Ce–M–M– Research Center for Molecular Medicine of the Austrian Academy of Sciences

Genetics of MPN: from initiation to progression

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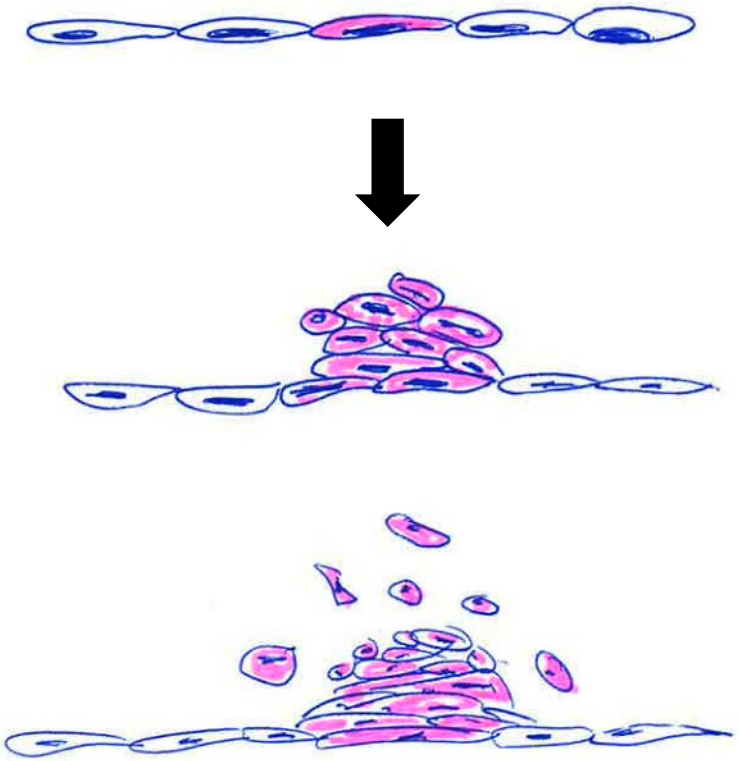
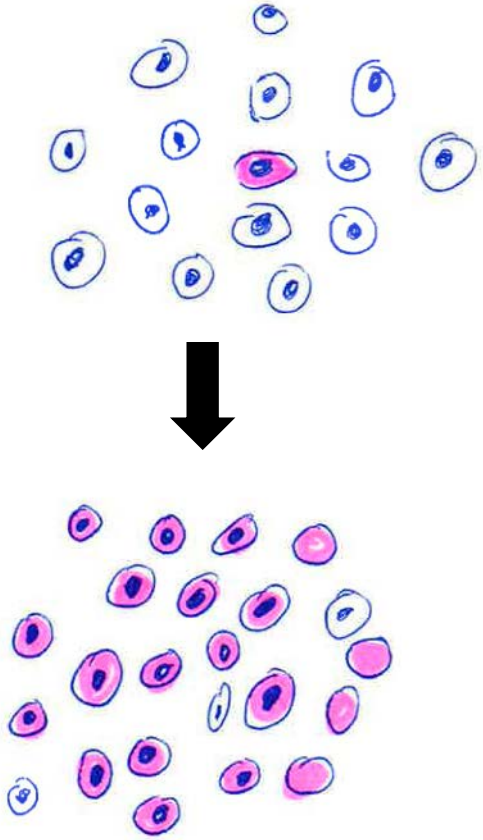
Solid and “liquid” tumors

