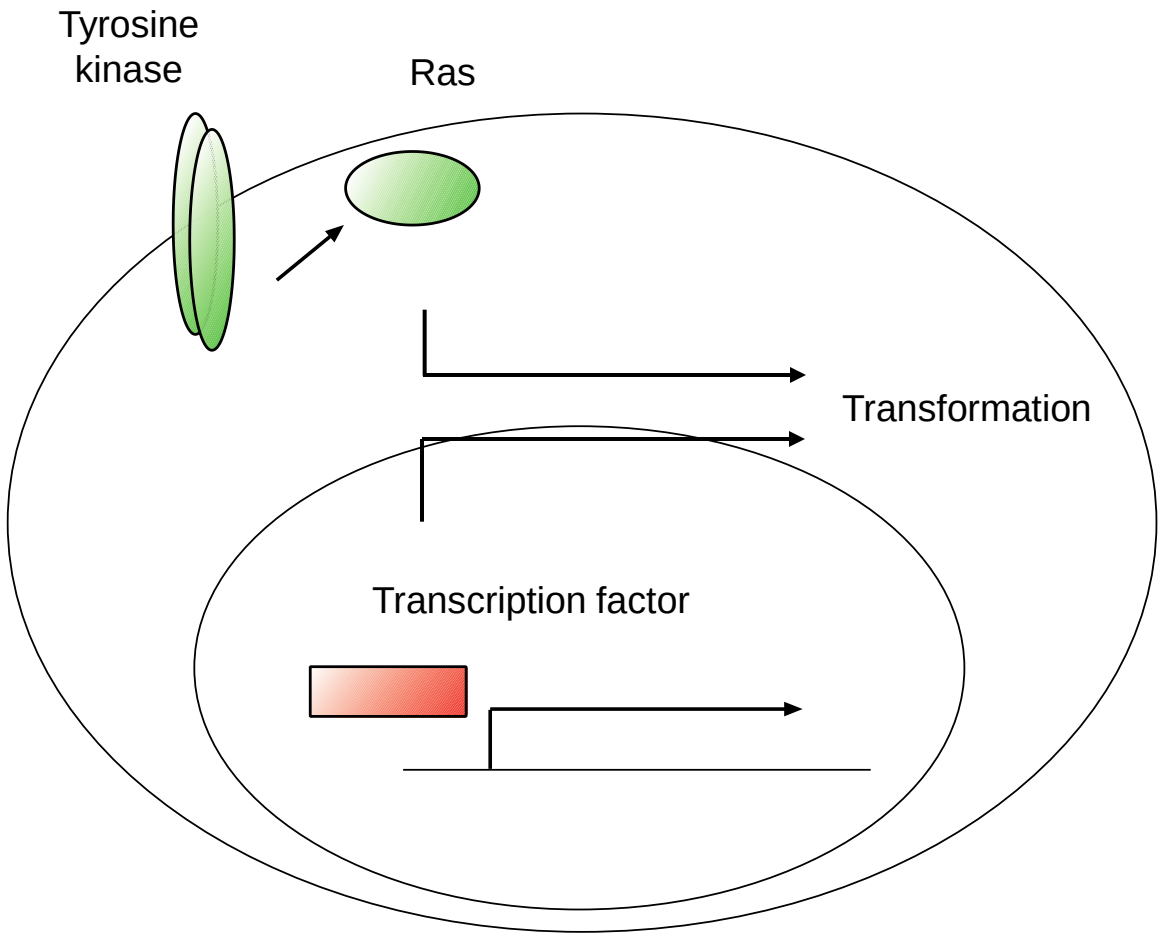
- Good correlation of immune staining and RQ PCR (high IHC)?
- Comparable therapy in all cases?
- Prognostic index w/o EZH2?
- Repression of which gene determines poor prognosis by EZH2?
- EZH2 may play a critical role in AML

The polycomb repressor and trithorax complexes



Mills, Nat Rev Cancer 2010

Acute myeloid leukemia



High Co-expression of CD135 and CD117 Predicts Poor Outcome in Acute Myeloid Leukemia: A Prospective Study

S.K. Sharawat

Department Of Medical Oncology , All India Institute of Medical Sciences

New Delhi -110029, INDIA

CD135 and CD117 co-expression in AML

- CD117
- C-kit: stem cell receptor
- Mutations activate TK
- „druggable“

- CD135
- FLT3: fms like tyrosine kinase 3
- TK mutations and internal tandem duplication activate TK
- „druggable“

