Evil in AML

Ruud Delwel

Papendal 2020

Conflict of Interest Disclosure Form

In accordance with the rules of the Health Care Inspectorate (IGZ)

Name: Ruud Delwel

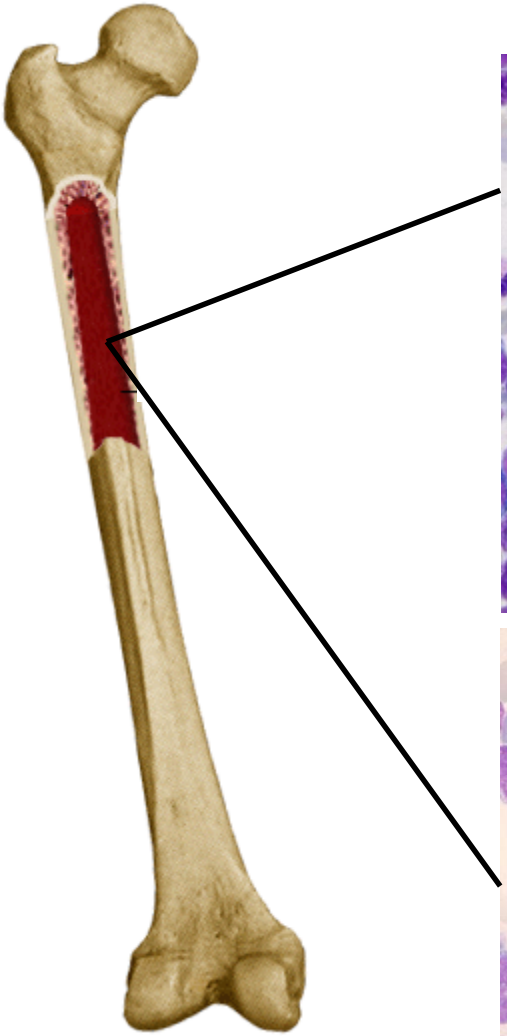
Affiliation: Erasmus MC

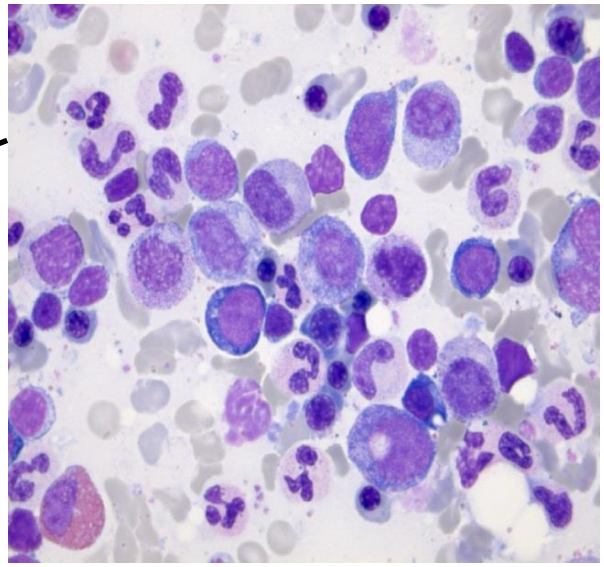
☑ I have no potential conflict of interest to report

☐ I have the following potential conflict(s) of interest to report

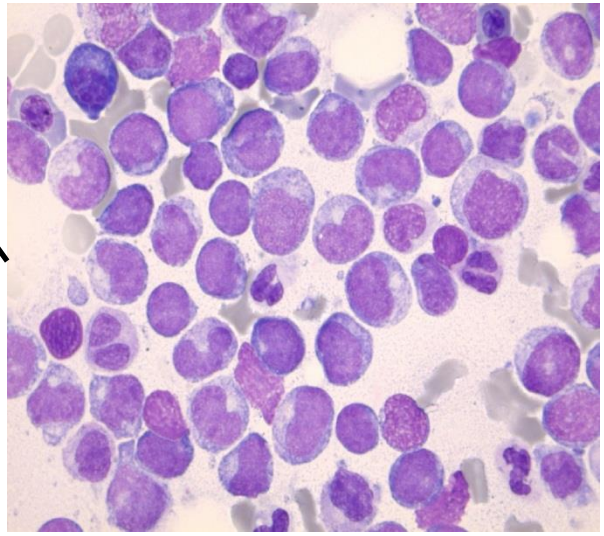
Type of affiliation / financial interest	Name of commercial company
Receipt of grants/research supports:	
Receipt of honoraria or consultation fees:	
Participation in a company sponsored speaker's bureau:	
Stock shareholder:	
Other support (please specify):	
Scientific advisory board	

Blood cell formation in bone marrow





Healthy bone marrow



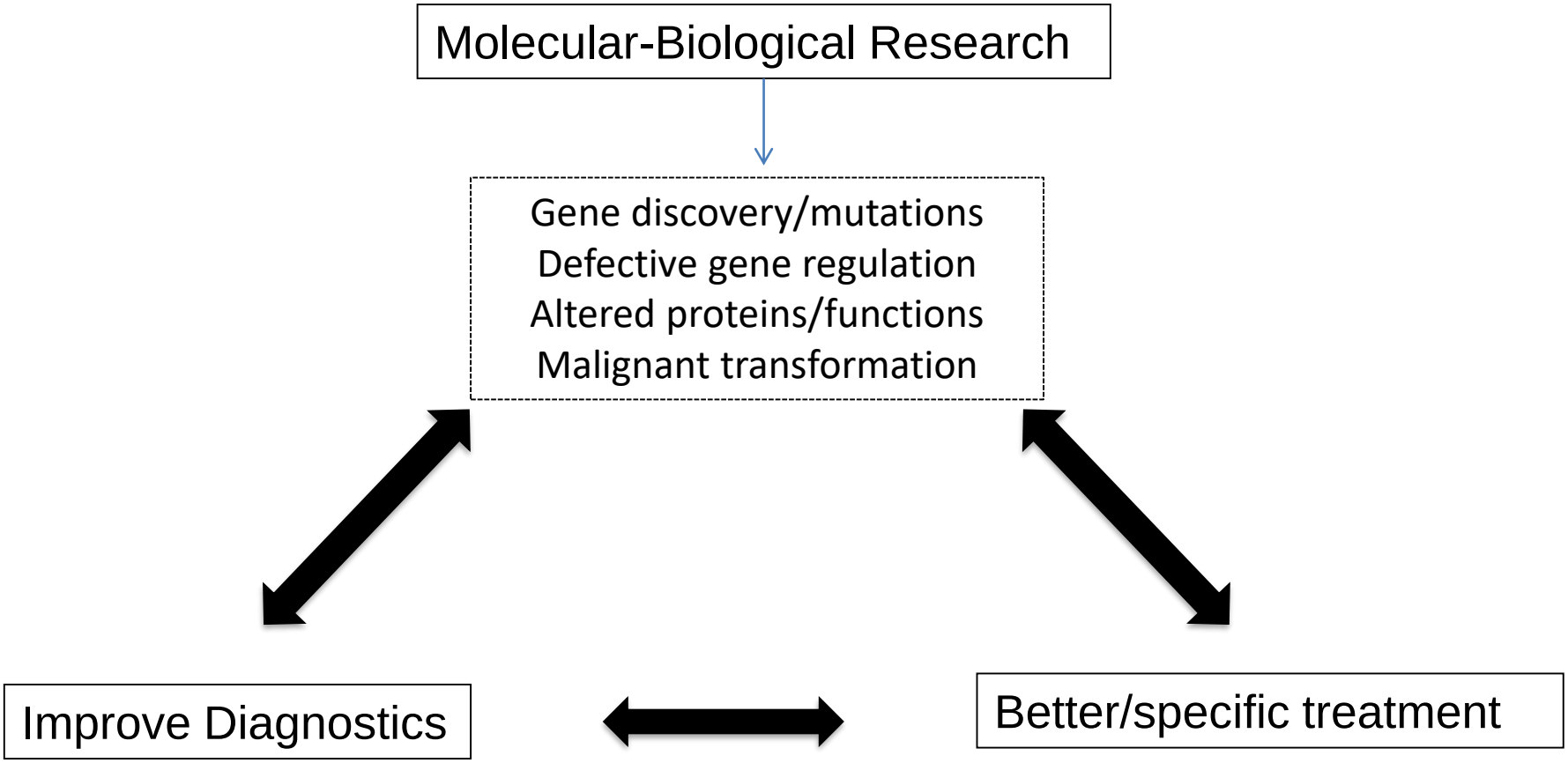
Acute Myeloid Leukemia (AML)

Acute Myeloid Leukemia is not one disease

- AML subtypes can be recognized by recurrent (cyto)genetic abnormalities
- Recurrent (cyto)genetic defects are predictive for therapy outcome
- Fusion-genes are generated at the breakpoints of most translocations

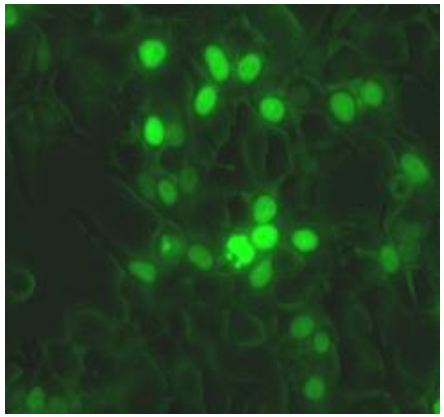
Acute Myeloid Leukemia is not one disease

- AML subtypes can be recognized by recurrent (cyto)genetic abnormalities
- Recurrent (cyto)genetic defects are predictive for therapy outcome
- Fusion-genes are generated at the breakpoints of most recurrent translocations
- *Evil* gene regulation is a hallmark of AML

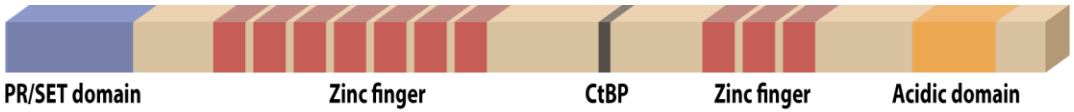

Molecular-Biological Research
Gene discovery/mutations
Defective gene regulation
Altered proteins/functions
Malignant transformation
Improve Diagnostics
Better/specific treatment

EVI1: an *Evil* gene in AML

- *EVI1 (ecotropic virus integration-1)* was first identified in mouse leukemia
- *EVI1* encodes a nuclear DNA binding zinc-finger protein
- *EVI1* is overexpressed in 3q26/3q21 AML



Nuclear expression

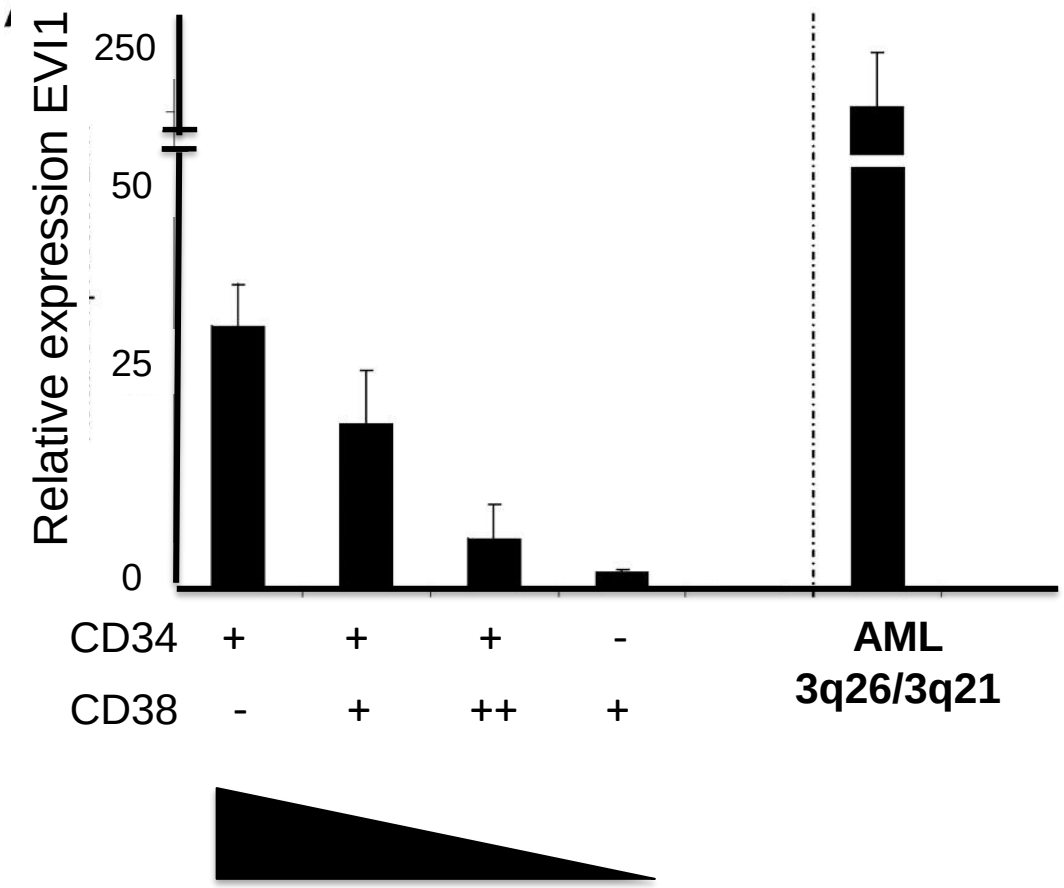


Zinc finger protein

EVI1 in AML:

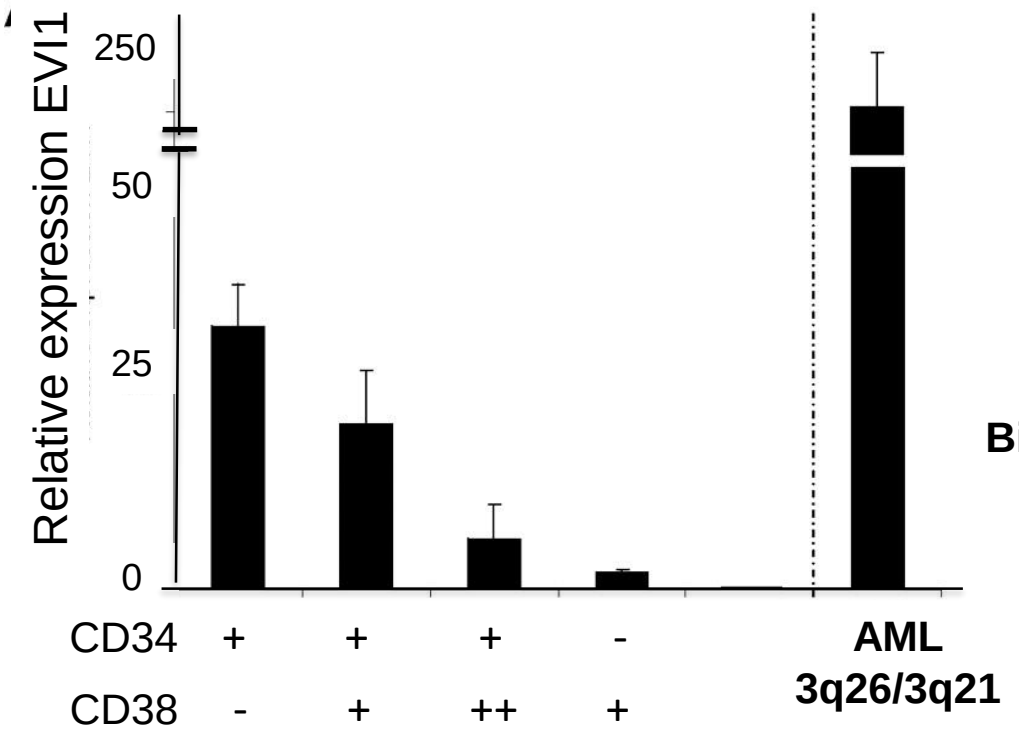
The Good Side of EVI1

EVI1 is a hematopoietic stem cell gene



Bindels et al. Blood 2012

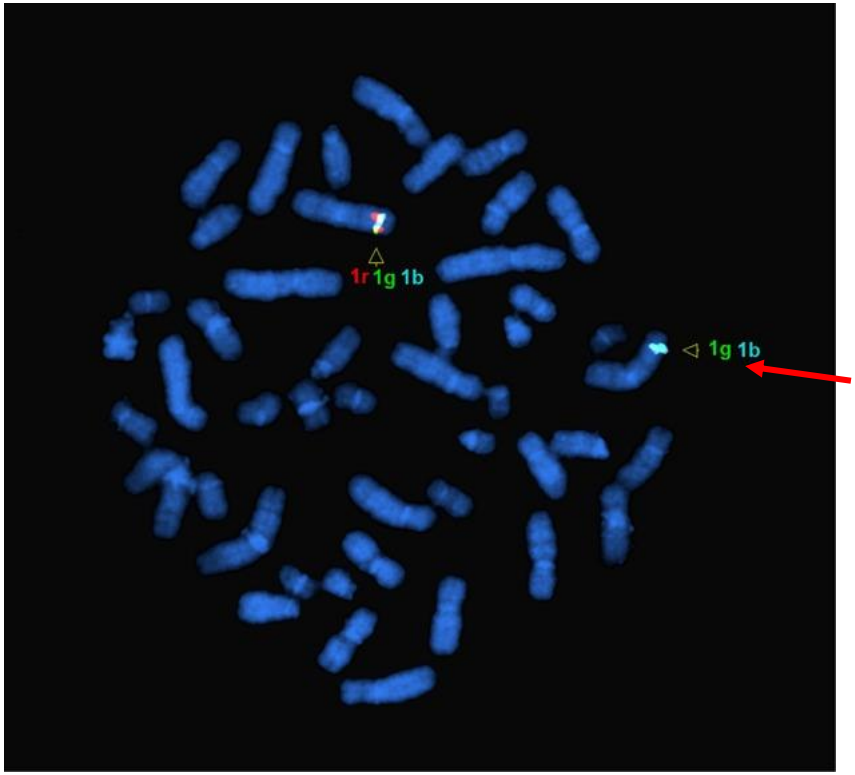
EVI1 is a hematopoietic stem cell gene



Bindels et al. Blood 2012

- ***EVI1* is expressed in dormant hematopoietic stem cells** (HSCs) in the mouse (Kataoka et al, J Exp Med, 2011)
- ***Evi1*$^{-/-}$ HSCs lose repopulating** ability in mice (Goyama et al, Cell Stem cell 2008)