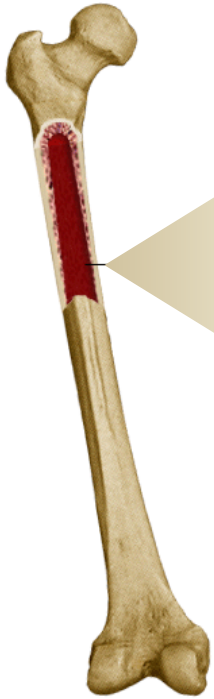
Solid tumor



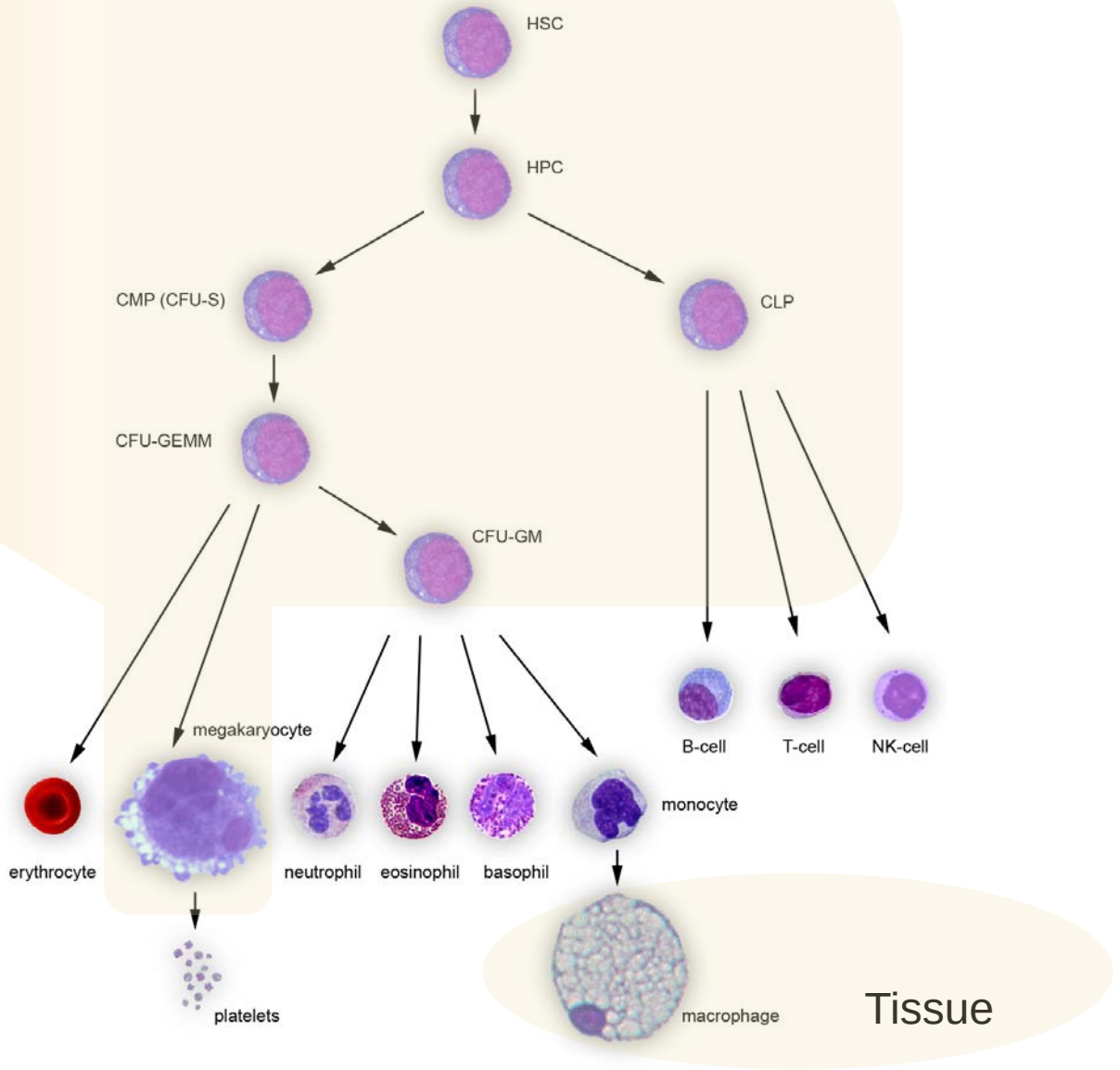
Leukemia



Blood production - hematopoiesis

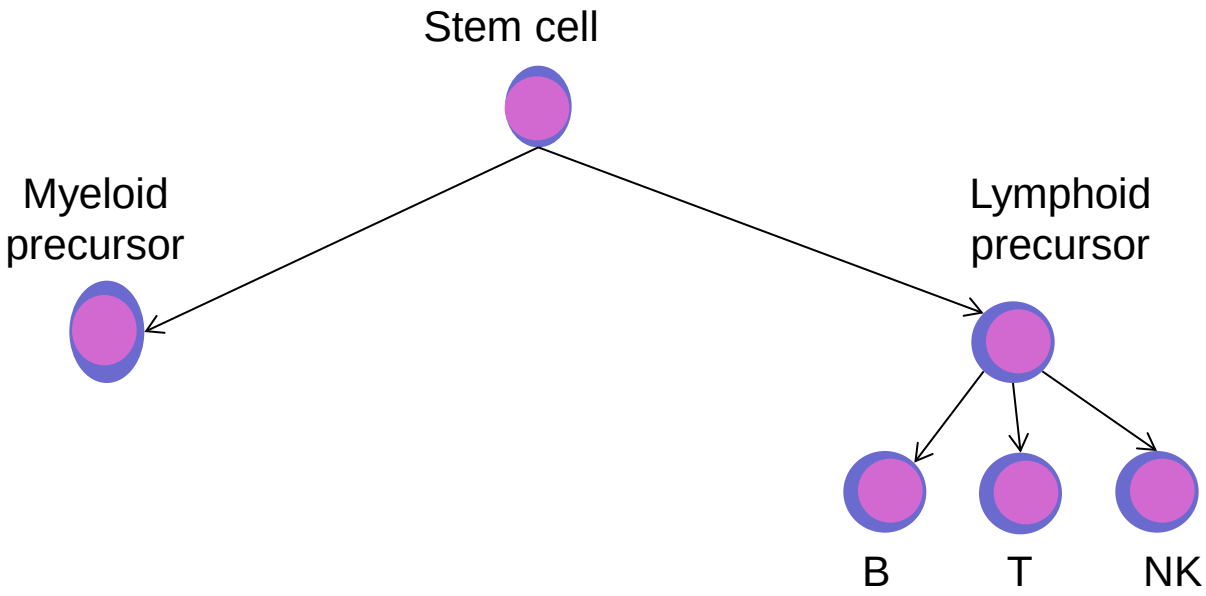


Bone marrow



Peripheral blood

Clinical presentations of hematological cancers



Disease type	Number of subtypes
Acute myeloid leukemia (AML)	16
Myelodysplastic syndromes (MDS)	7
Myeloproliferative neoplasms (MPN)	8
Mixed MPN/MDS neoplasms	5

Disease type
B lymphoblastic leukemia
T lymphoblastic leukemia
Mature B-cell neoplasms
Mature T-cell and NK-cell neoplasms
Hodgkin lymphoma
Histiocytic and dendritic cell neoplasms
Post-transplant lymphoproliferative disorders

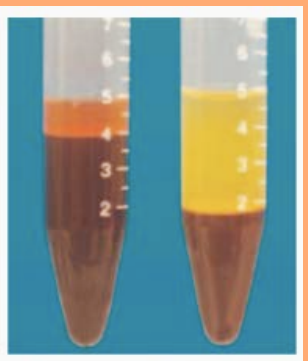
Myeloid malignancies

Myeloproliferative neoplasms

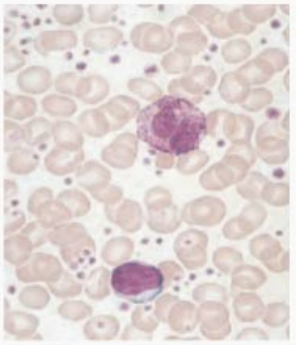
Chronic myelogenous leukemia – BCR-ABL
Polycythemia vera
Essential thrombocythemia
Primary myelofibrosis
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia
Hypereosinophilic syndrome
Mast cell disease
MPNs, unclassifiable