Study objective and design

- To evaluate to prognostic role of CD135 and CD117 co-expression in AML

- N=115 AML (>16 years?) from 4/2008 - 5/2010
- Multiparameter FACS on $CD45^{dim}$
- N=20 (17%): FLT3 - ITD

Sharawat et al., ESMO 2012

Results of study

- CD135, CD117 and CD135/CD117 were expressed in 95 (82%), 104 (90%) and 74 (64%) patients
- Patients with high co-expression had inferior EFS (P=0.0002) and OS (P=0.0003).
- In step-wise Cox regression multivariable analysis, low hemoglobin, high WBC count and increased CD135/CD117 co-expression predicted EFS.
- High co-expression of CD135/CD117 may be an independent predictor of poor outcome in AML

Sharawat et al., ESMO 2012

Discussion Sharawat et al.

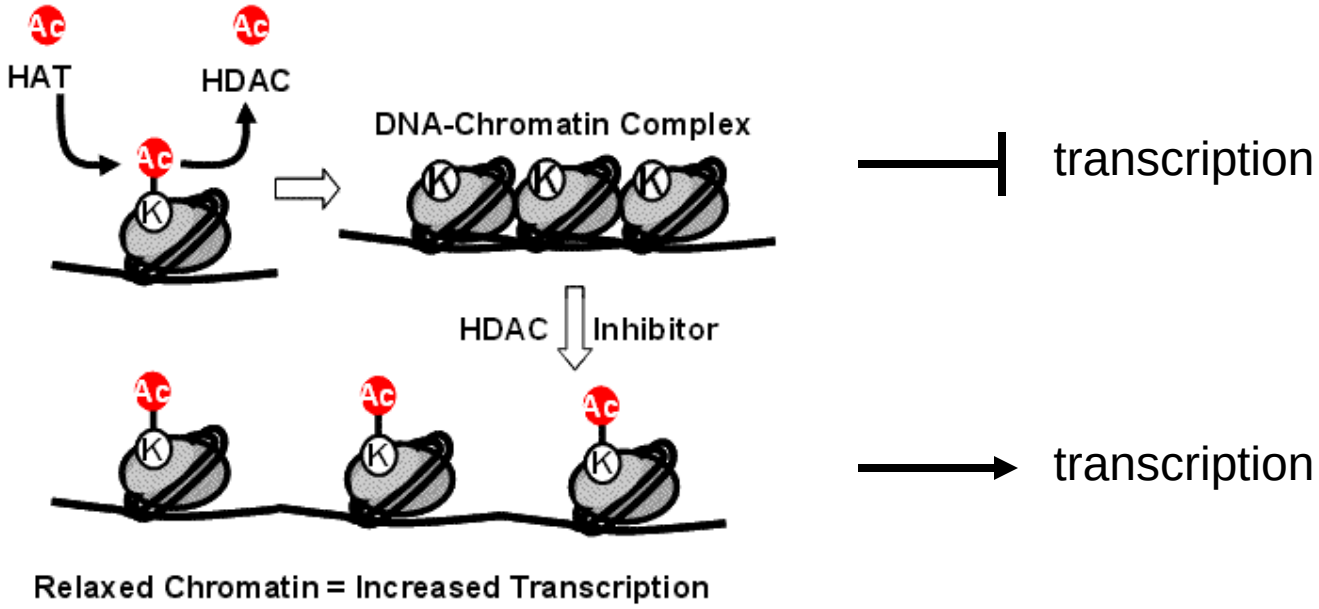
- FACS: simple, fast, available
- Multi-institutional trial?
- AML: comparable therapy?
- Co-expression of two TK genes: what advantage for AML cells?
- Co-expression of CD135 and CD117: what genetic background?
- If corroborated: targeted therapy with TK inhibitors such as sorafenib or sunitinib?

Results of a phase I/II trial of belinostat in combination with idarubicin in AML – favorable impact on mainly intermediate cytogenetic risk AML can be predicted by gene expression profiling

Lars Bullinger
Department of Internal Medicine III,
University Clinic, Ulm, Germany

ClinicalTrials.gov ID: NCT00878722

Histone acetyltransferases and histone deacytelases



Modified: http://mktg.biotek.com/images/figure1.gif

Study objectives and design

- To evaluate gene expression profiles and response to HDAC inhibitor belinostat in AML

- Phase I/II study on 41 AML patients
  - >60y: refractory / relapsed AML
  - 18-60y: 2nd relapse / refractory to 2 AML courses
  - >60y: high risk AML
  - >60y: MDS > 10% blasts in marrow
- Gene expression profiles