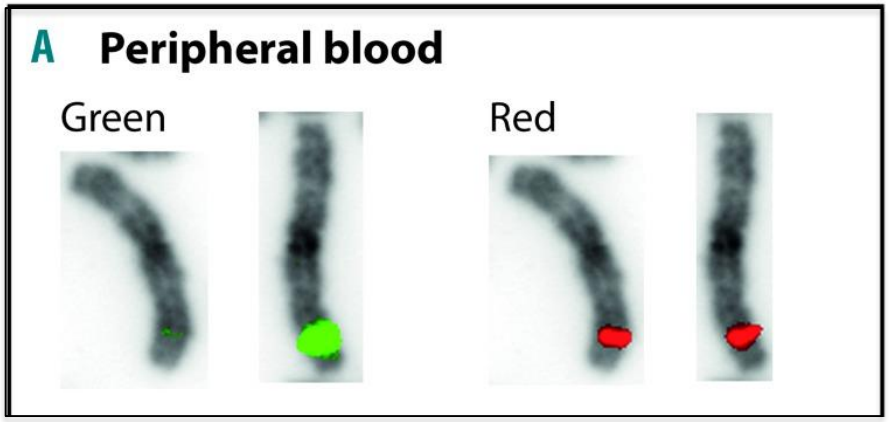
Germline loss of *EVI1*: Bone marrow failure



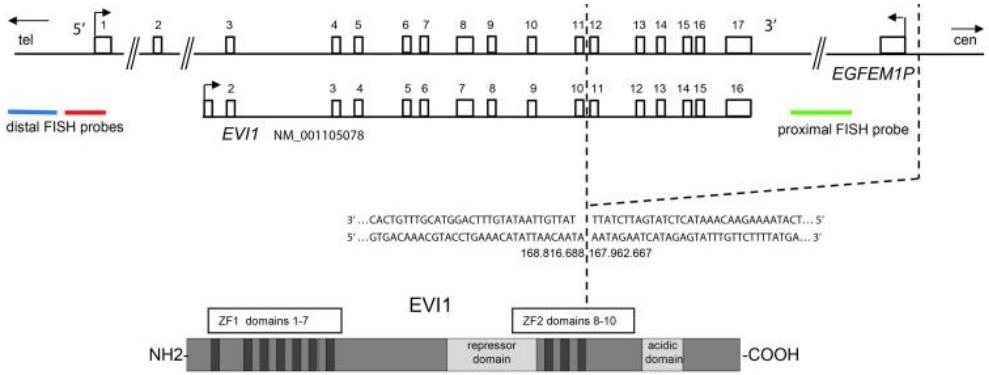
Loss of **EVI1**

Bouman and others. Am. J. of Med. Gen., 2015

Germline *EVI1* deletions in human: Bone marrow failure



Nielsen M et al. J Med Genet., 2012



Bone marrow failure and germline EVI1 deletion in humans

Deletion of the 3q26 region including the EVI1 and MDS1 genes in a neonate with congenital thrombocytopenia and subsequent aplastic anaemia.
Nielsen M and others. J Med Genet., 2012

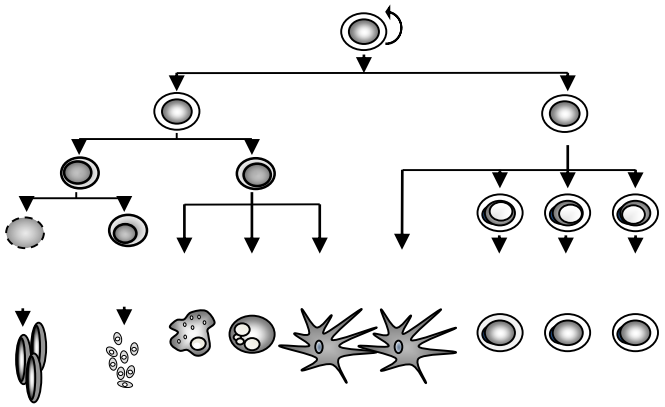
Congenital thrombocytopenia in a neonate with an interstitial microdeletion of 3q26.2q26.31
Bouman and others. Am. J. of Med. Gen., 2015

Lethal neonatal bone marrow failure syndrome with multiple congenital abnormalities, including limb defects, due to a constitutional deletion of 3' MECOM.
Buijs and others. Haematologica, 2018

Congenital hypoplastic bone marrow failure associated with a de novo partial deletion of the MECOM gene at 3q26.2.
Kjeldsen E and others. Gene. 2018

EVI1 balance is essential for HSC function

***EVI1*$^{+}$ HSC**



Healthy

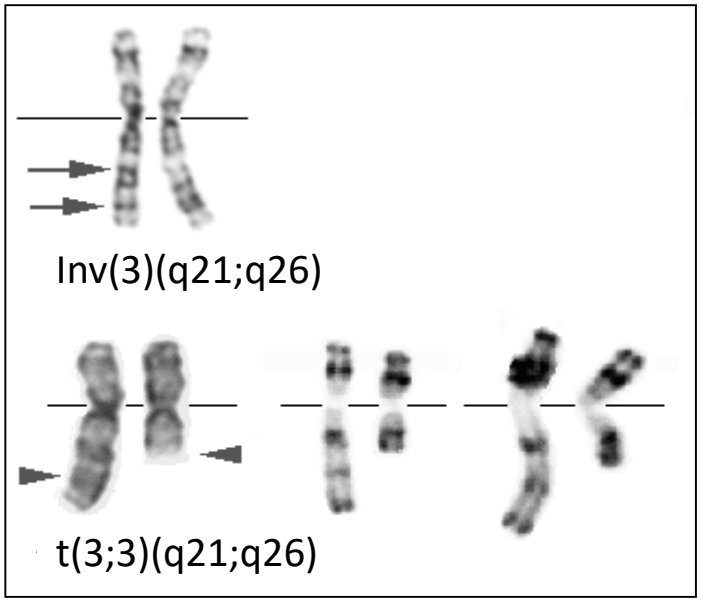
***EVI1*$^{-}$ HSC**

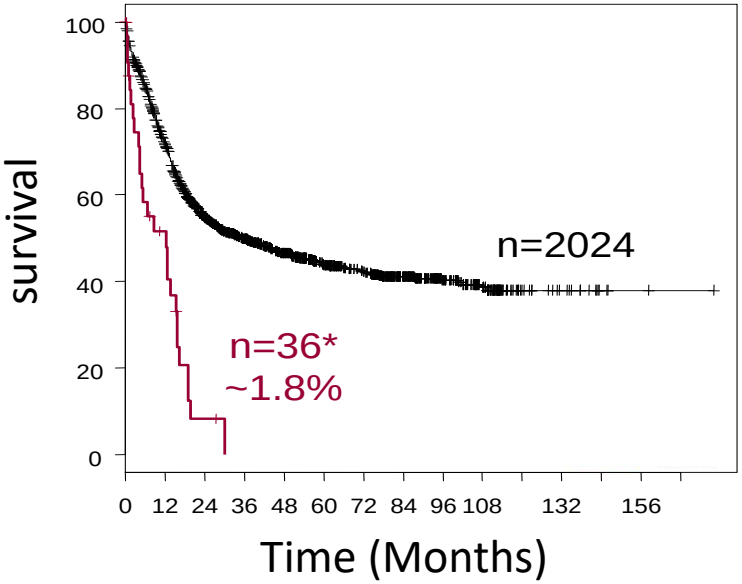
No blood cell formation

EVI1 in AML:

The Evil Side of Evi1

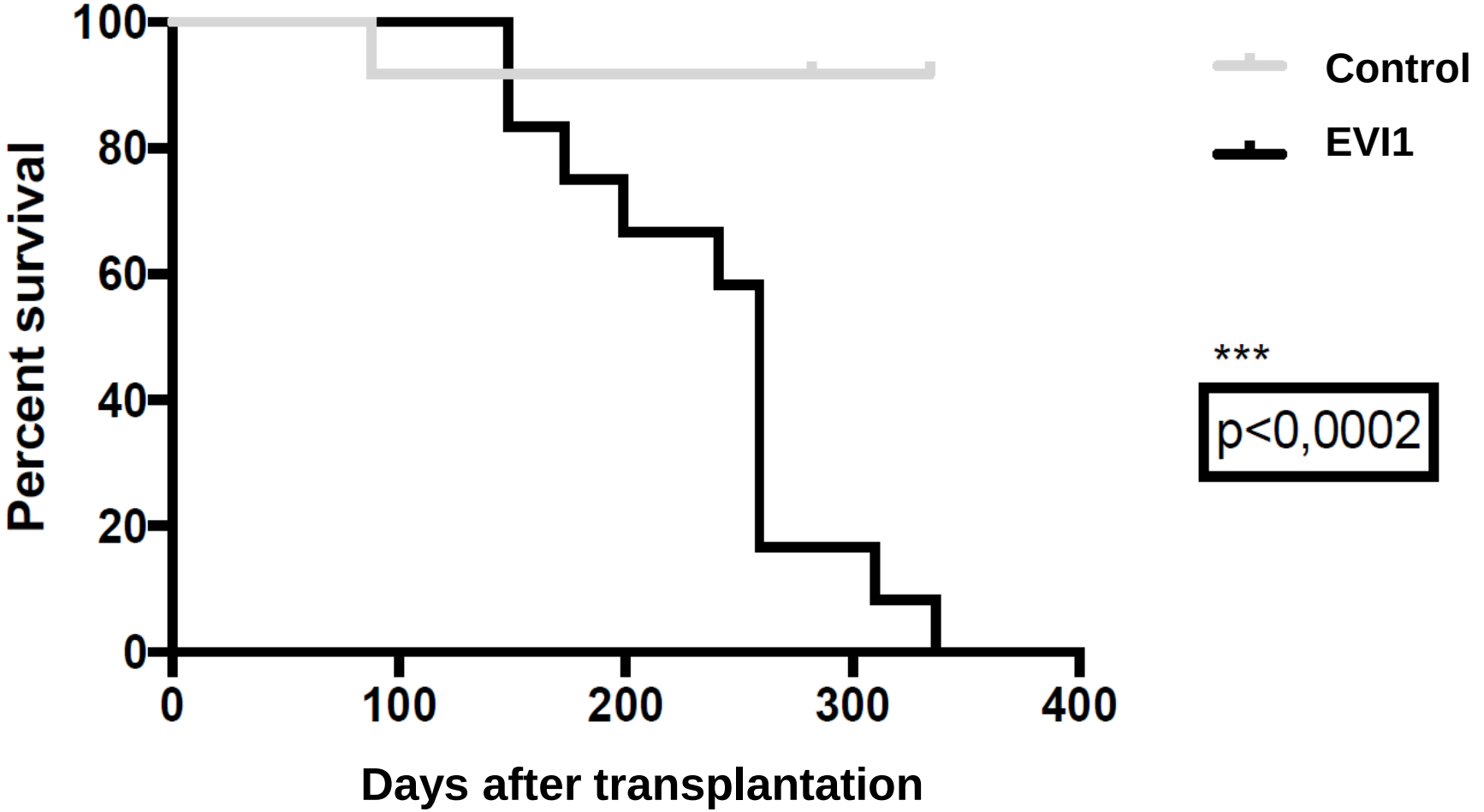
AML with chromosome 3q26/3q21 aberrations





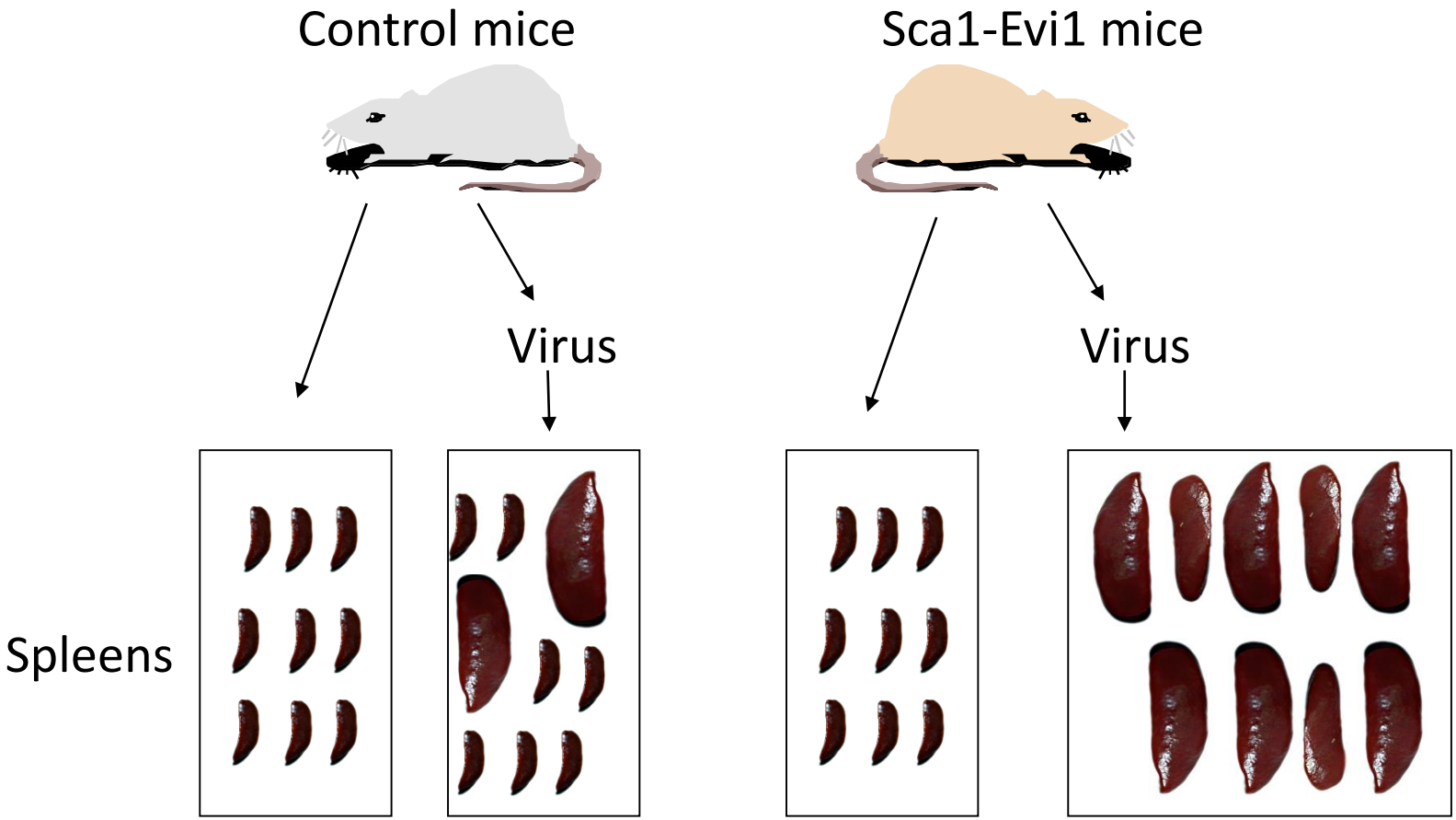
- ***EVI1*** located on chromosome 3q26 is overexpressed
- ***RPN1*** (Ribophorin-1) resides on 3q21
- WHO 2008 : ***RPN1-EVI1*** **AML**