BCR-ABL negative

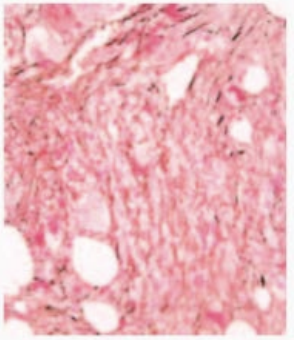
Polycythemia vera



Essential thrombocythemia



Primary myelofibrosis

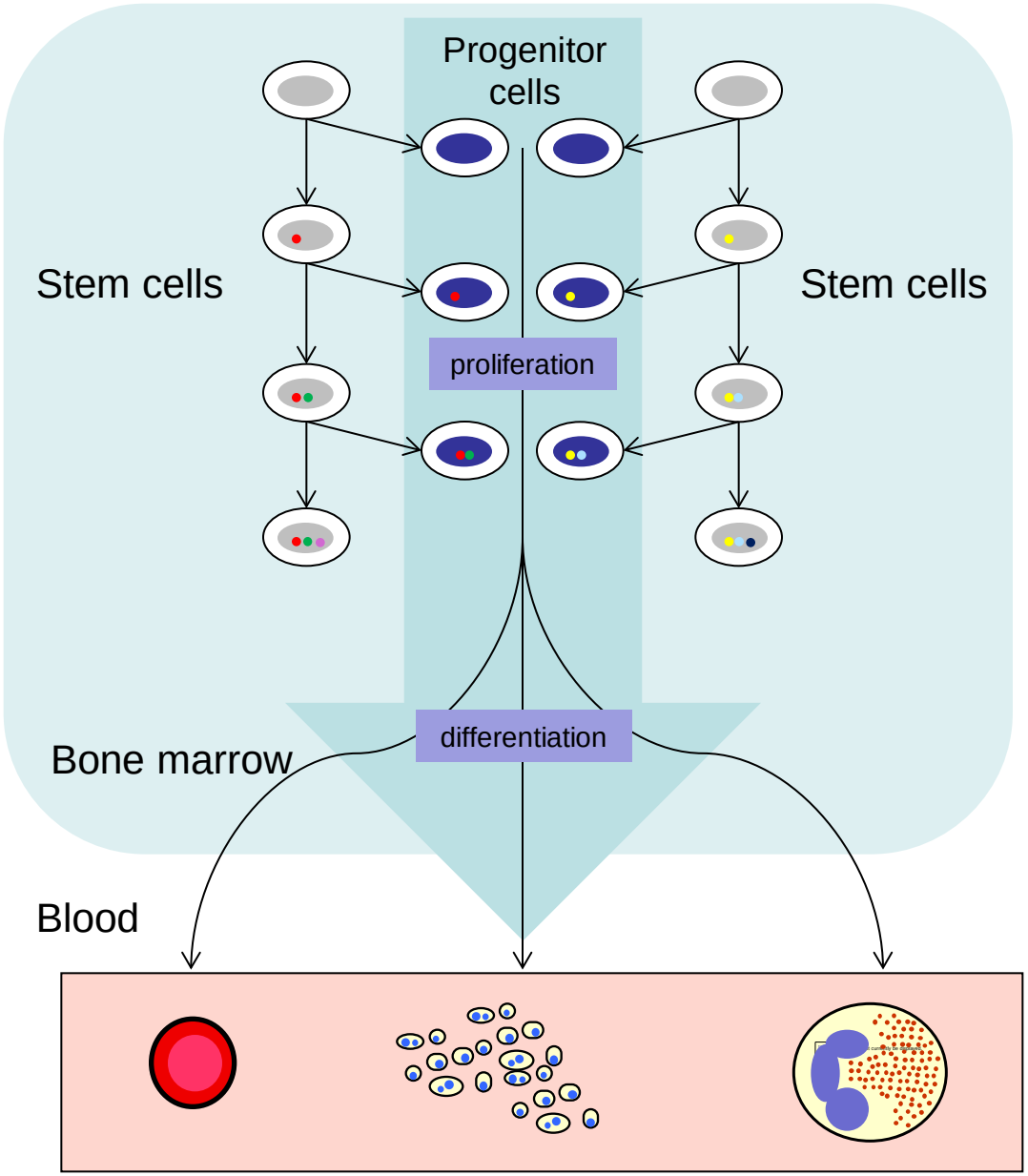


Acute myeloid leukemia

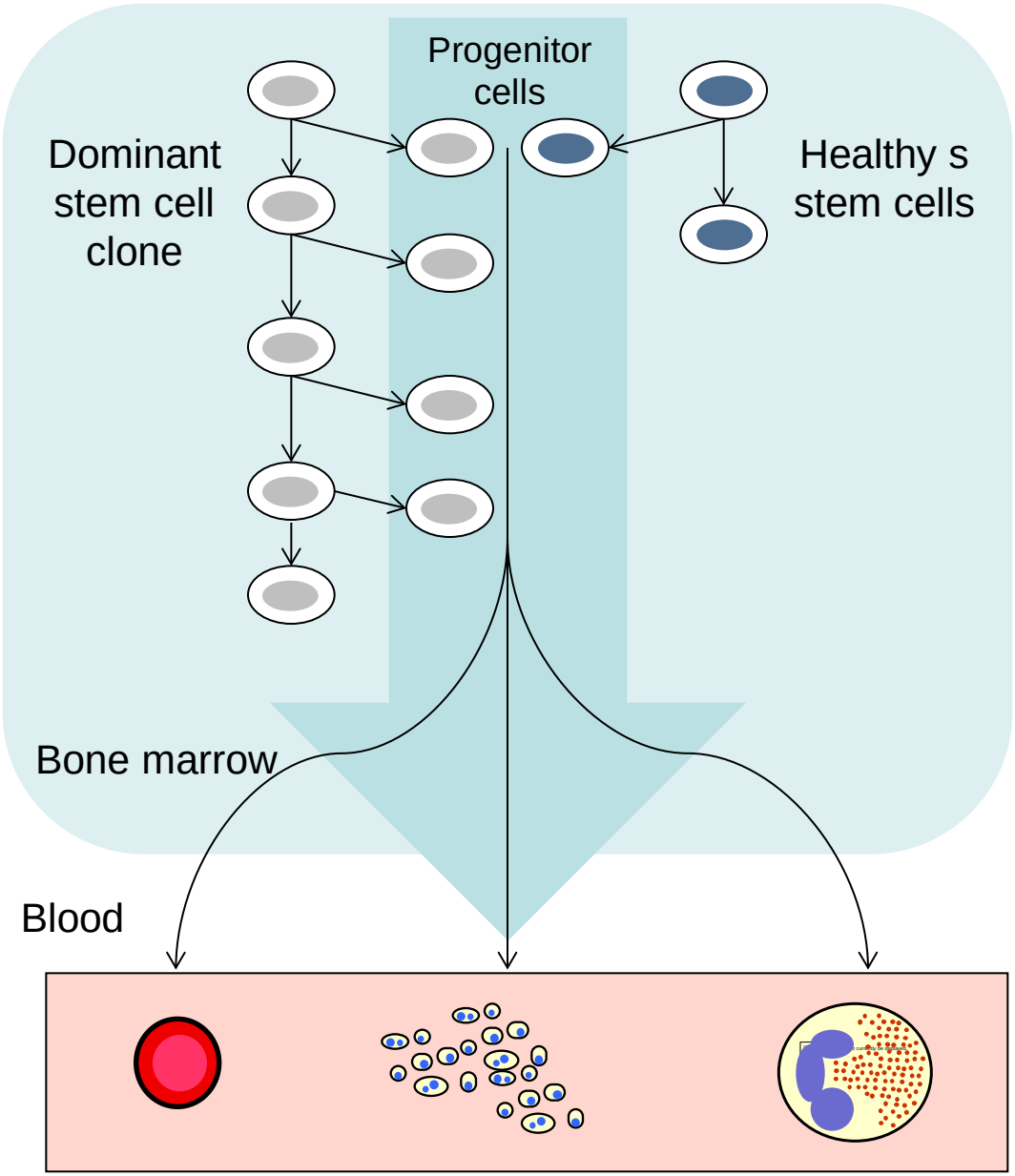
de novo
secondary

Myelodysplastic syndromes

Hematopoiesis

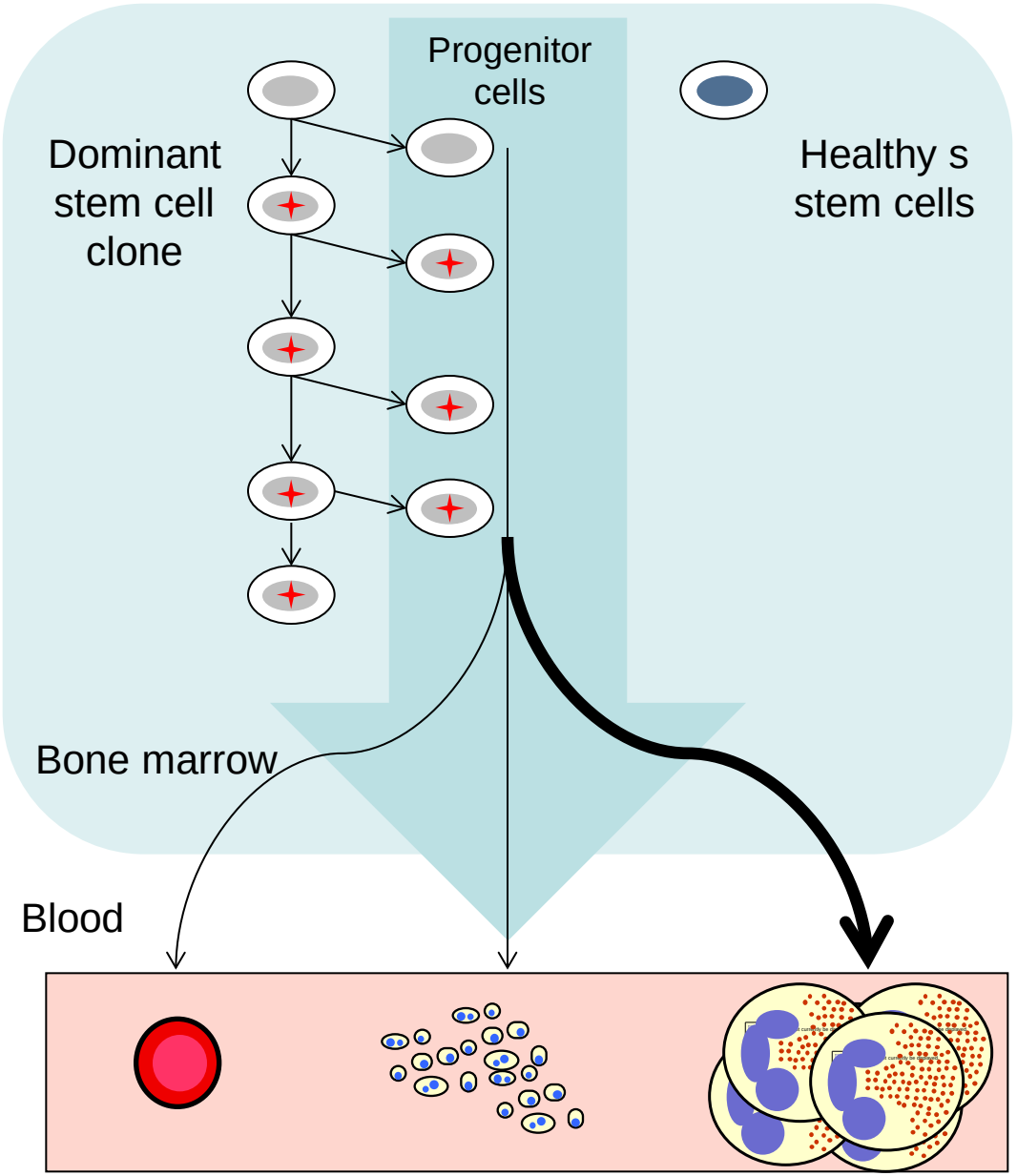


Shifted monoclonal hematopoiesis