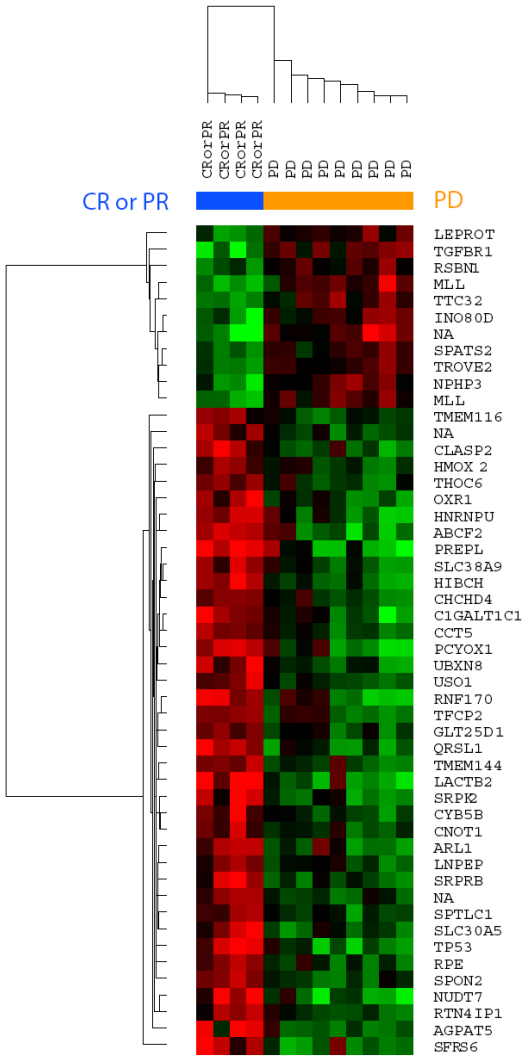
Bullinger et al., ESMO 2012

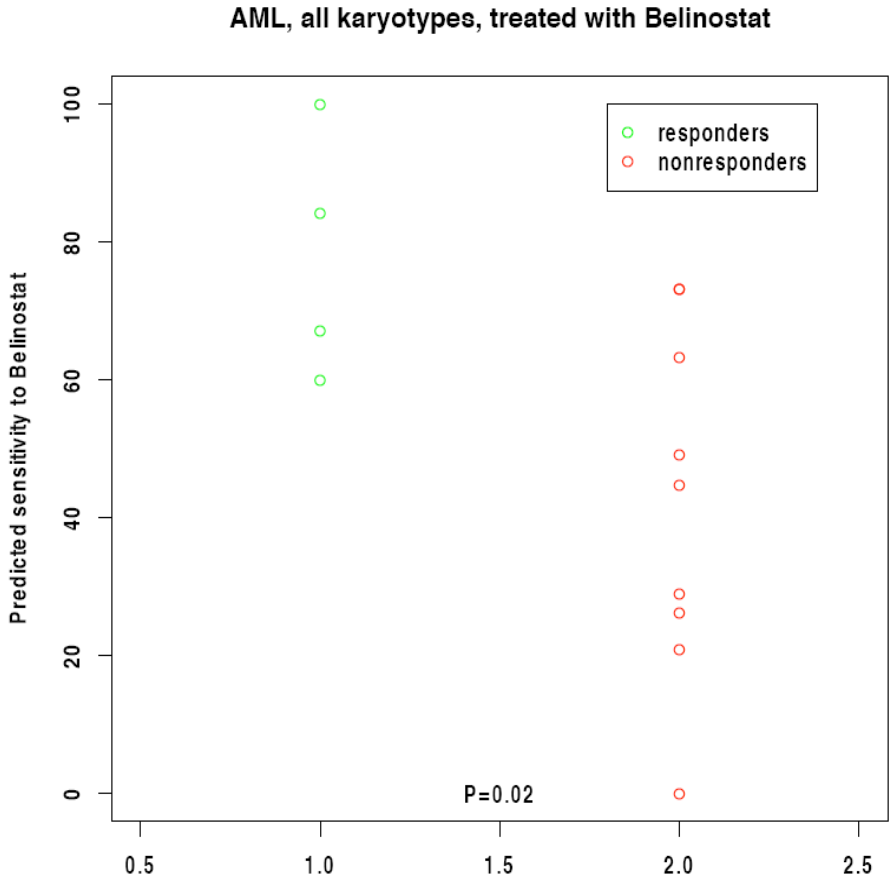
Results Bullinger (I)