EVI1 overexpression in HSCs causes AML



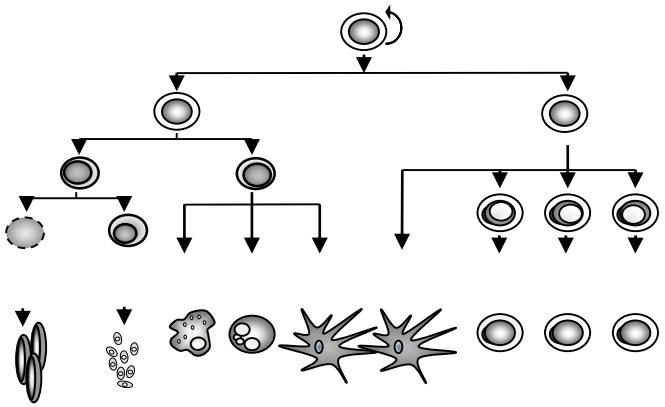
With Mick Milsom, DKFZ, Heidelberg

Sca1-*EVI1* transgenic mice are predisposed to develop AML



EVI1 balance is essential for HSC function

***EVI1*$^{+}$ HSC**

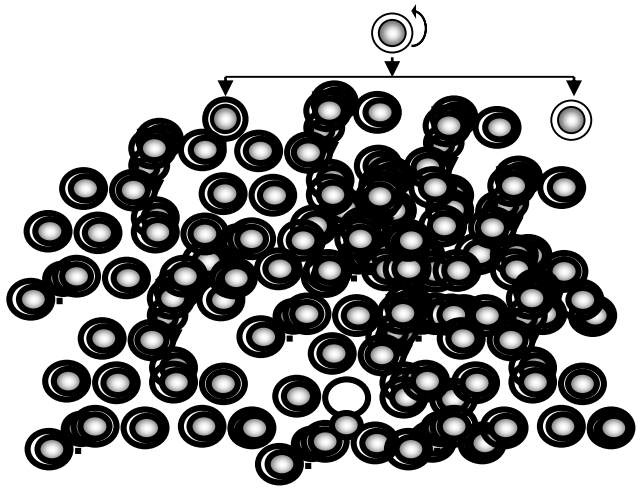


Healthy

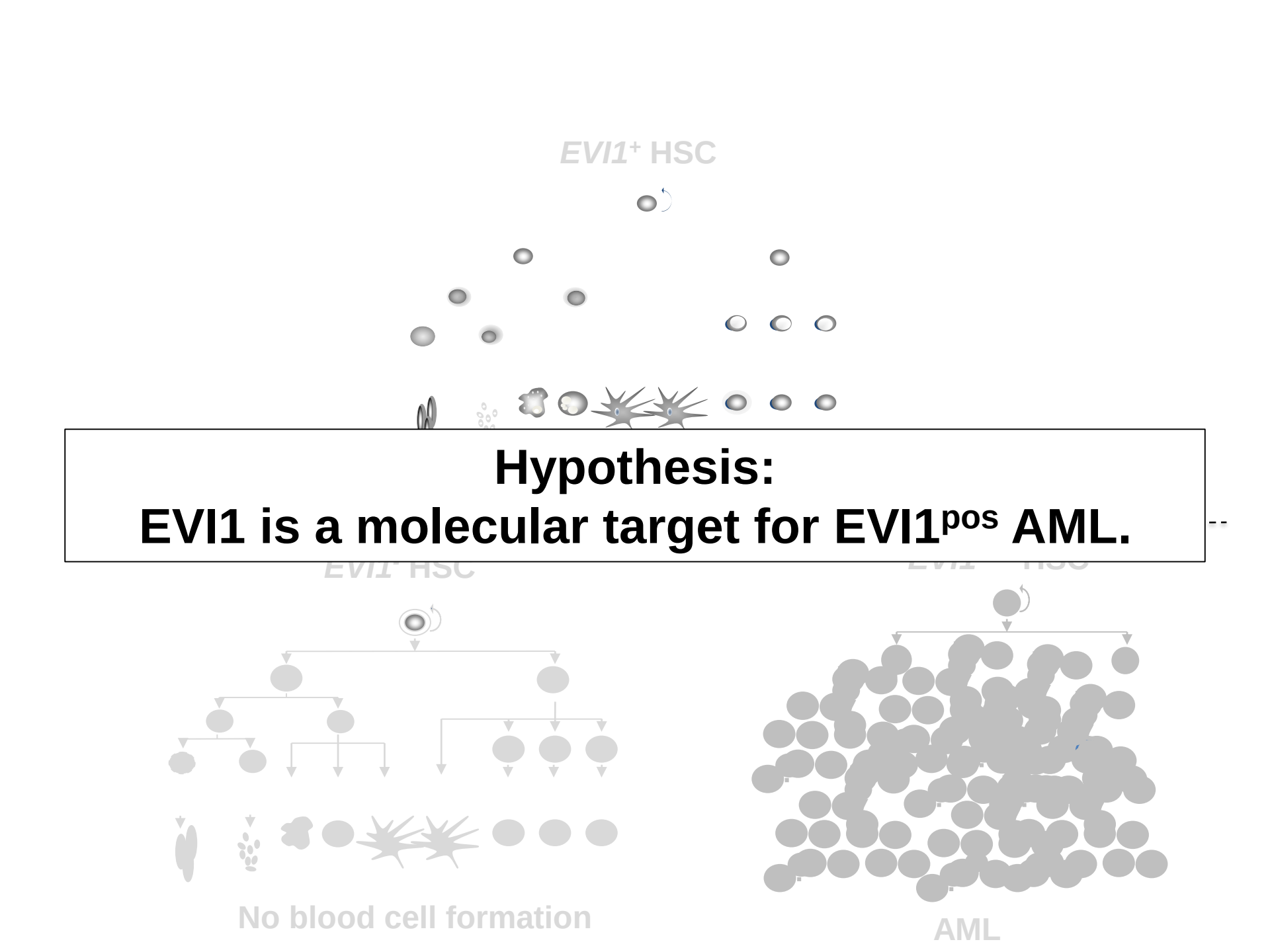
***EVI1*$^{-}$ HSC**

No blood cell formation

***EVI1*$^{+++}$ HSC**



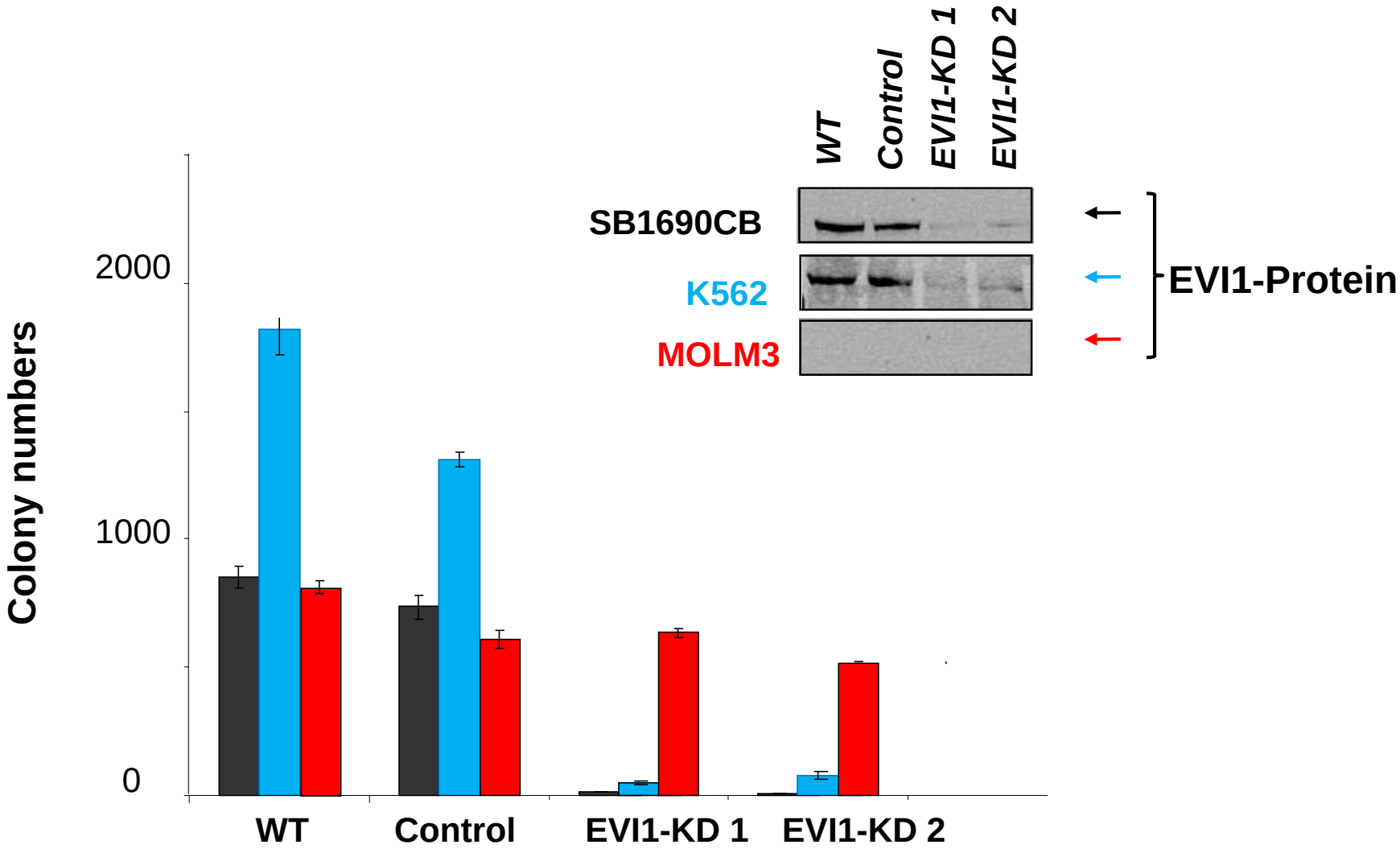
AML


EVI1+ HSC
Hypothesis:
EVI1 is a molecular target for EVI1pos AML.
No blood cell formation
AML

Hypothesis:
EVI1 is a molecular target for treatment of EVI1^{pos} AML.

Question:
Is EVI1 essential for AML proliferation and survival?

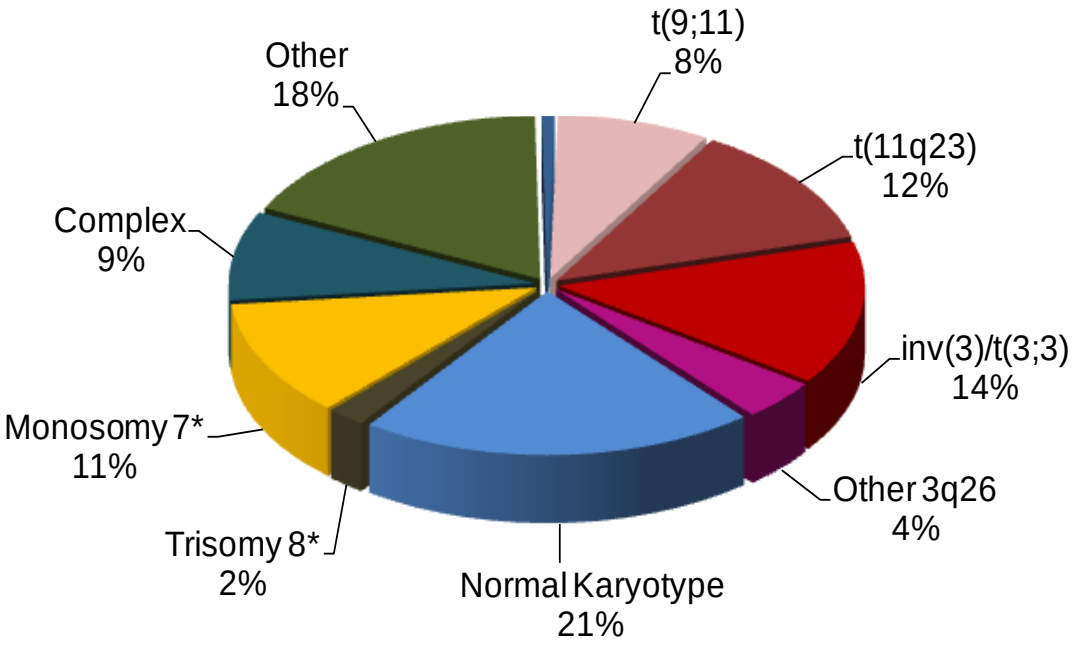
EVI1^{pos} AML: EVI1 is essential for in vitro proliferation: knock-down (KD) experiment



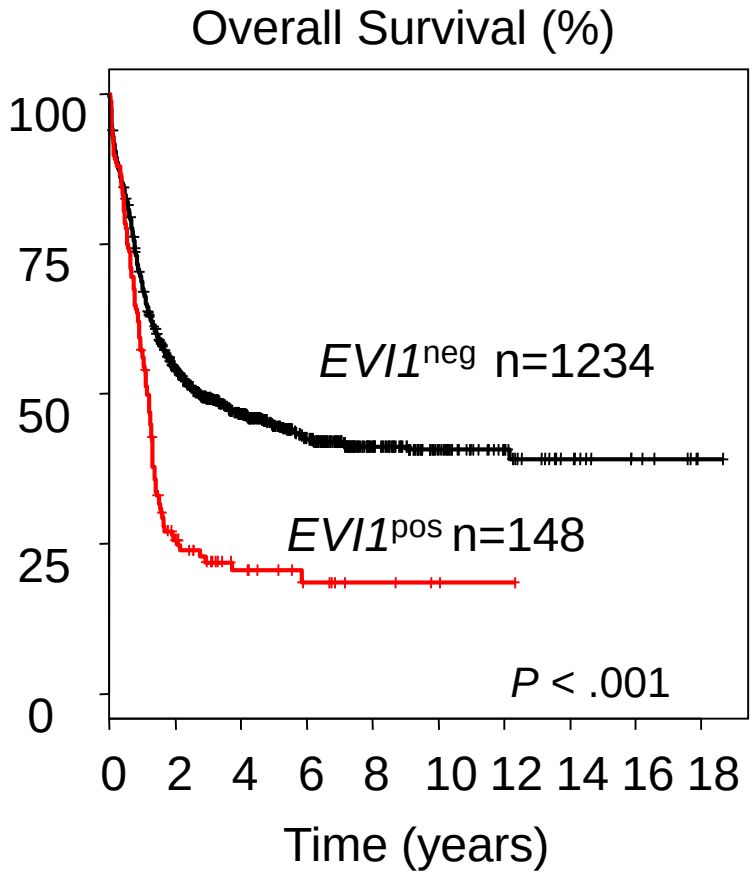
Questions:

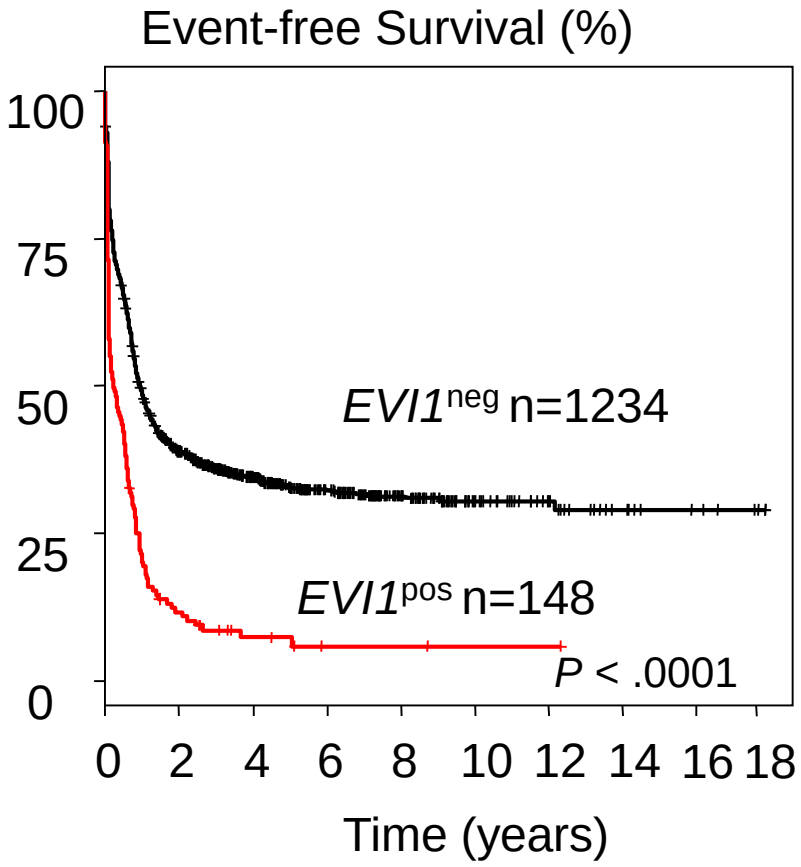
What is the incidence of EVI1 overexpression in AML?