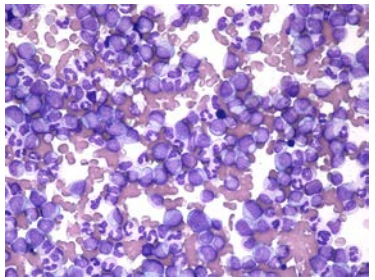


Monoclonal hematopoiesis

BCR-ABL

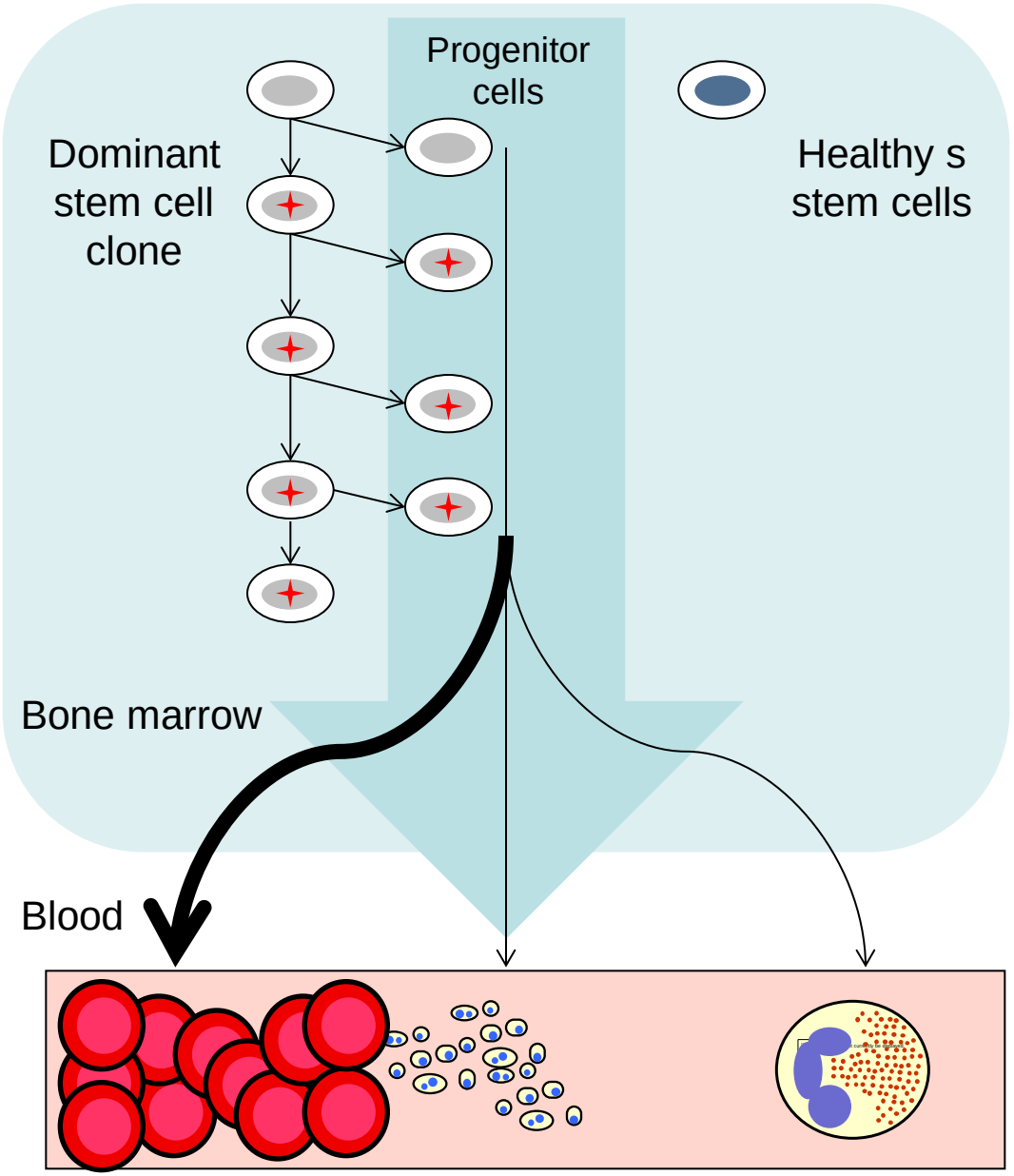


Chronic myeloid leukemia

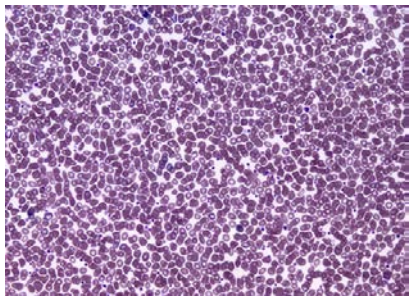


Monoclonal hematopoiesis

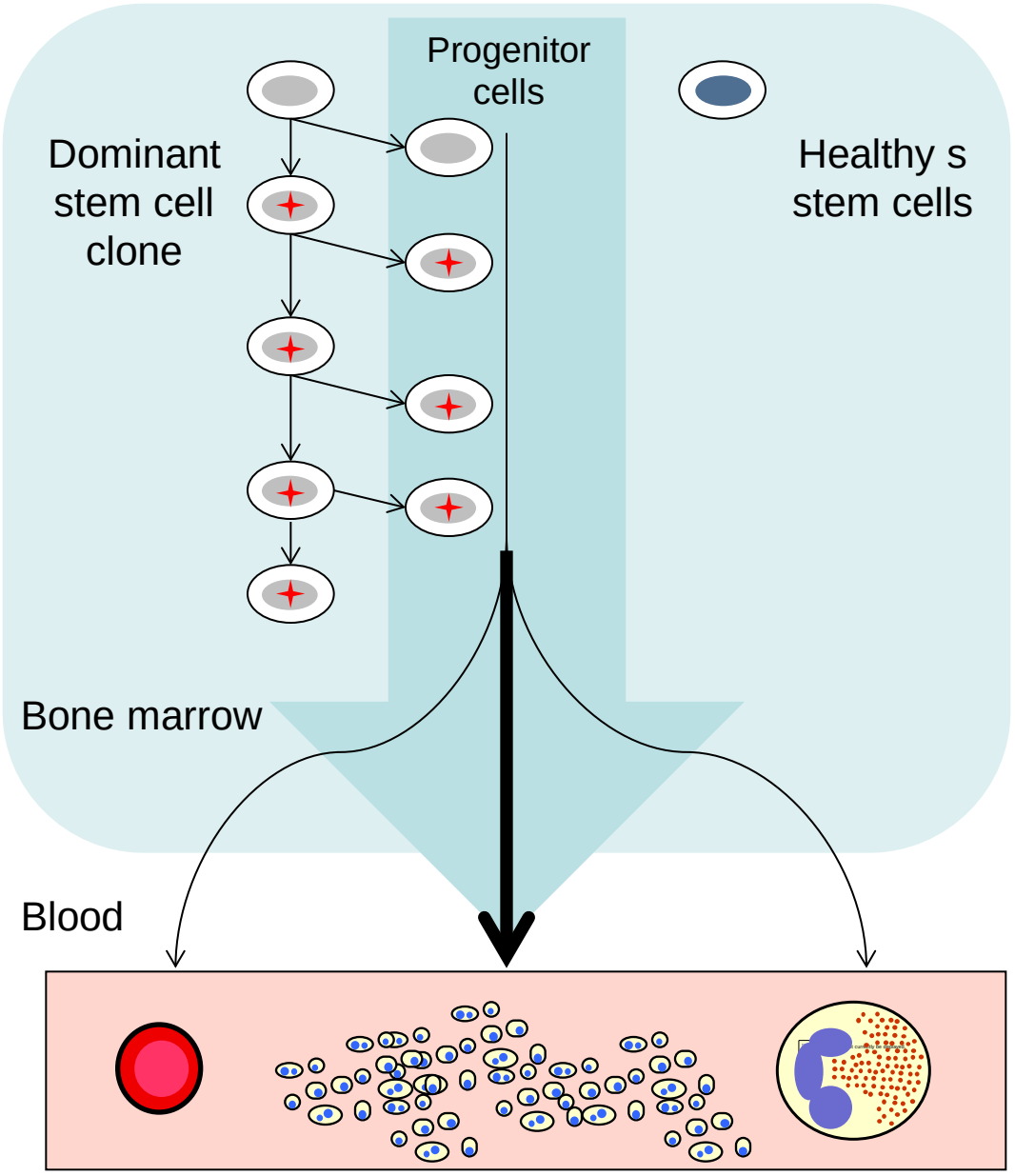
JAK2-V617F



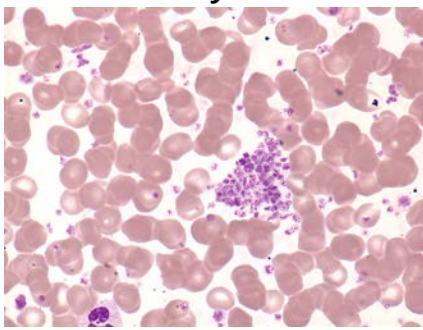
Polycythemia vera



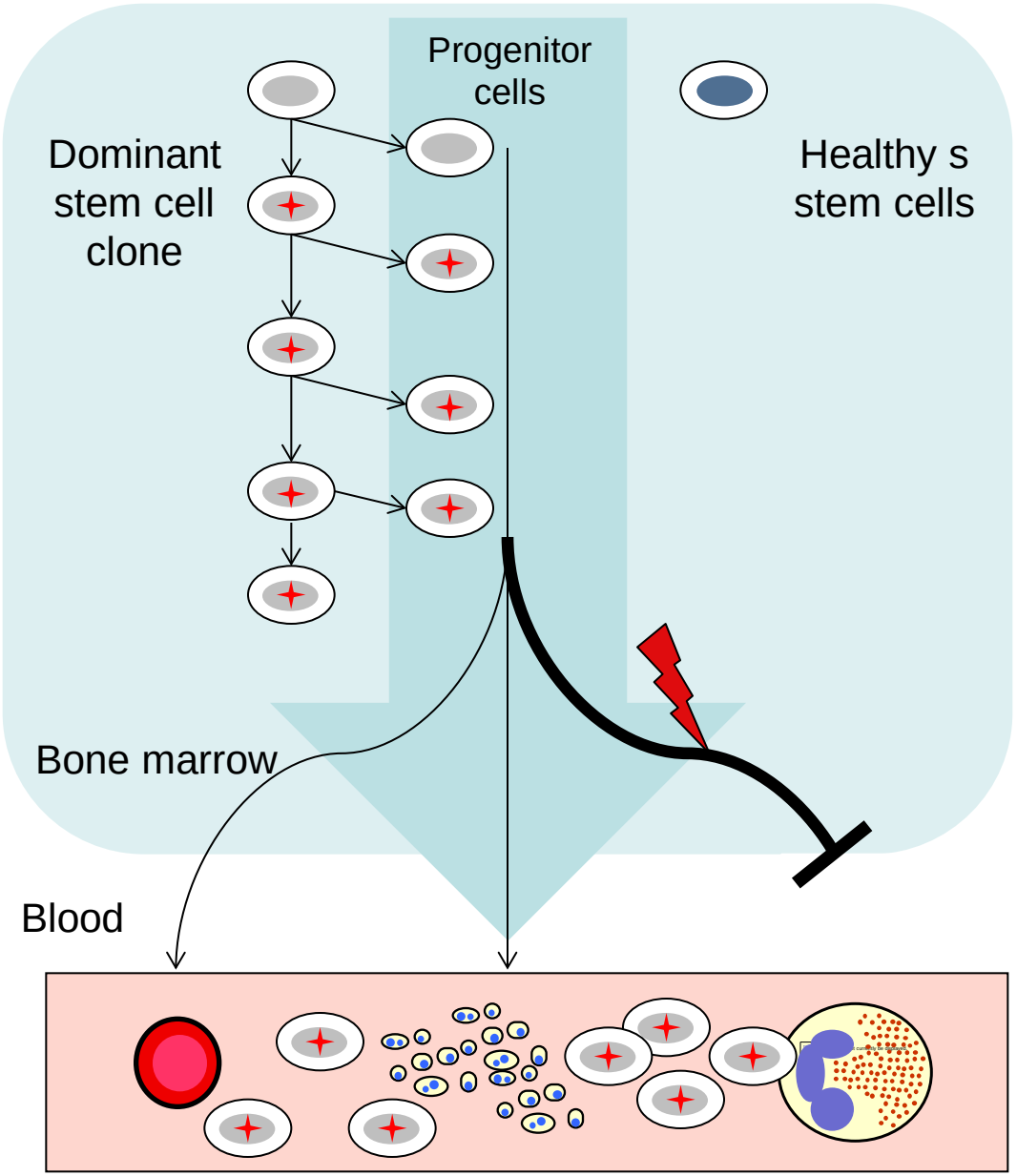