- 9 / 41 AML patients responded (22%)
  - 7 / 25 complete / partial response low / intermediate risk (28%)
  - 2 / 16 PR high risk AML (13%)

Schedule	Step	Patient	Best Overall Response	Response Duration (Weeks)	Event Free Survival (Weeks)	AML Type
A	2	033-006	CR	26.3	28.7	De novo
A	3	030-008	CR	20.6	28.3	After MDS
A	4	030-016	CRi	28.1	28.1 (2.7)*	After MDS
B	6	011-056	PR	5.4	11.4	De novo MDS
B	7	033-057	CR	30.3	32.1	After MDS
B	8	033-065	PR	19.6	26	After MDS
B	9	032-072	CR	6.1	8.1	After MDS
B	9	033-067	PR	3	3.9	After MDS
B	9	033-073	PR	3.9	4.3	Treatment AML

Response to HDAC inhibitor belinostat correlates with gene expression pattern and may be predicted by an *in vitro* score





Bullinger et al., ESMO 2012

Conclusion Bullinger et al.

- HDAC inhibitor belinostat is active in high risk / relapsed AML
- Very heterogeneous patient cohort, different treatments
- Response to belinostat is associated with specific gene expression pattern, e.g. p53, MLL pathway
- Which gene **downstream** of this is responsible?
- Different risk profiles possible in AML patients treated with HDAC inhibitors?
- “high risk” cases may still benefit from an epigenetic treatment

Summary Lin, Sharawat, Bullinger ESMO 2012

- AML is composed of aberrant tyrosine kinases plus nuclear lesions
- Prognosis determined by:
  - Polycomb repressor complex / EZH2
  - Co - expression of essential tyrosine kinases
- Epigenetic knowledge may offer new treatment options in AML

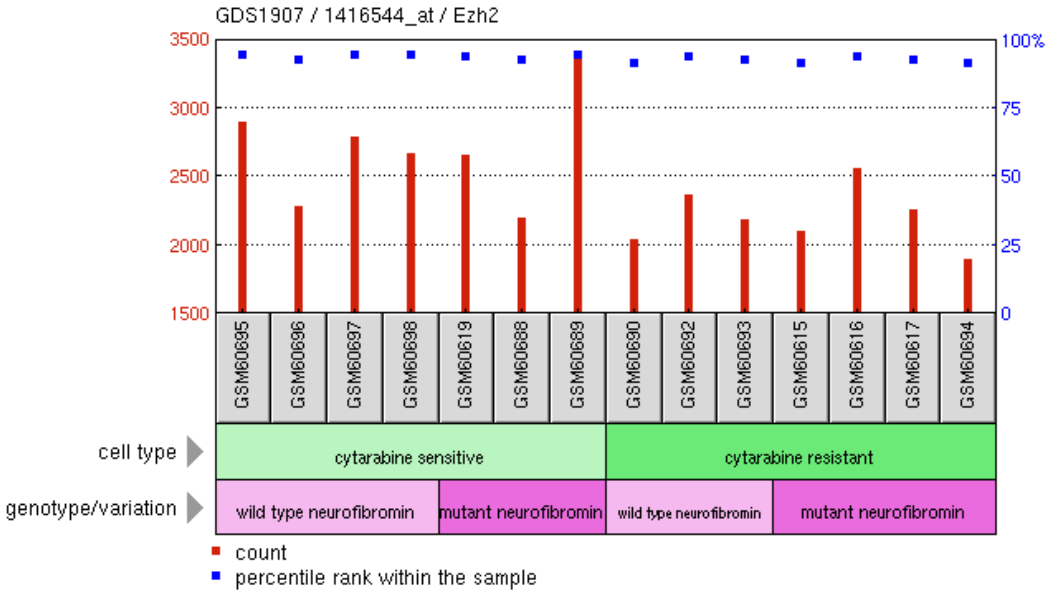
Interaction of drug dose and oncogenic *Ras*

	Less drug	More drug
Wildtype *Ras*	-	-
oncogenic *Ras*	--	++

Neubauer et al., Blood 1994
Neubauer et al., J Clin Oncol 2008
Meyer et al. PLoS One 2009

EZH2 and AML: Expression and resistance to ara-C

Profile GDS1907 / 1416544_at / Ezh2
Title Acute myeloid leukemia cells resistant to cytarabine
Organism Mus musculus



http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS1907:1416544_at