EVI1 is overexpressed in 10% of human AML



EVI1 expression is an independent prognostic factor



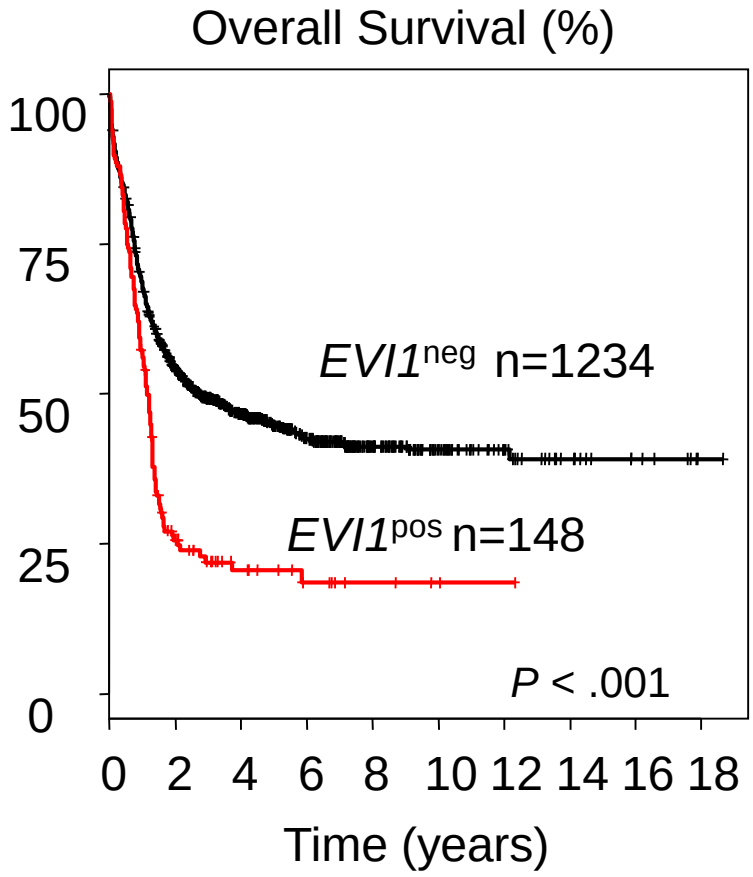


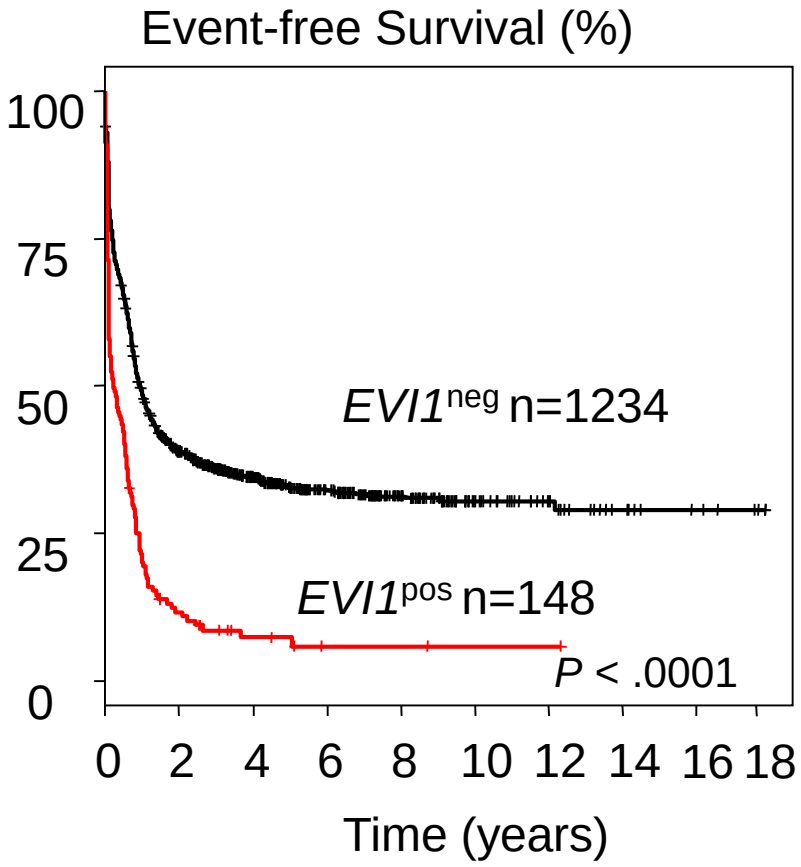
STUDY AML GROUP

Erasmus MC University Medical Center Rotterdam

HOVON

EVI1 expression is an independent prognostic factor



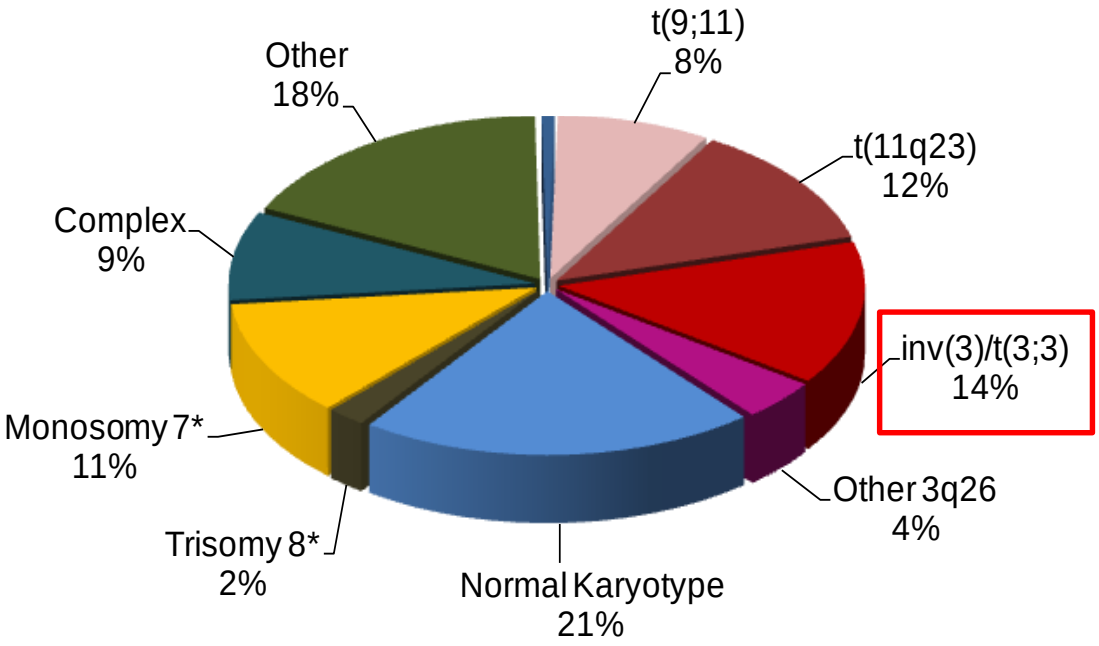


***EVI1*-pos: Chemotherapy + Allogeneic Stem Cell Transplantation**

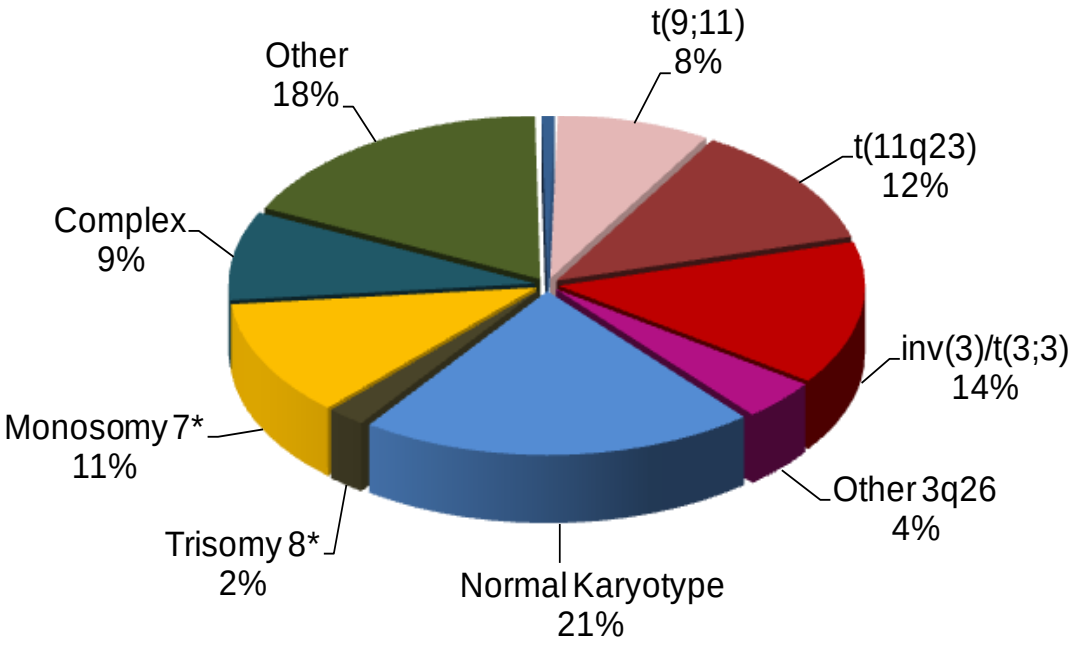
HOVON

Question:
Which AML subtypes express EVI1 and which not?

Distribution of EVI1 expression among AML subtypes.



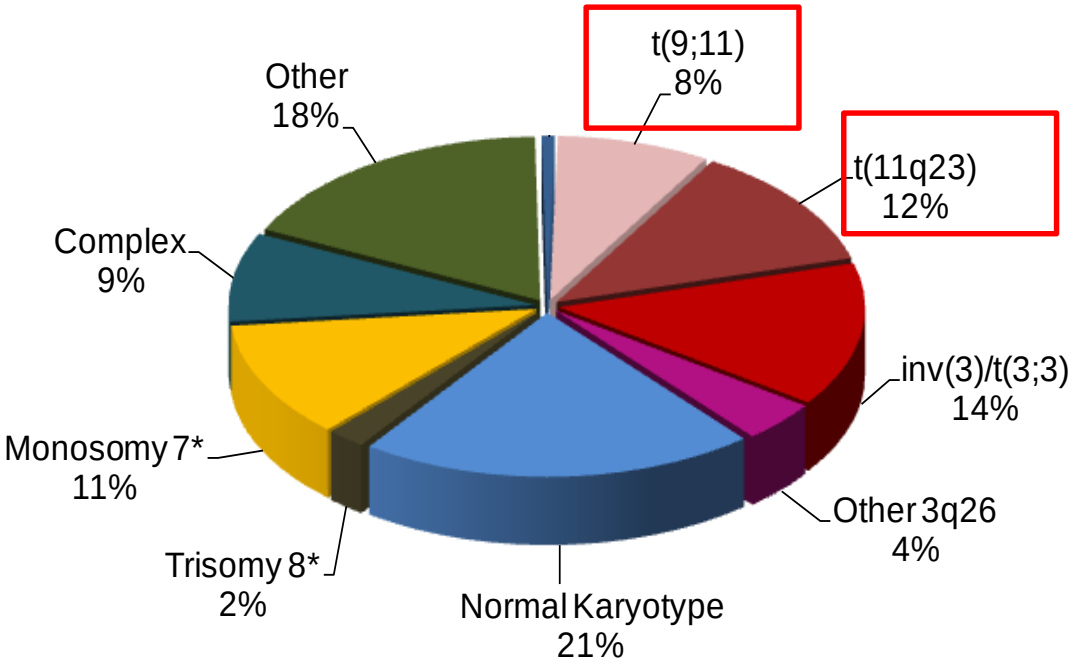
Distribution of EVI1 expression among AML subtypes.



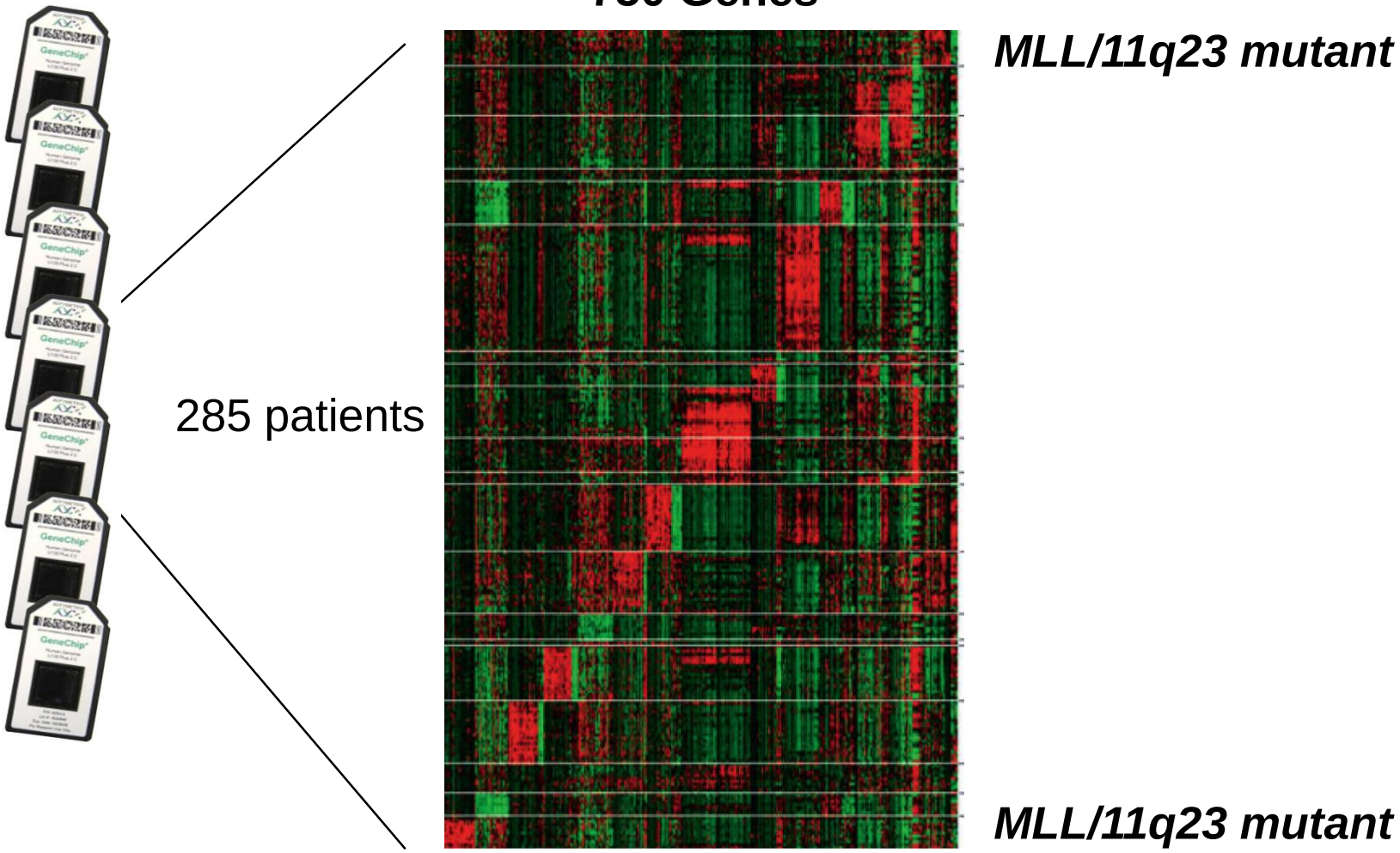
EVI1 is not expressed in AML with
t(8;21), inv(16), t(15;17), CEBPA mutated, NPM1 mutated.

Question:
What are mechanisms of EVI1 overexpression in AML?

EVI1 expression in AML with chromosome 11q23/MLL rearrangements.

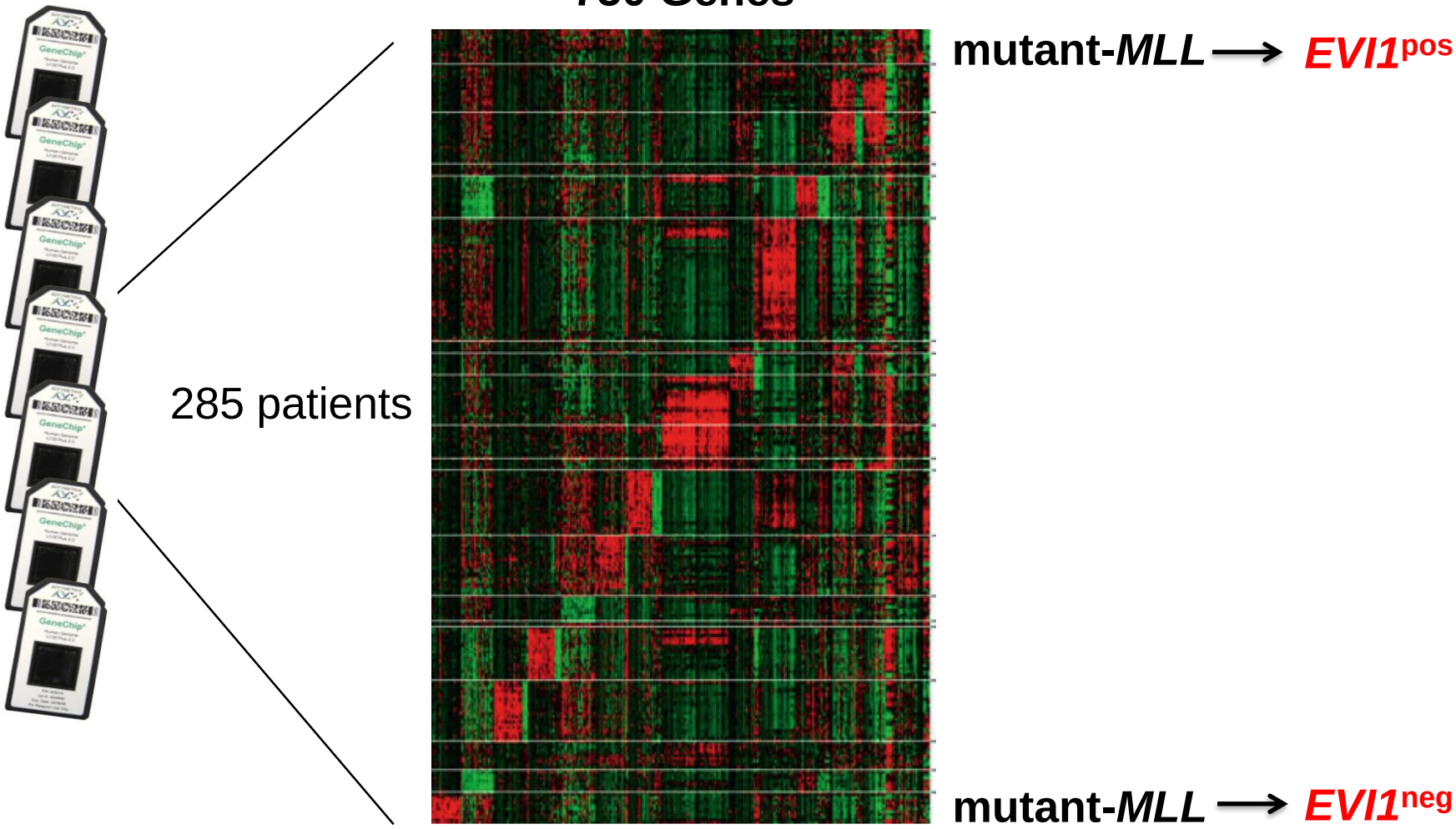


Gene expression signatures correlate with mutations



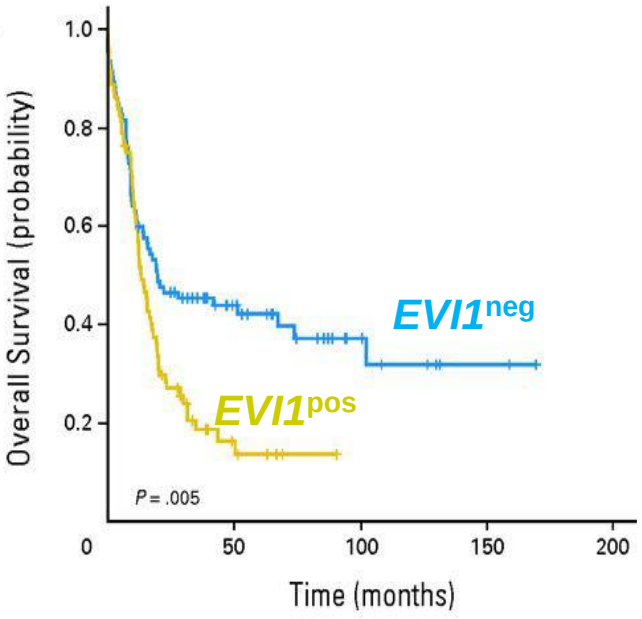
Valk et al, NEJM, 2004

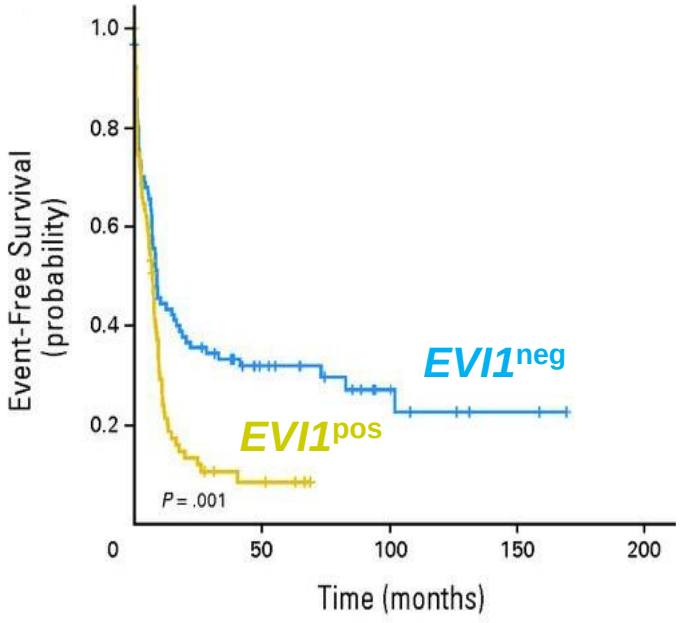
Gene expression signatures correlate with mutations



Valk et al, NEJM, 2004

EVI1^{pos} *MLL*-Rearranged AML: poor survival





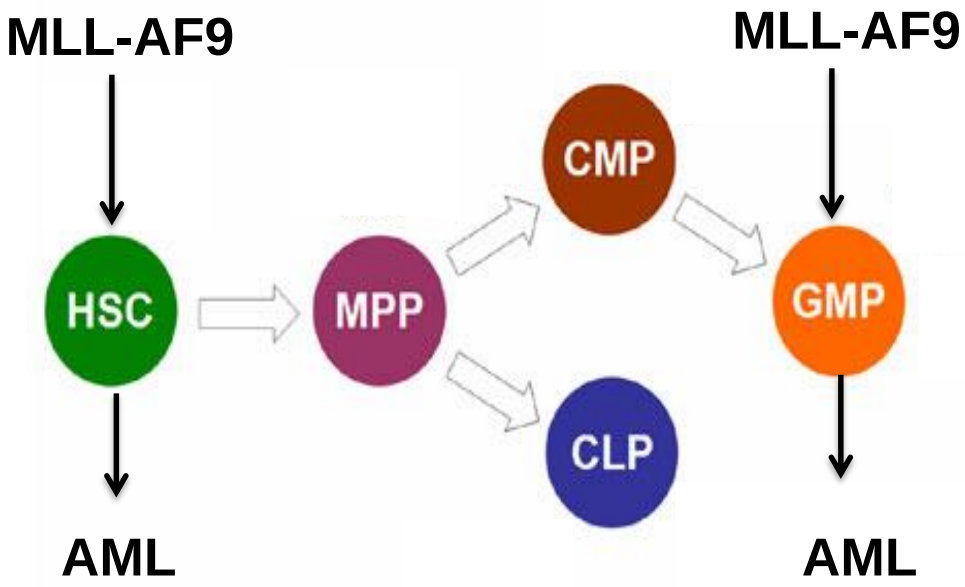
Gröschel et al. J Clin Oncol 2013

No *EVI1* rearrangements have been found in 11q23/*MLL*-rearranged AMLs

Question

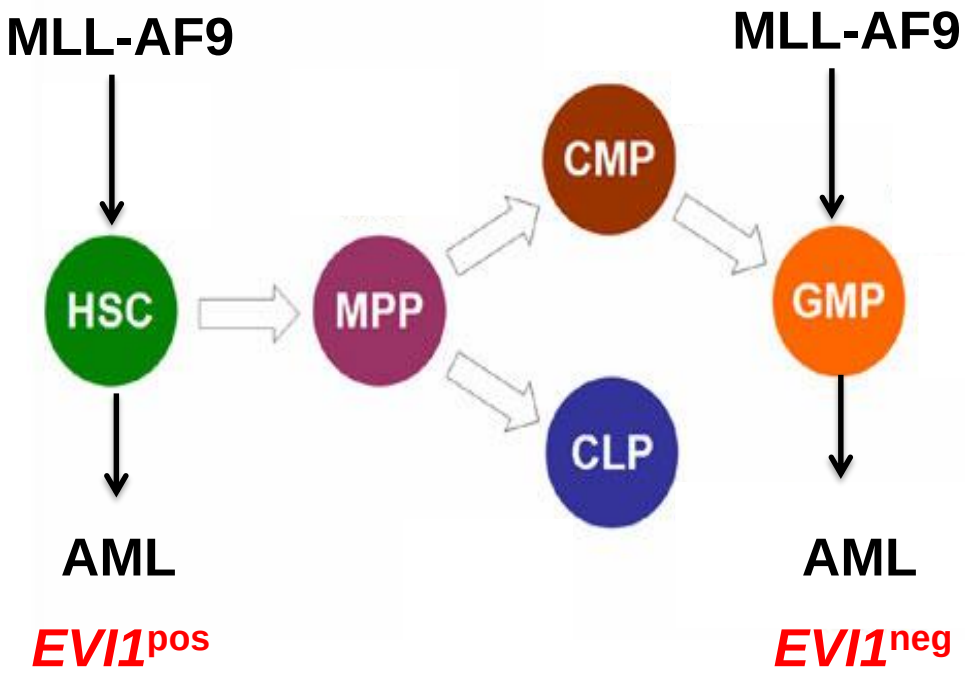
Why is EVI1 expressed in a subset of *MLL*-rearranged AML?