Monoclonal hematopoiesis



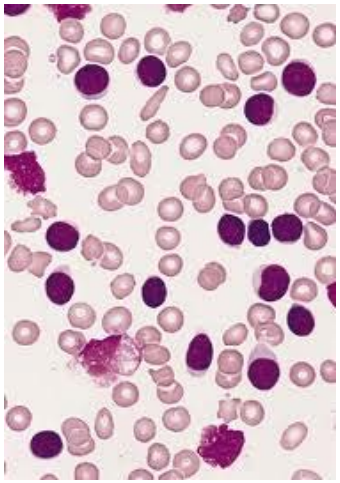
Throbocythemia



Monoclonal hematopoiesis

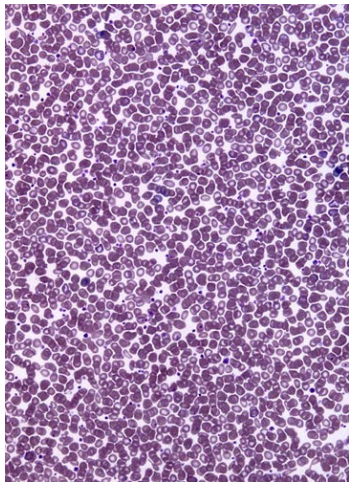


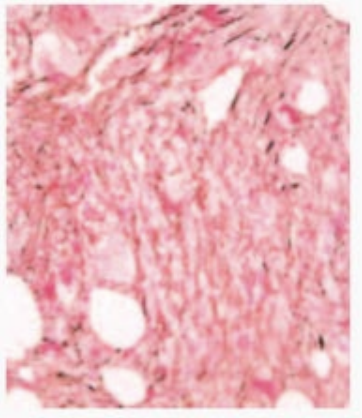
Acute myeloid leukemia

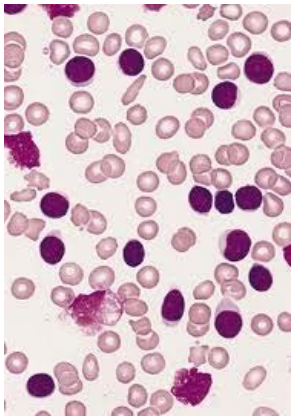


Disease progression in MPN

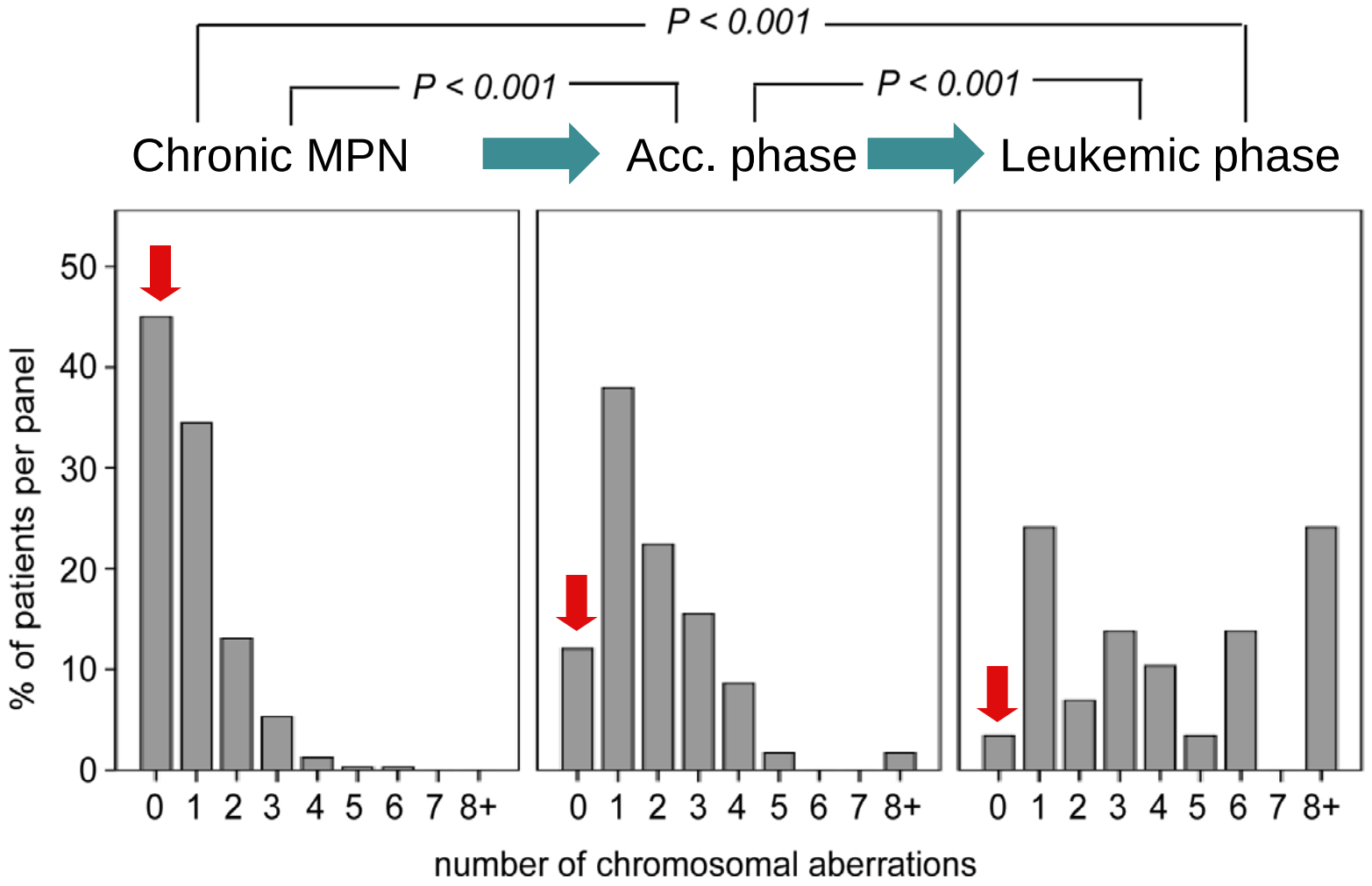
Chronic phase → Accelerated phase → Leukemic phase