MLL-AF9 transforms *EVI1*^{pos} HSCs and *EVI1*^{neg} GMPs



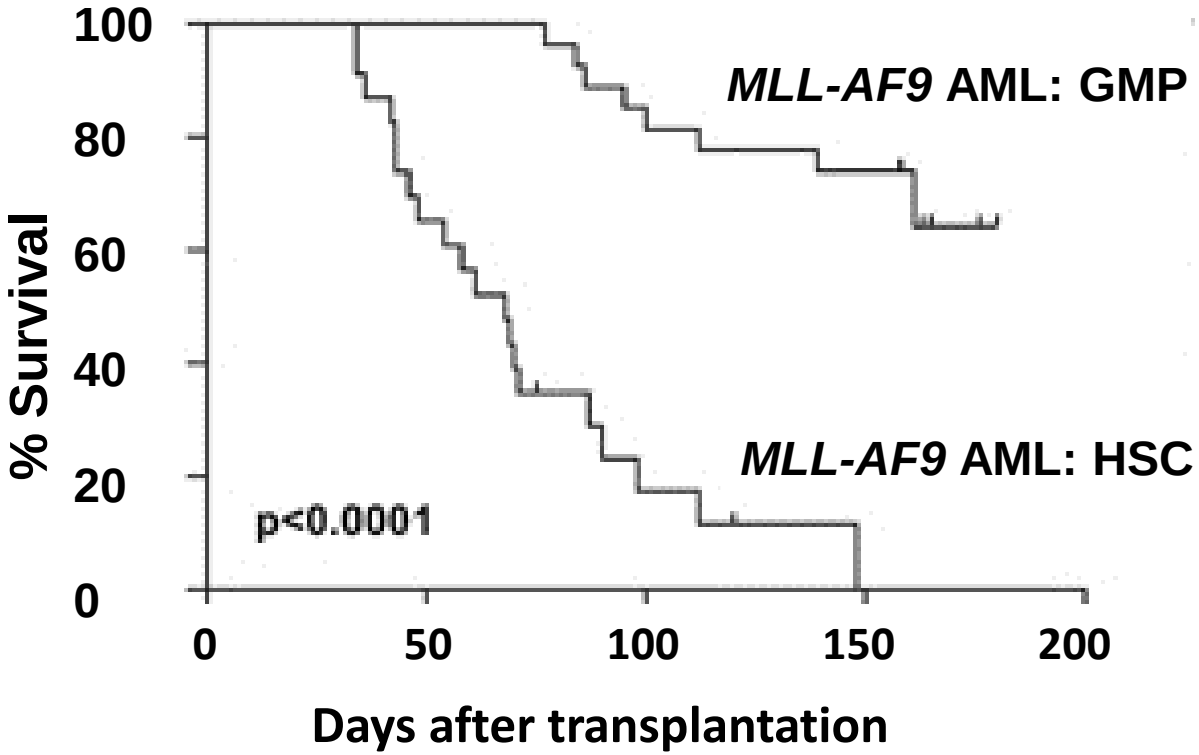
Adapted from Krivtsov et al. Leukemia 2013

MLL-AF9 transforms *EVI1*^{pos} HSCs and *EVI1*^{neg} GMPs



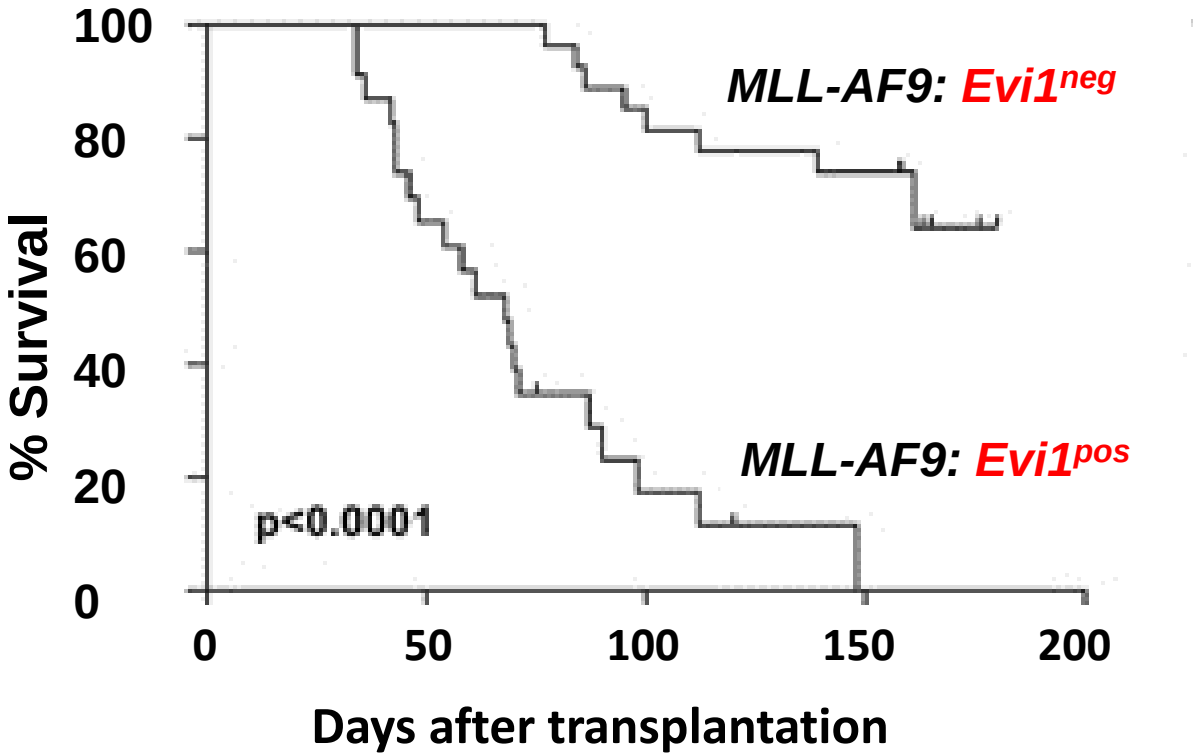
Adapted from Krivtsov et al. Leukemia 2013

Leukemia development of *MLL-AF9 transformed HSCs versus GMPs in mouse*



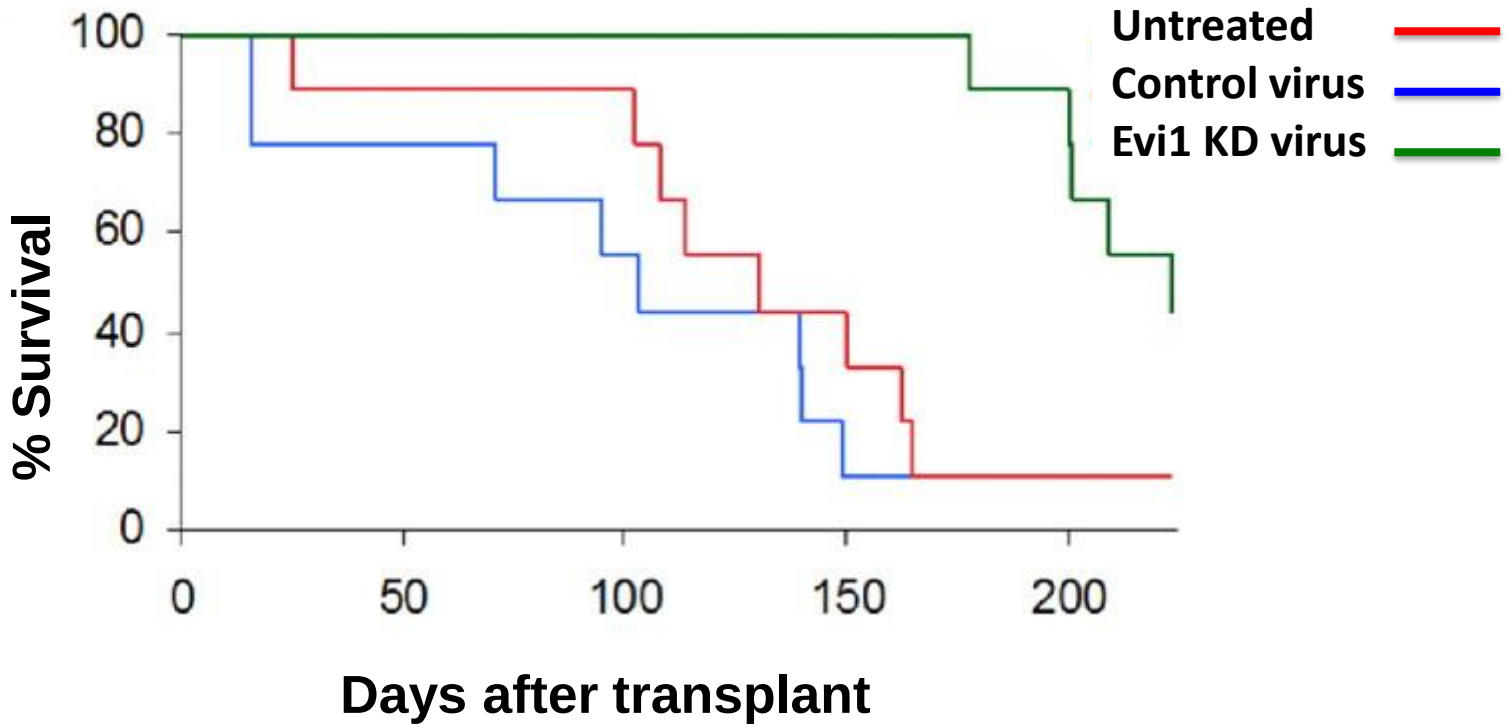
Krivtsov AV et al. Leukemia 2013

MLL-AF9 transformed HSCs express *EVI1.*



Krivtsov AV et al. Leukemia 2013

In vivo growth inhibition of MLL-AF9 mouse AML upon *EVI1* elimination.



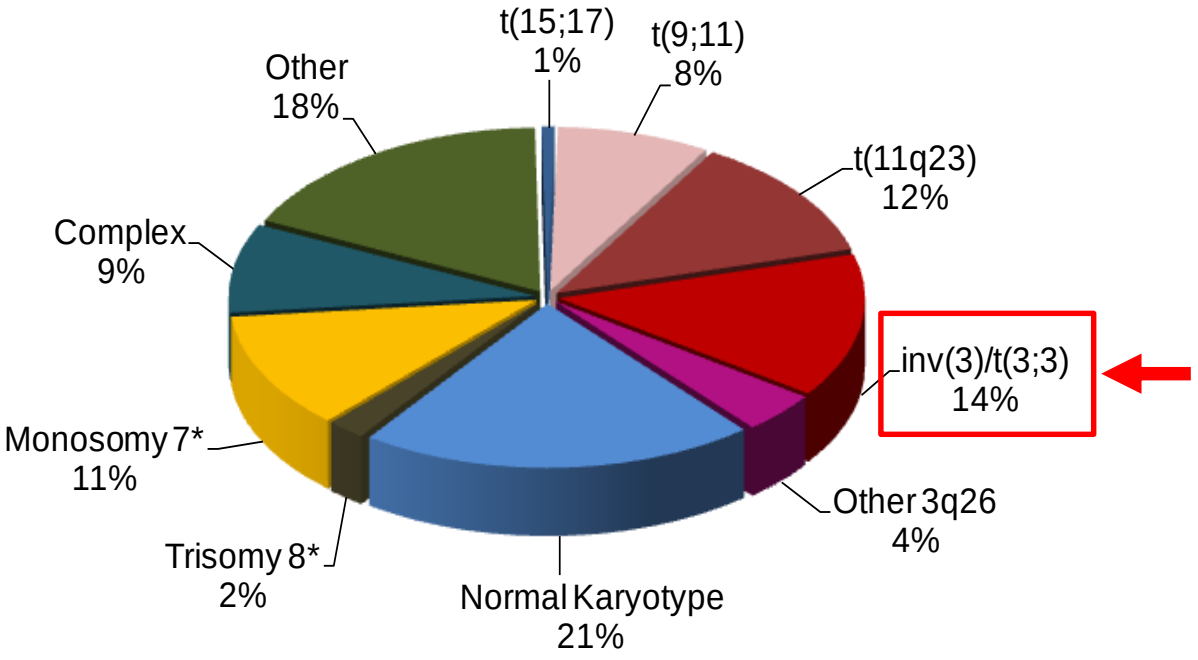
E. M. J. Bindels et al. Blood 2012

EVI1 overexpression in MLL-rearranged AML

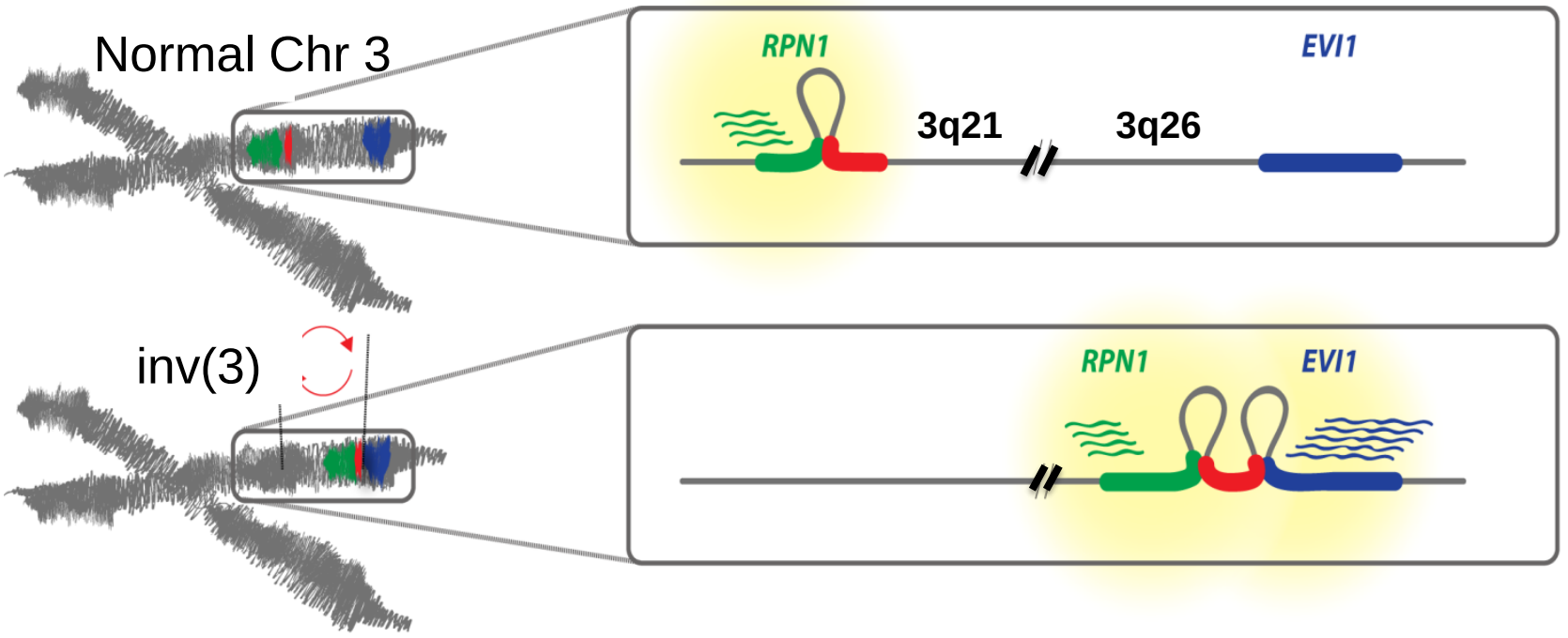
Conclusions

- **MLL-fusion genes transform *EVI1*^{pos} HSCs.**
- **MLL-fusion maintains *EVI1* expression of transformed HSCs**
- **These leukemia cells depend on *EVI1***
- ***EVI1* is a potential target for treatment of MLL-rearranged AML**

Question:
What are the mechanism of aberrant *EVI1* expression in AML?