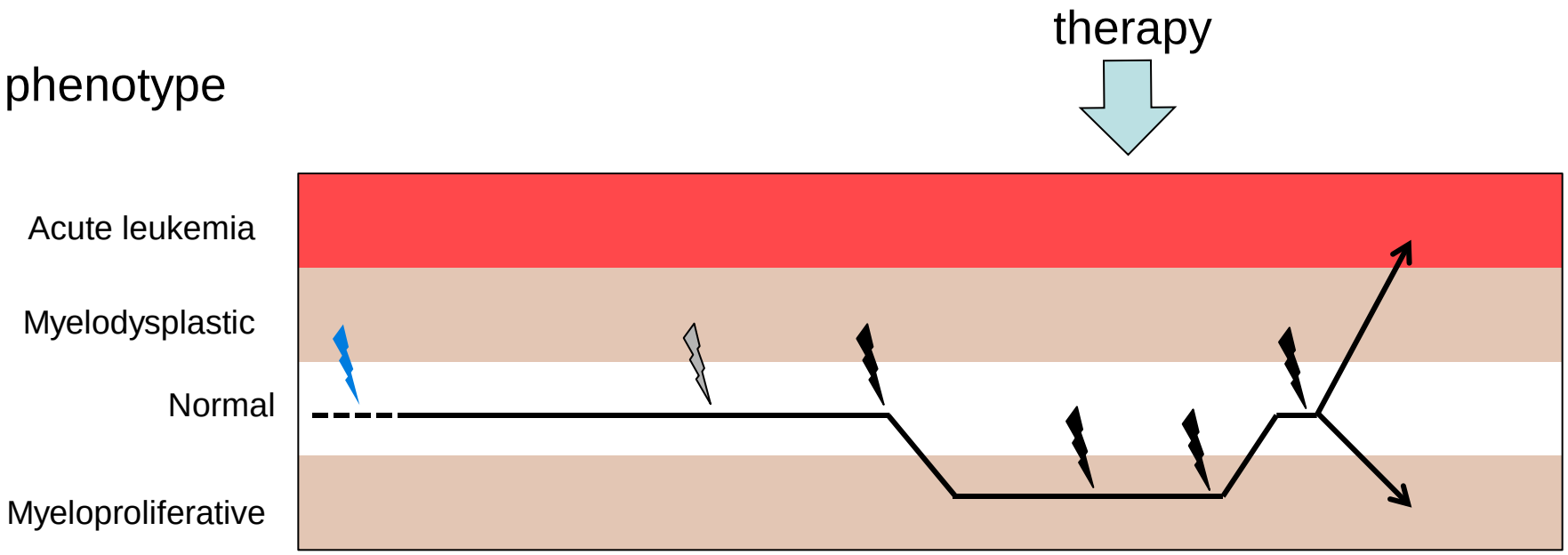


Cytogenetic lesions in MPN disease progression



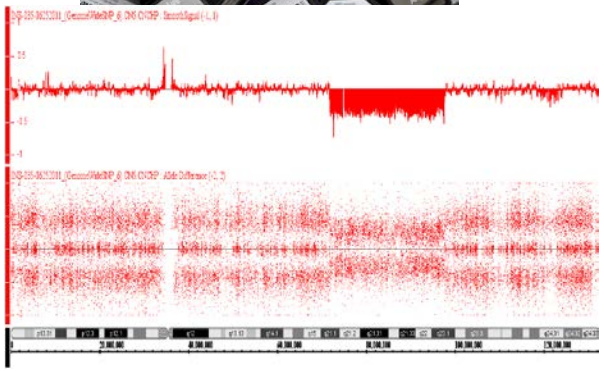
Klampfl et al., Blood, 2011

Mutations during disease evolution in MPN



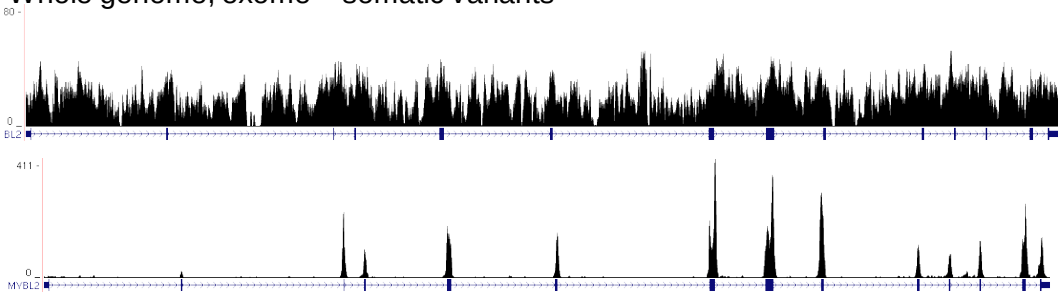
Technologies used in mutation discovery

SNP array genotyping

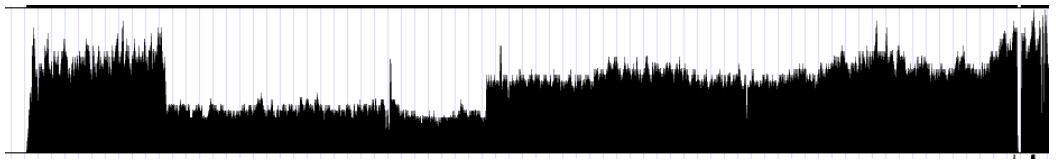


Deep sequencing

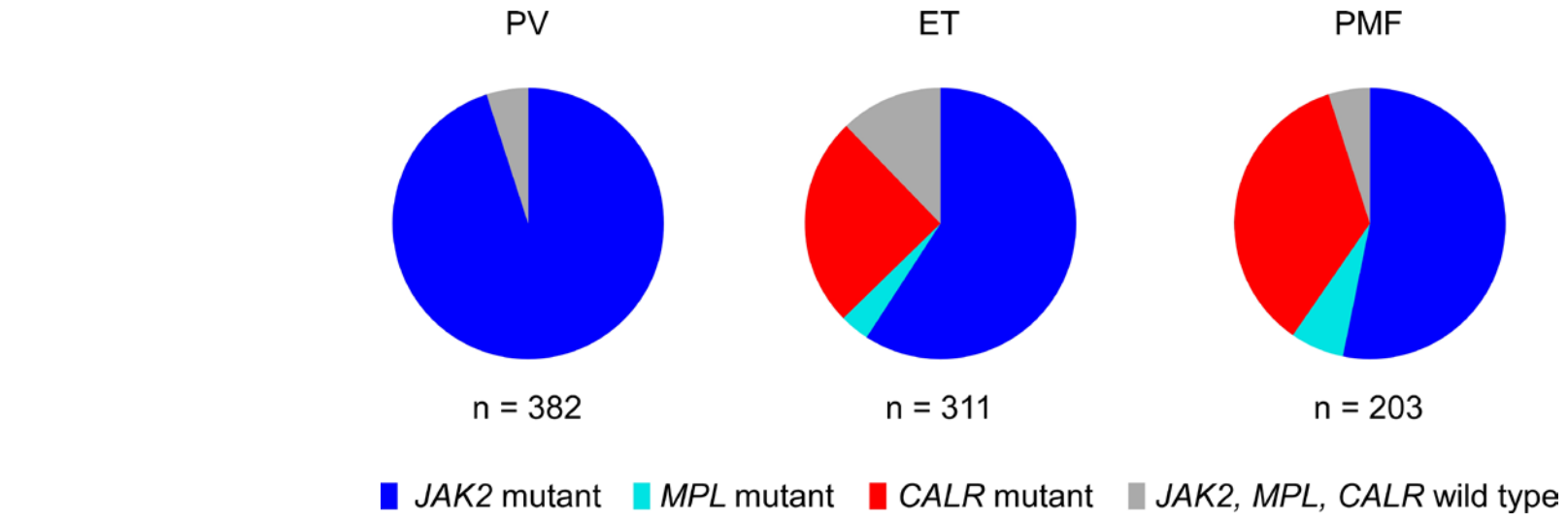