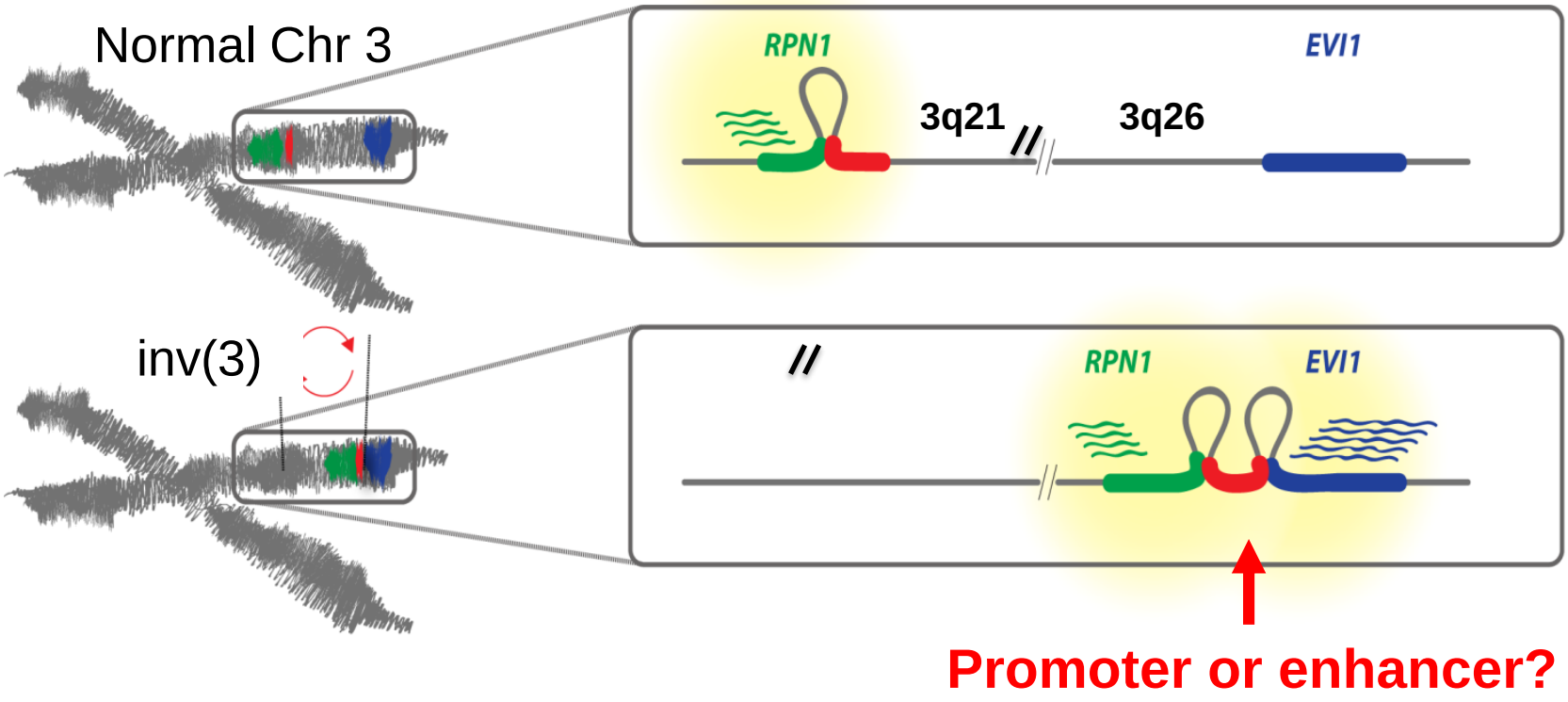


Proposed mechanism of EVI1 activation in AML with 3q26/3q21 aberrations



- Inv(3) causes aberrant EVI1 expression
- Inv(3) does not affect RPN1 expression
- No RPN1-EVI1 fusion-gene generated

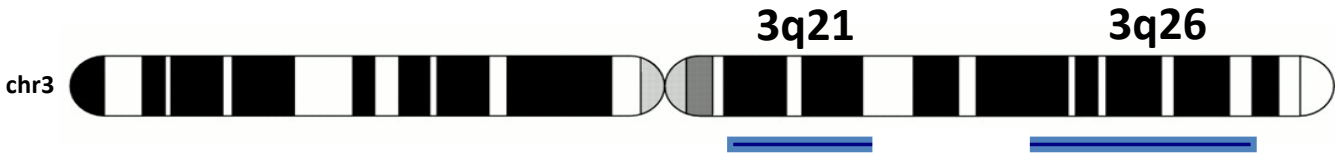
Proposed mechanism of *EVI1* activation in AML with 3q26/3q21 aberrations



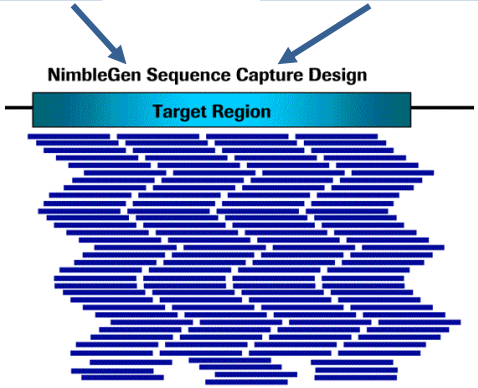
Question:
Where are the breakpoints near *EVI1* and *RPN1* in AML with with 3q26/3q21 abnormalities?

Breakpoint identification in AML with 3q26/3q21 abnormalities

DNA from 41 AML samples with 3q26/3q21 aberrations

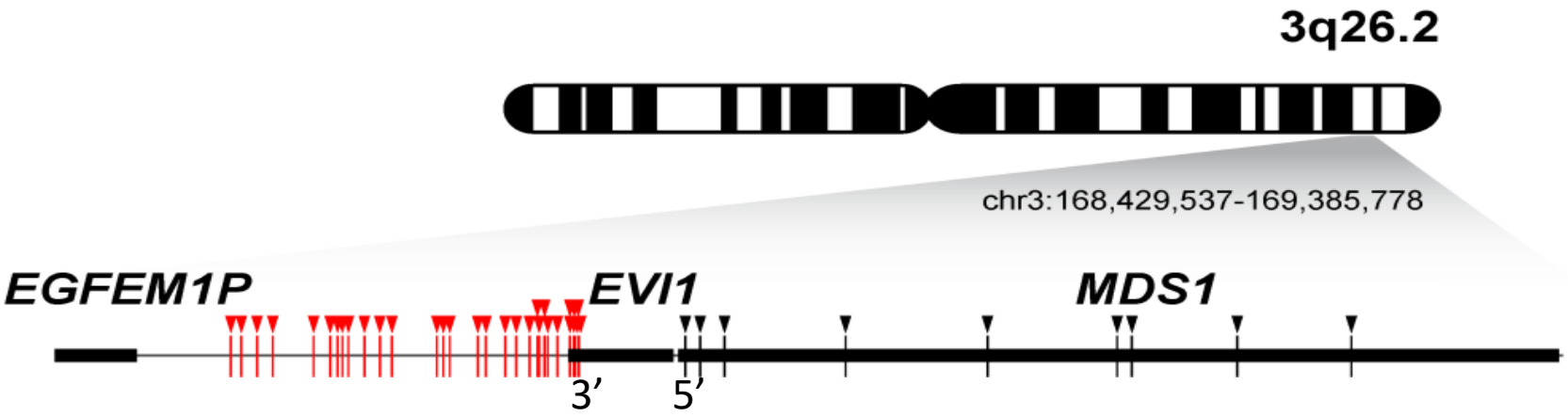


Capture Target:



Next Generation Sequencing

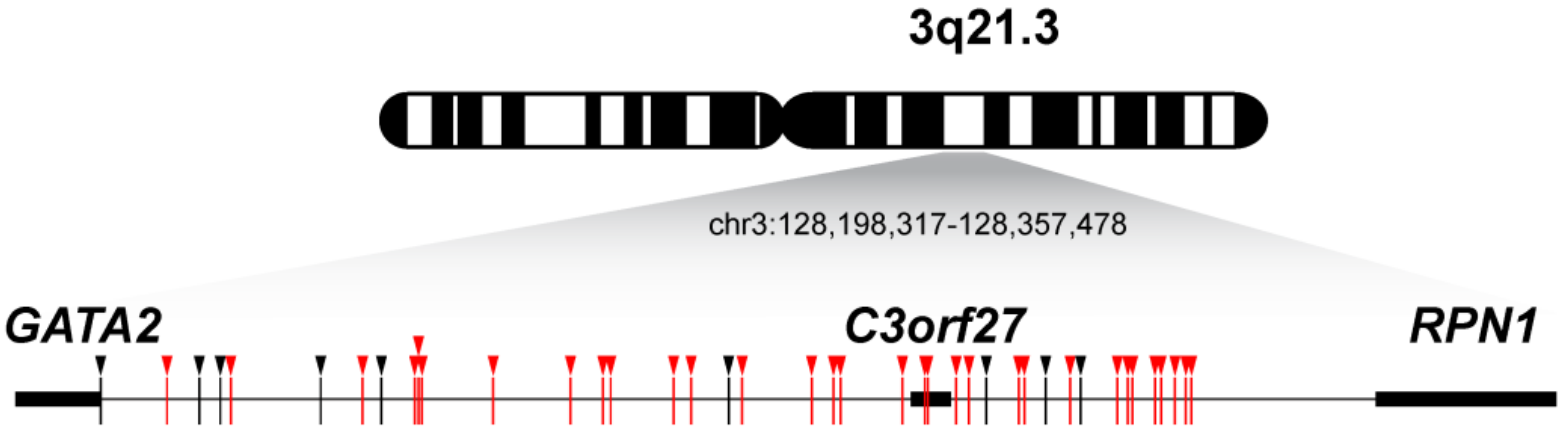
Breakpoints near *EVI1* at 3q26



Inv(3) breakpoints: 3’ of *EVI1*

t(3;3) breakpoints: 5’ of *EVI1*

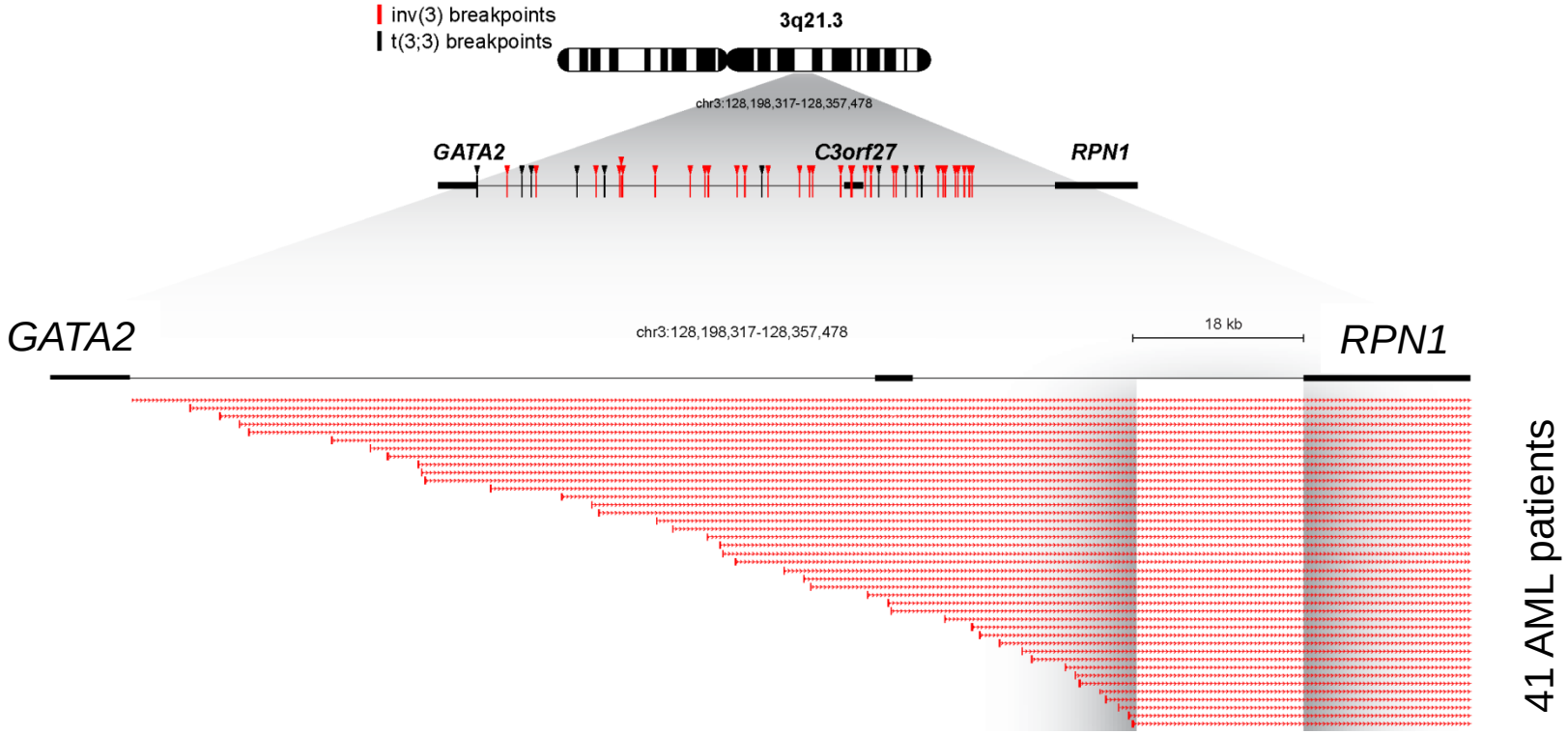
Breakpoints at 3q21 near *RPN1*



Inv(3) breakpoints

t(3;3) breakpoints

Breakpoint architecture discloses minimal translocated region of 18 kb



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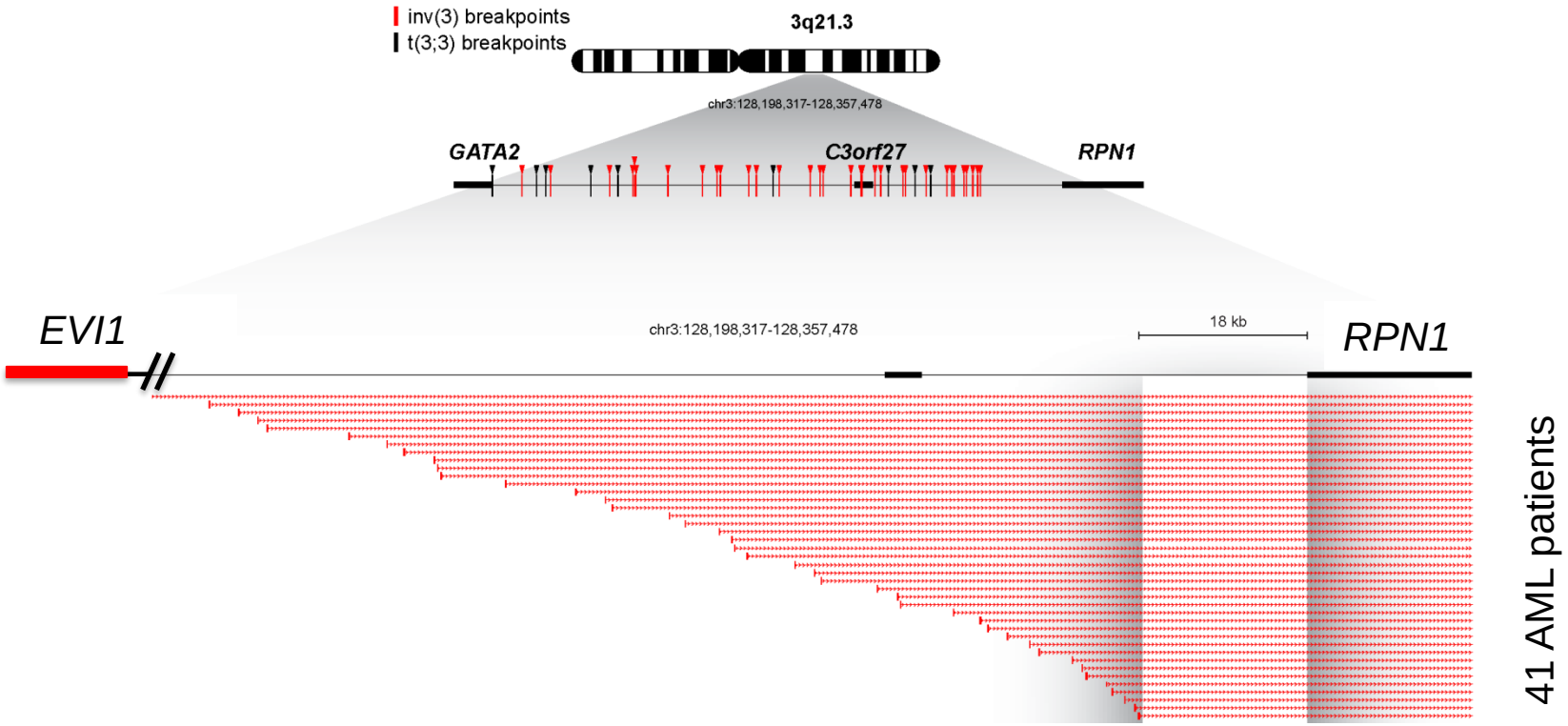
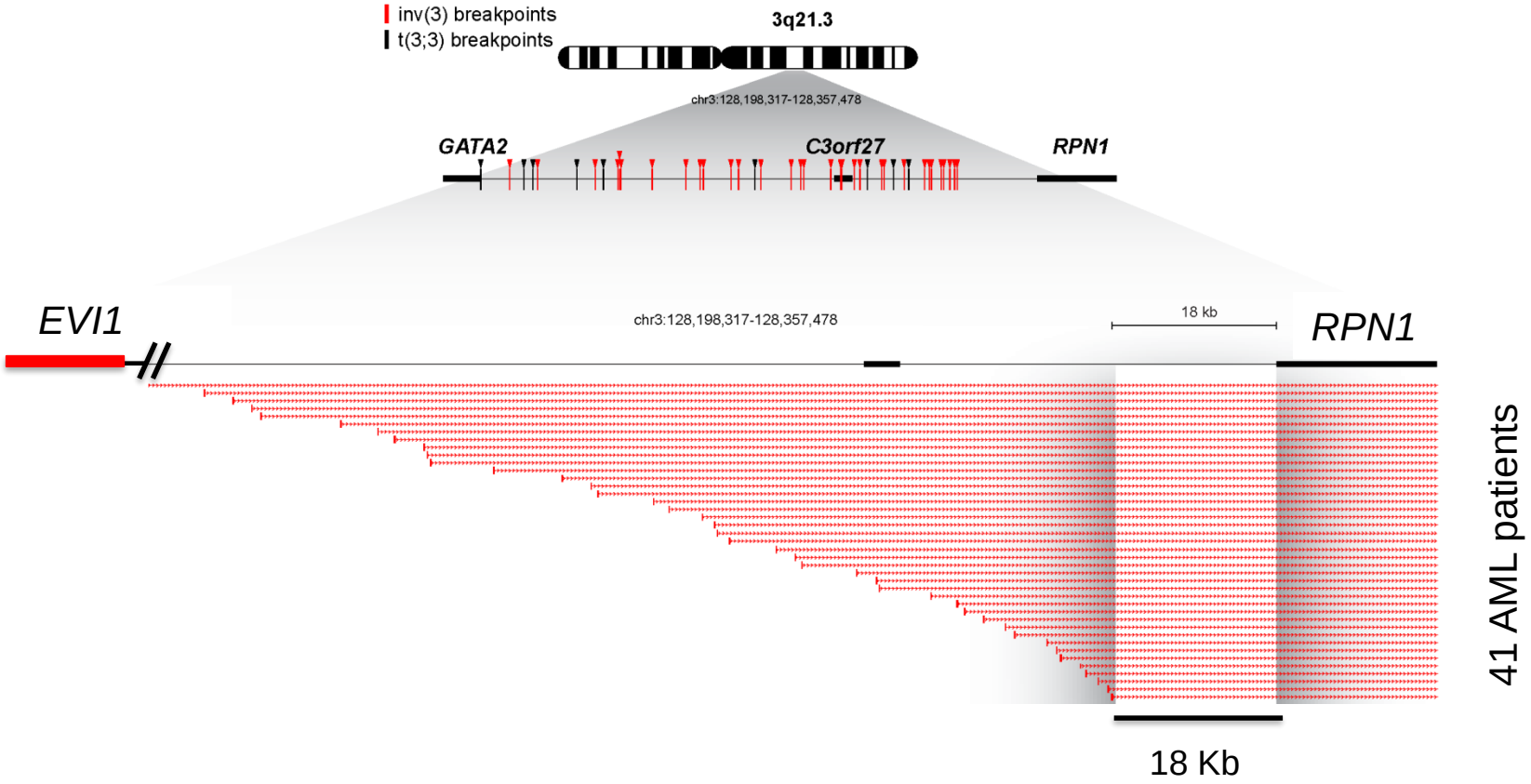
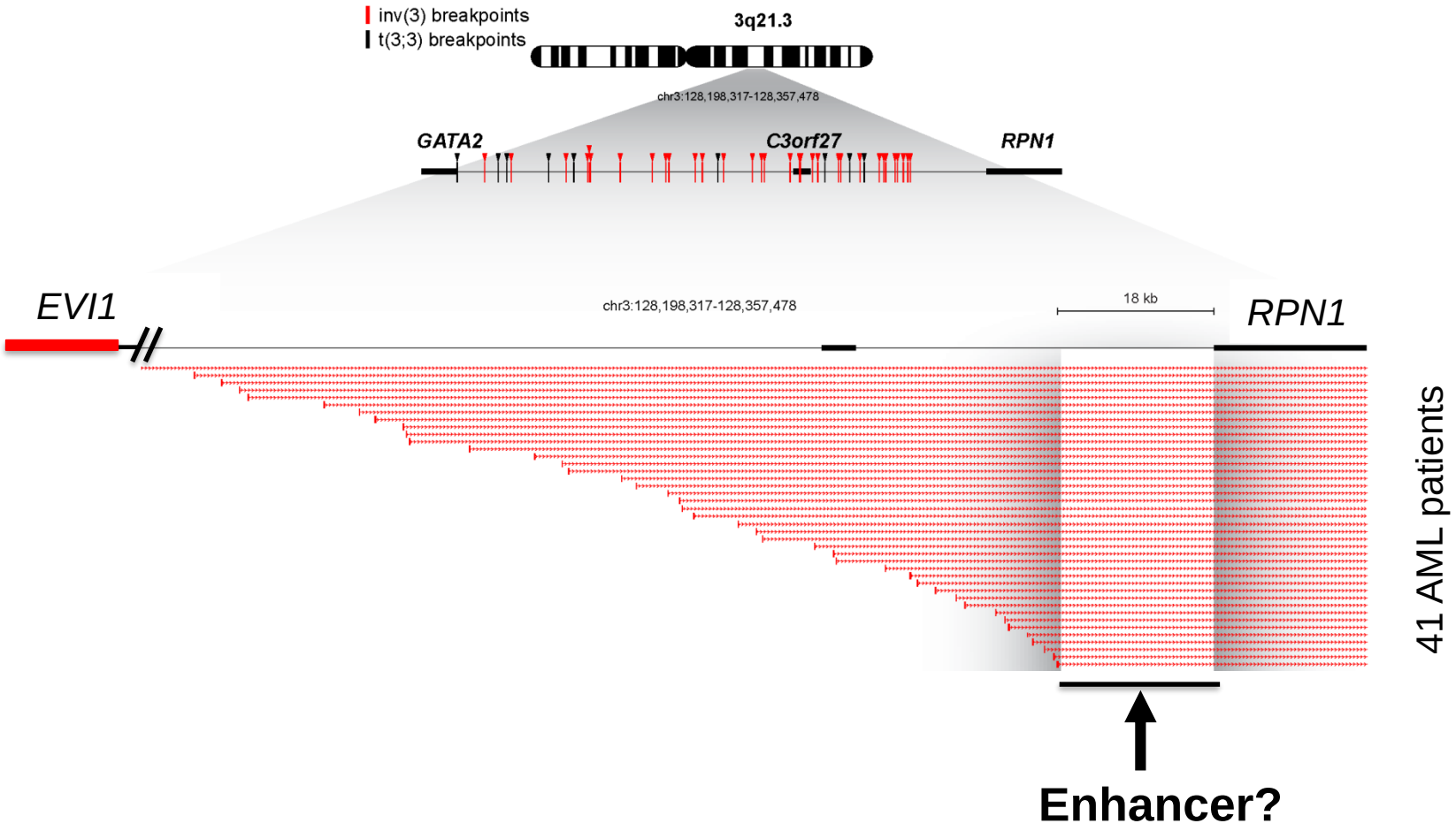


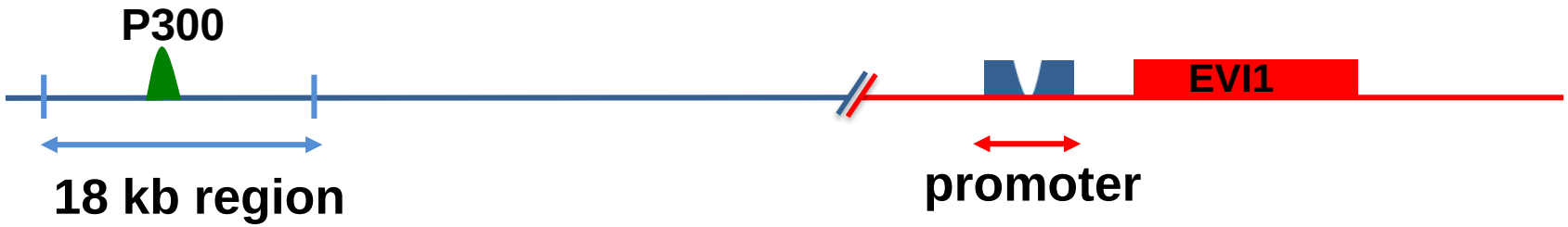
Breakpoint architecture discloses minimal translocated region of 18 kb



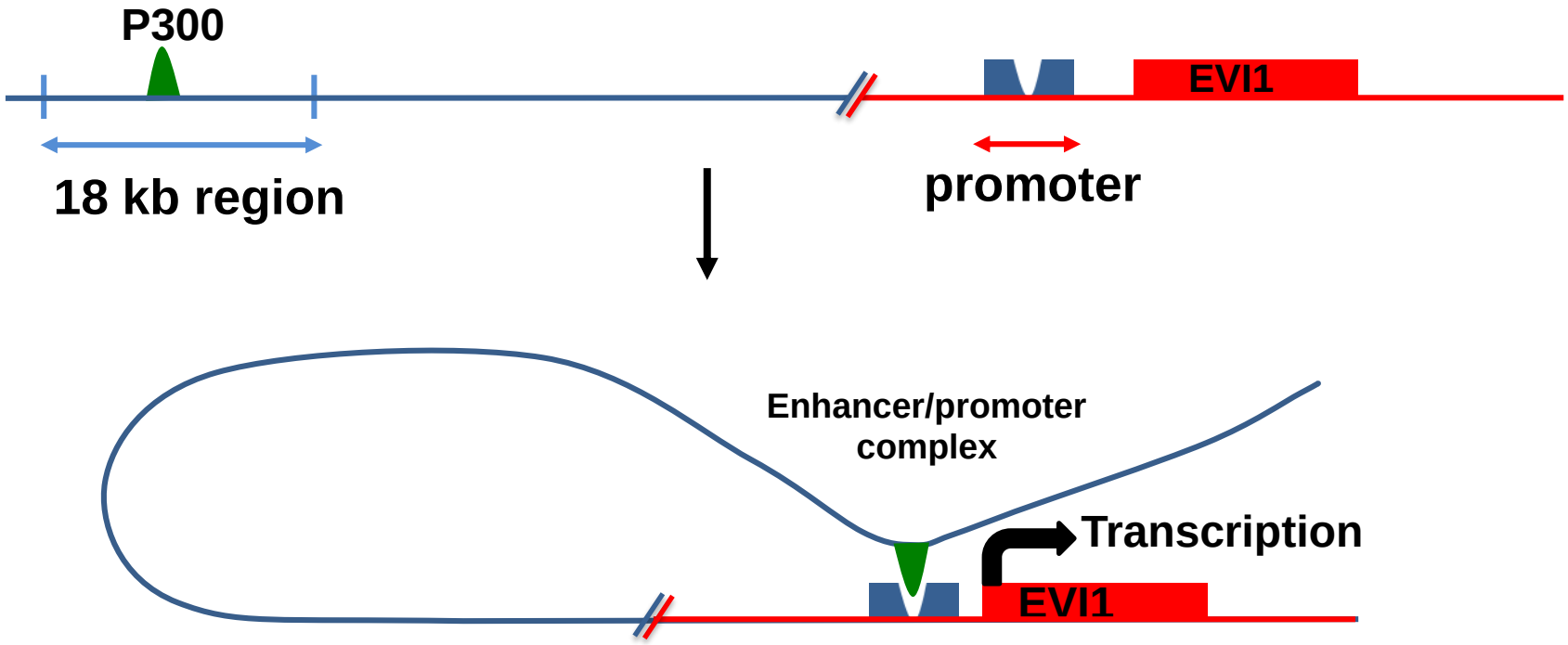
Breakpoint architecture discloses minimal translocated region of 18 kb



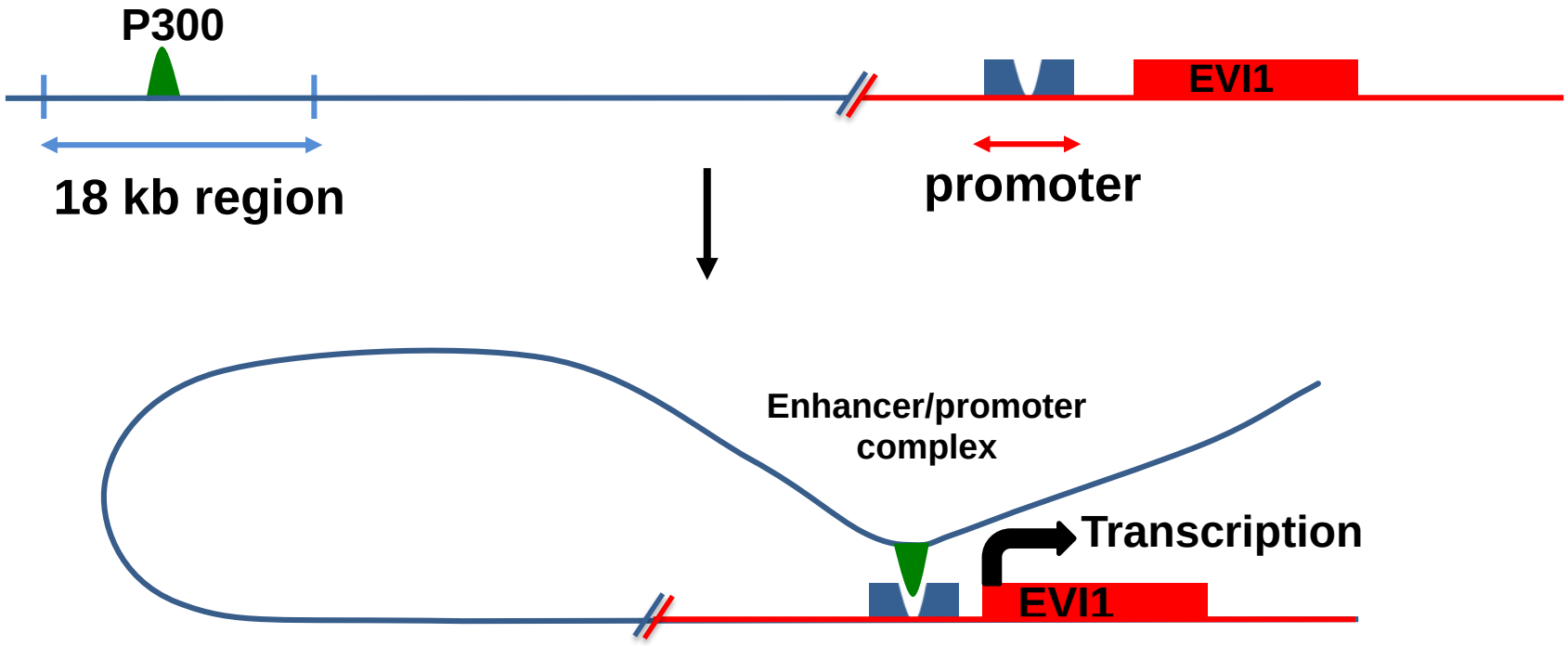
Active enhancers and promoters form a complex