Whole genome, exome – somatic variants

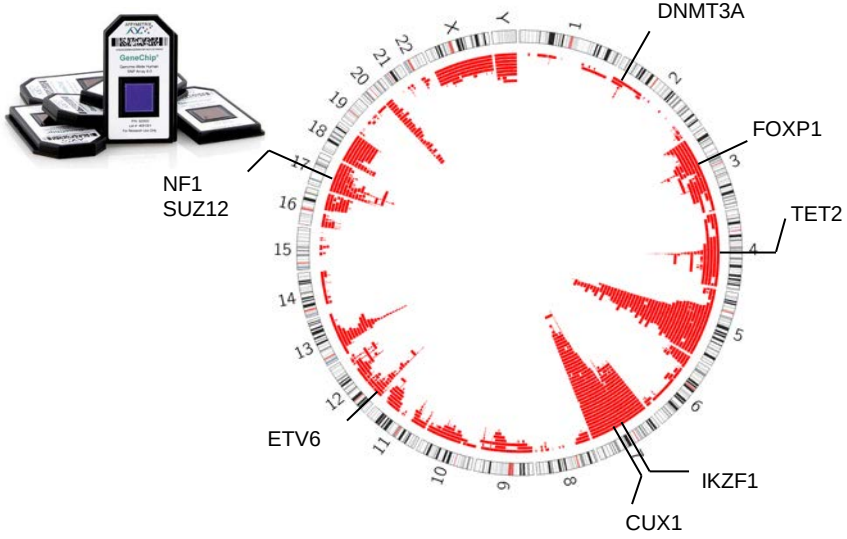


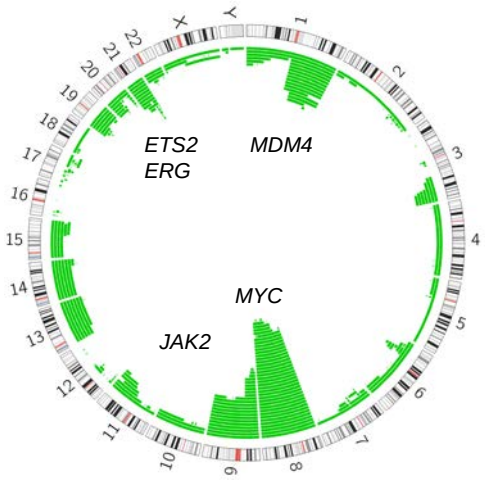
Whole genome – structural variants

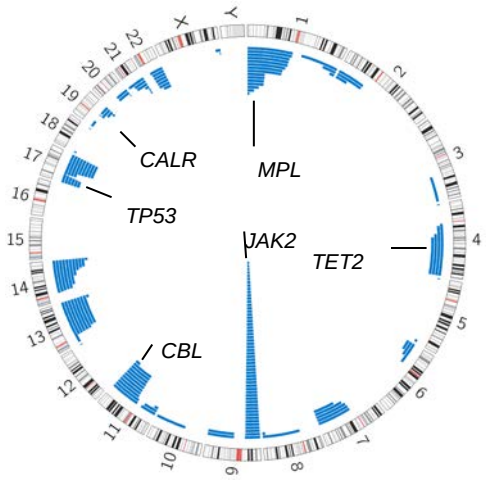


Somatic mutations in MPN

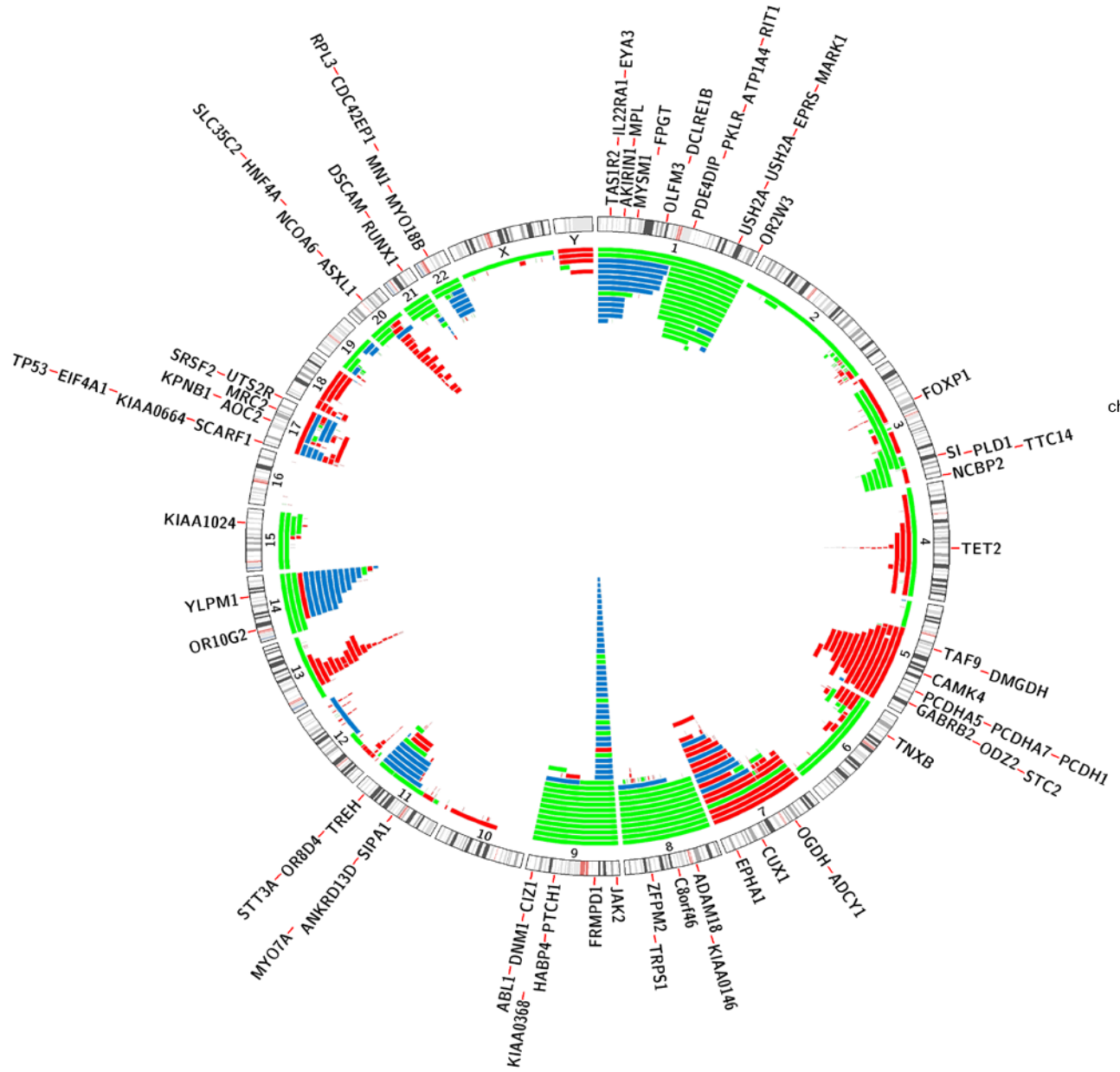






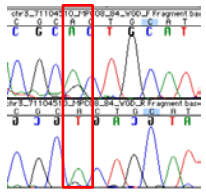


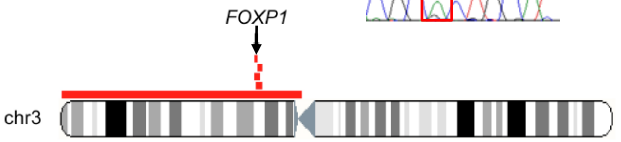
Overlap of mutations and chromosomal lesions