Active enhancers and promoters form a complex

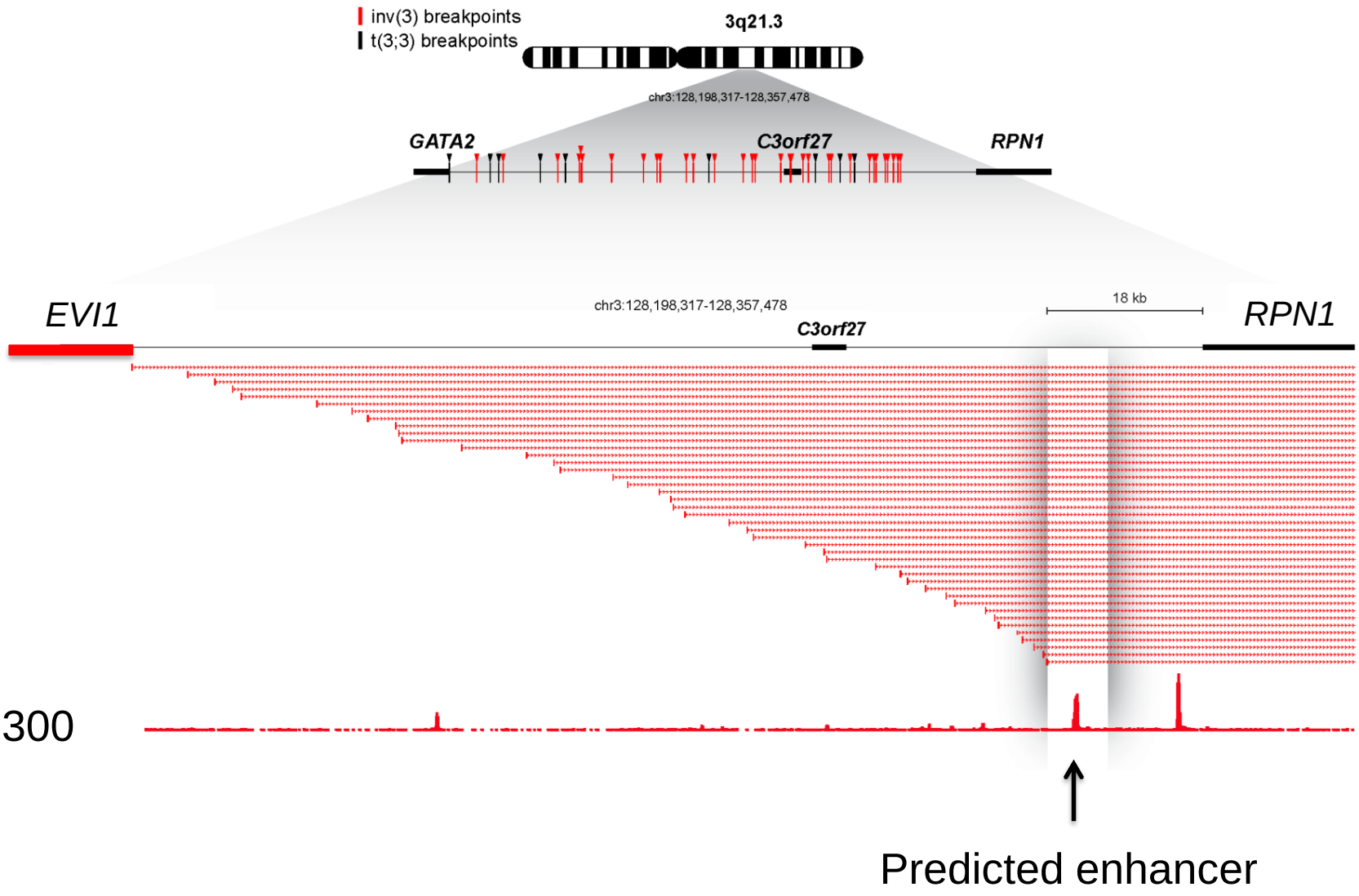


Active enhancers and promoters form a complex



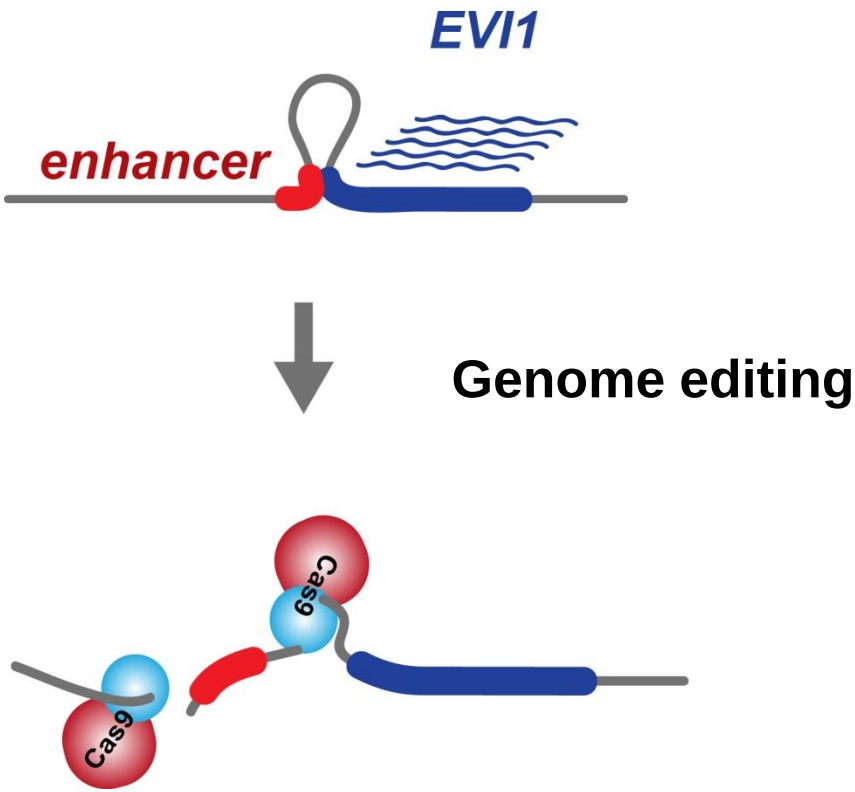
P300 Chromatin immuno precipitation

P300 binding region of identified



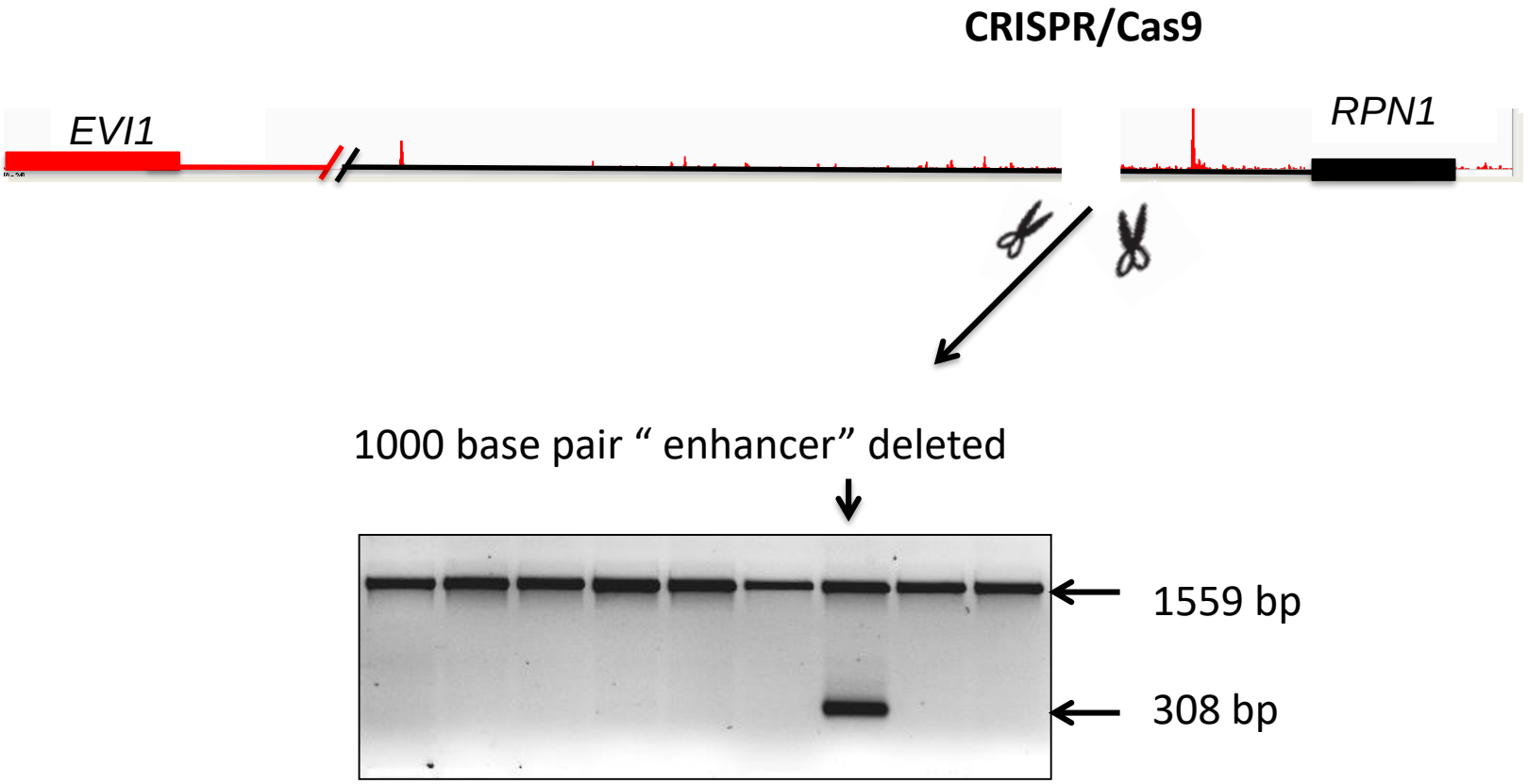
Does the predicted 1000 bp enhancer activate EVI1 expression in inv(3) AML cells ?

Genome-editing in inv(3) AML - Hypothesis

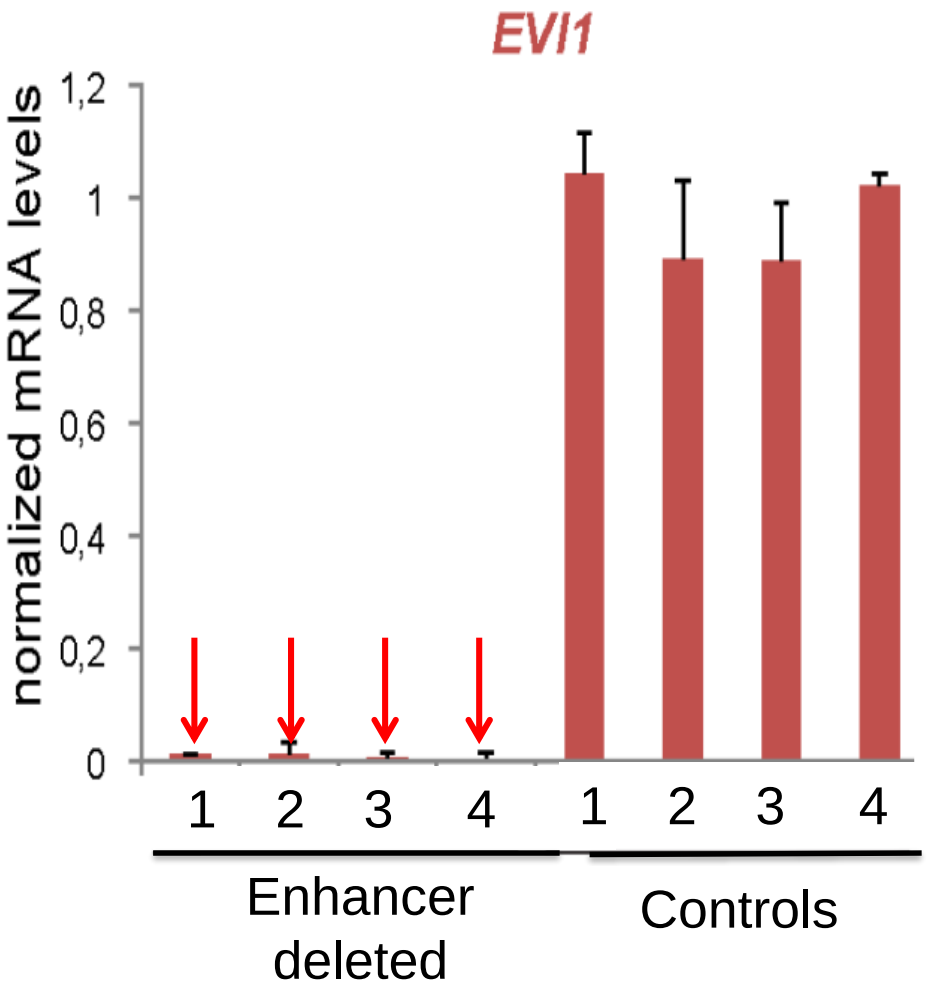


Enhancer deleted clones generated using genome editing

Enhancer deleted clones generated using genome editing



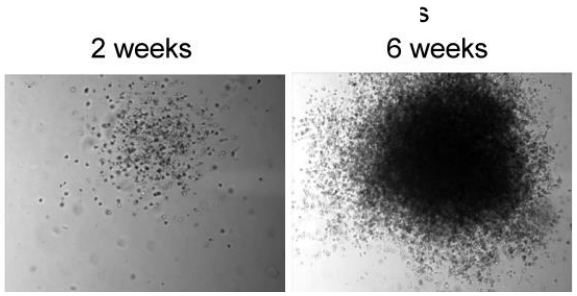
Enhancer deleted MUTZ-3 clones lose *EVI1* expression

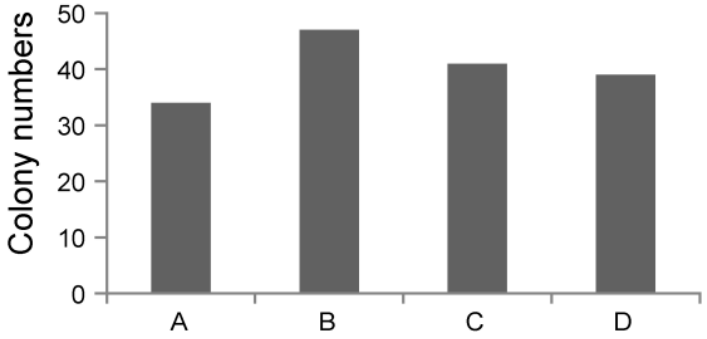


Question:
What is the biological relevance of enhancer deletion?

Colony growth of enhancer deleted clones is impaired

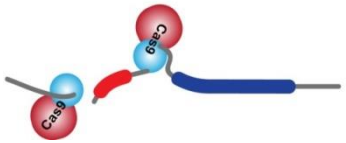
Control 3q26/3q21

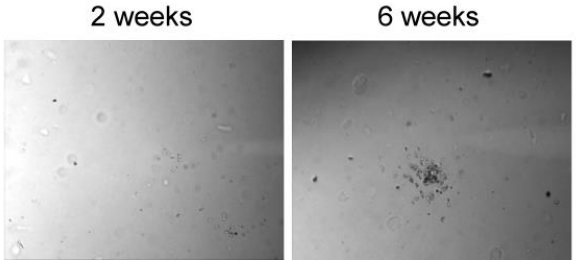


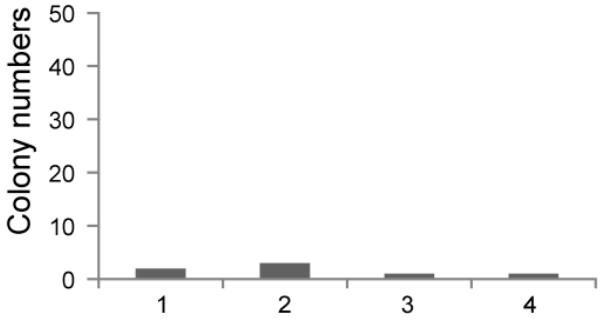


Enhancer deleted clones

Enhancer deleted 3q26/3q21

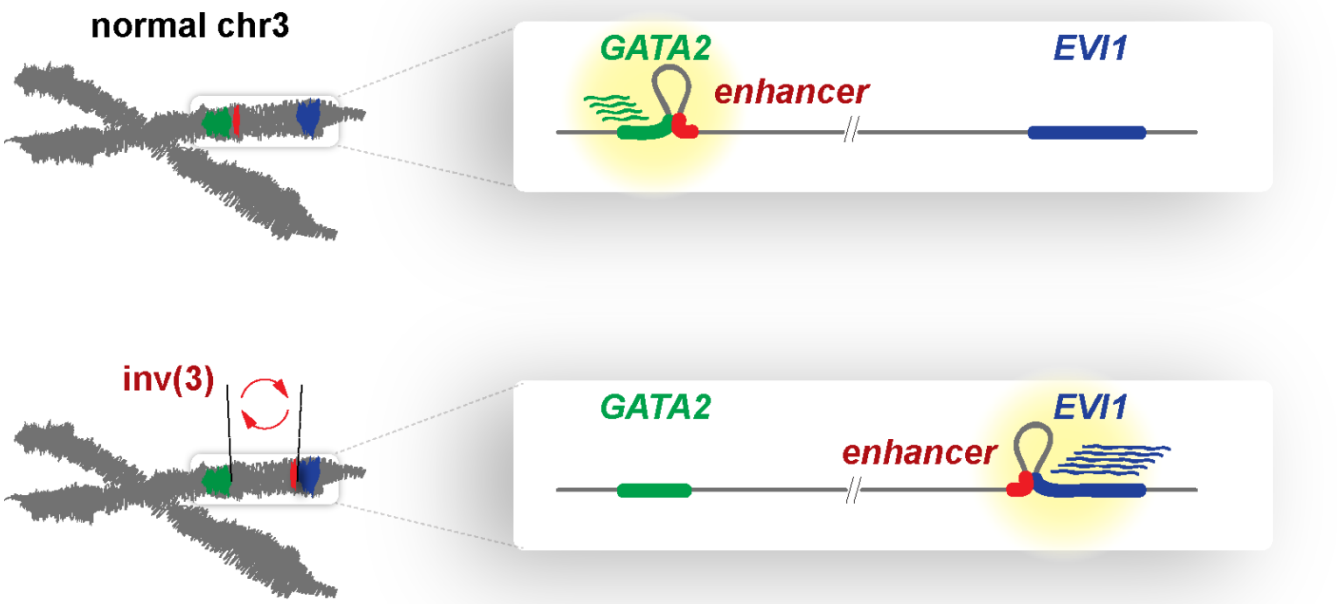




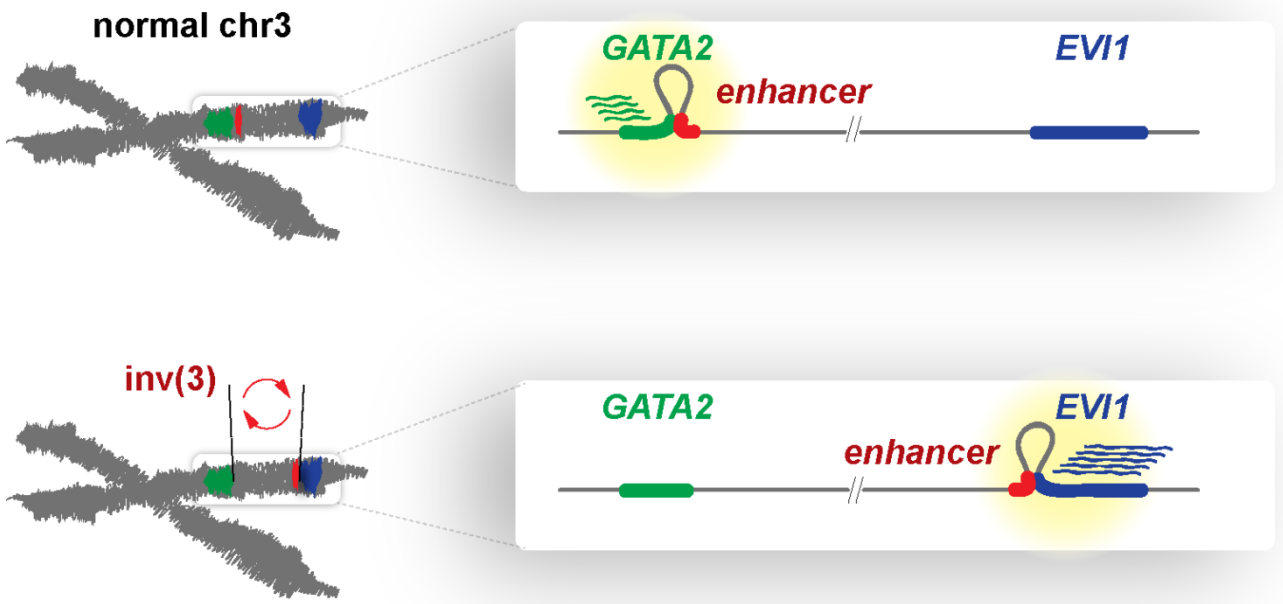


Control clones

Enhancer dislocation causes altered expression of two genes in AML with inv(3)

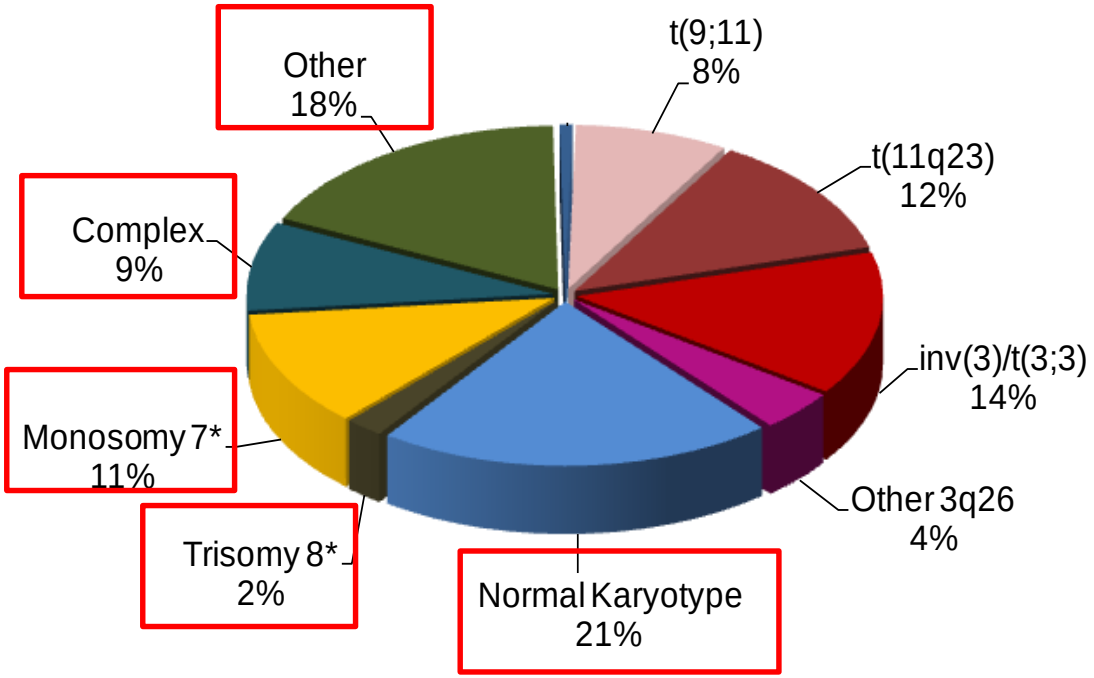


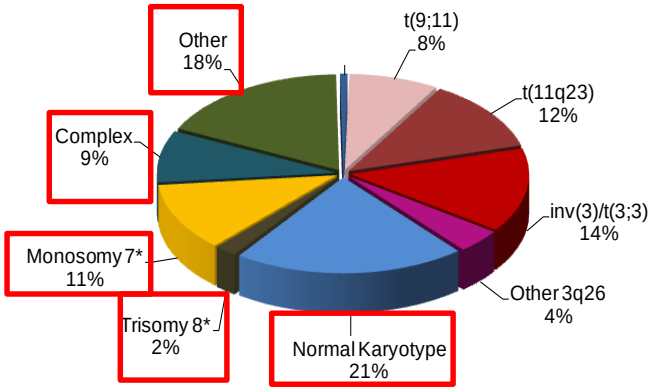
WHO: *RPN1-EVI1* AML ⟶ *GATA2-EVI1* AML



HOW DOES THE ENHANCER DRIVE *EVI1* OVEREXPRESSION?

Question:
What are the mechanism of aberrant *EVI1* expression in AML?





EVI1 expression in remaining subsets

- **Cryptic translocations (10 – 15%)***
- **EVI1 amplifications (5-10%)***
- **Unknown (75-80%)**

* Enhancer rearrangement predicted

Perspective

Molecular-Biological Research ⟶ Specific treatment

- AML is a disease of defective gene regulation
- Understanding of the molecular mechanisms is needed for every AML subtype
- EVI1 is a potential molecular target for treatment

Perspective

Molecular-Biological Research ⟶ **Specific treatment**

CML	BCR-ABL	Kinase inhibitors
APL	PML-RAR	ATRA/Arsenic
Prostate Cancer	Testosterone levels	LHRH agonists
Breast Cancer	Estrogen receptors	ER-antagonist
Breast Cancer	HER2 protein	Herceptin

Perspective

Molecular-Biological Research ⟶ Specific treatment

CML	BCR-ABL	Kinase inhibitors
APL	PML-RAR	ATRA/Arsenic
Prostate Cancer	Testosterone levels	LHRH agonists
Breast Cancer	Estrogen receptors	ER-antagonist
Breast Cancer	HER2 protein	Herceptin
Aim and hope:		
AML	**EVI1**	**????**

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