FOXP1

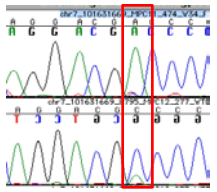
V515A

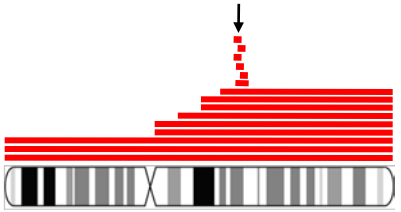




CUX1

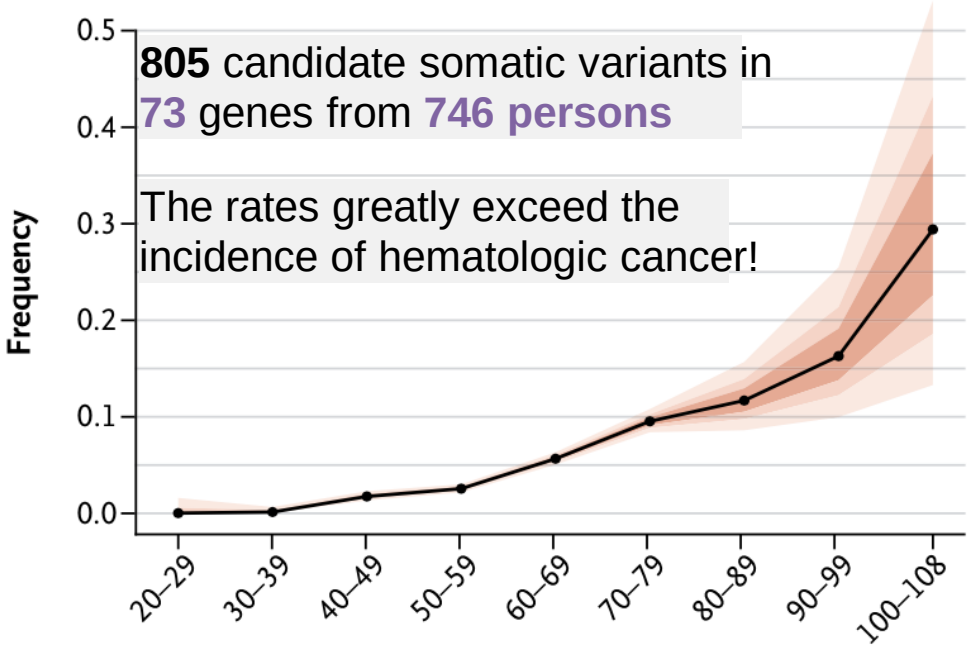
A802D





Prevalence of somatic mutations according to age

Siddhartha Jaiswal et al.



Implicated in hematologic cancers!

No. with Mutation	0	1	50	138	282	219	37	14	5
Total	240	855	2894	5441	5002	2300	317	86	17

Figure 1. Prevalence of Somatic Mutations, According to Age.

Colored bands, in increasingly lighter shades, represent the 50th, 75th, and 95th percentiles.

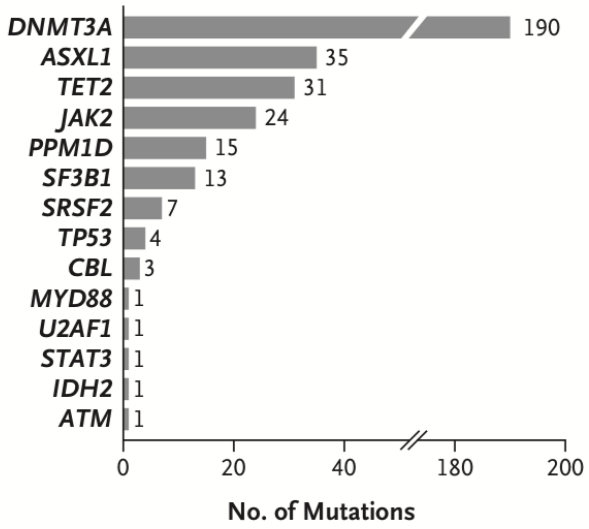
Mutated genes, mutation types

A

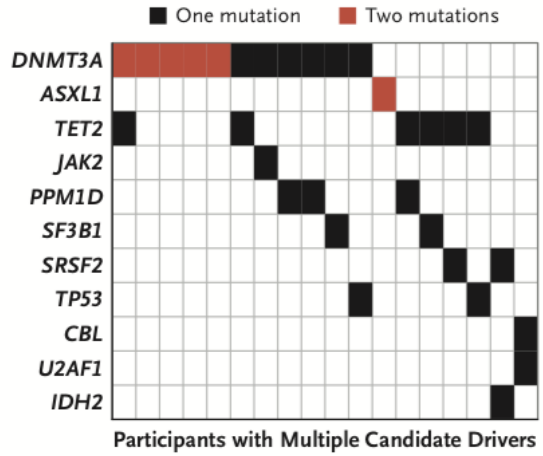
Giulio Genovese et al. - Figure 2

Gene	Disruptive Mutation	Missense Mutation	Synonymous Mutation	P Value
All genes — % of mutations	6	64	31	
Specific genes — no. of mutations				
DNMT3A	33	47	1	4.4×10^{-26}
TET2	20	2	2	8.8×10^{-15}
ASXL1	15	4	0	6.0×10^{-13}
PPM1D	10	0	0	7.0×10^{-9}

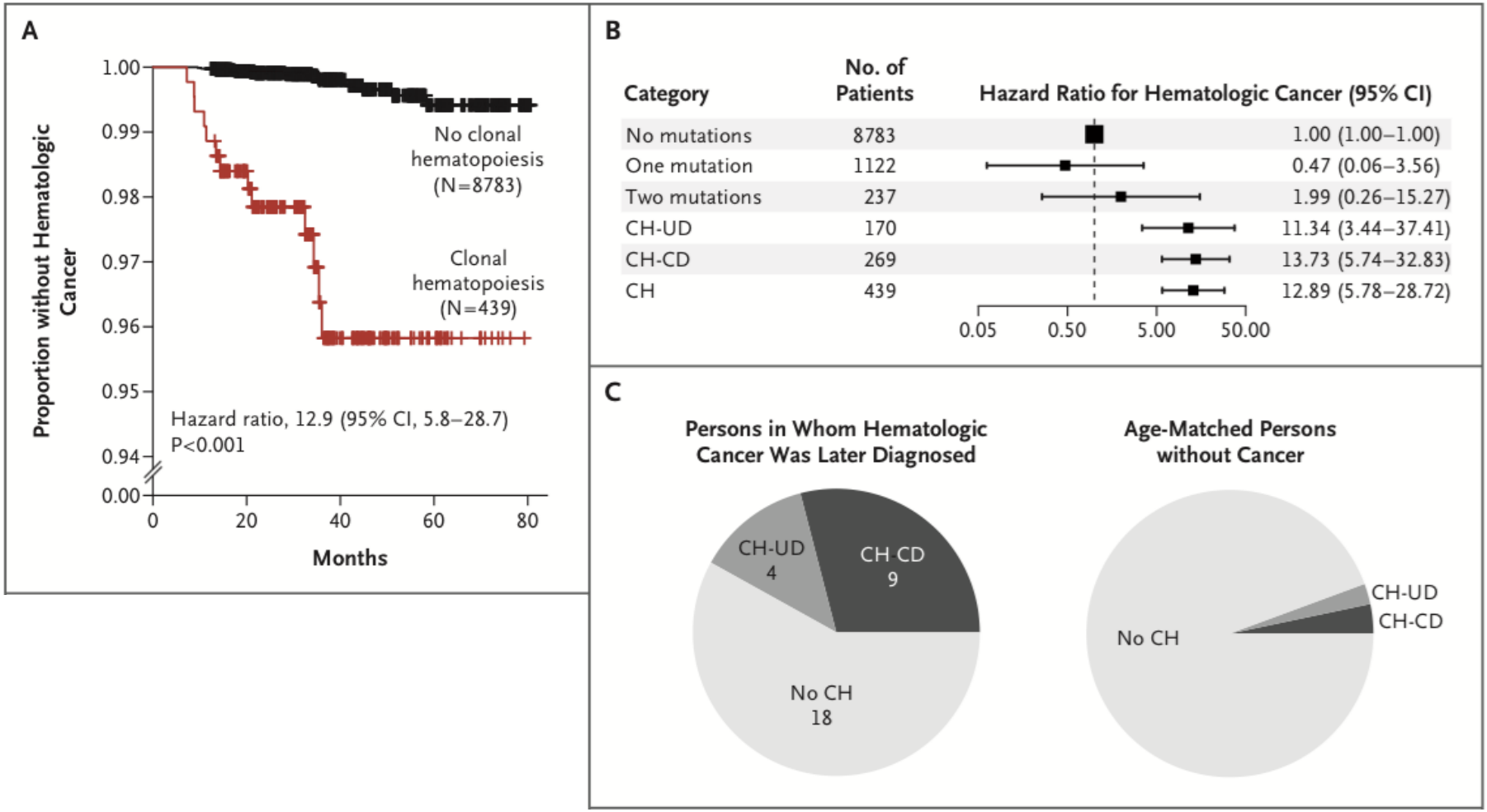
B



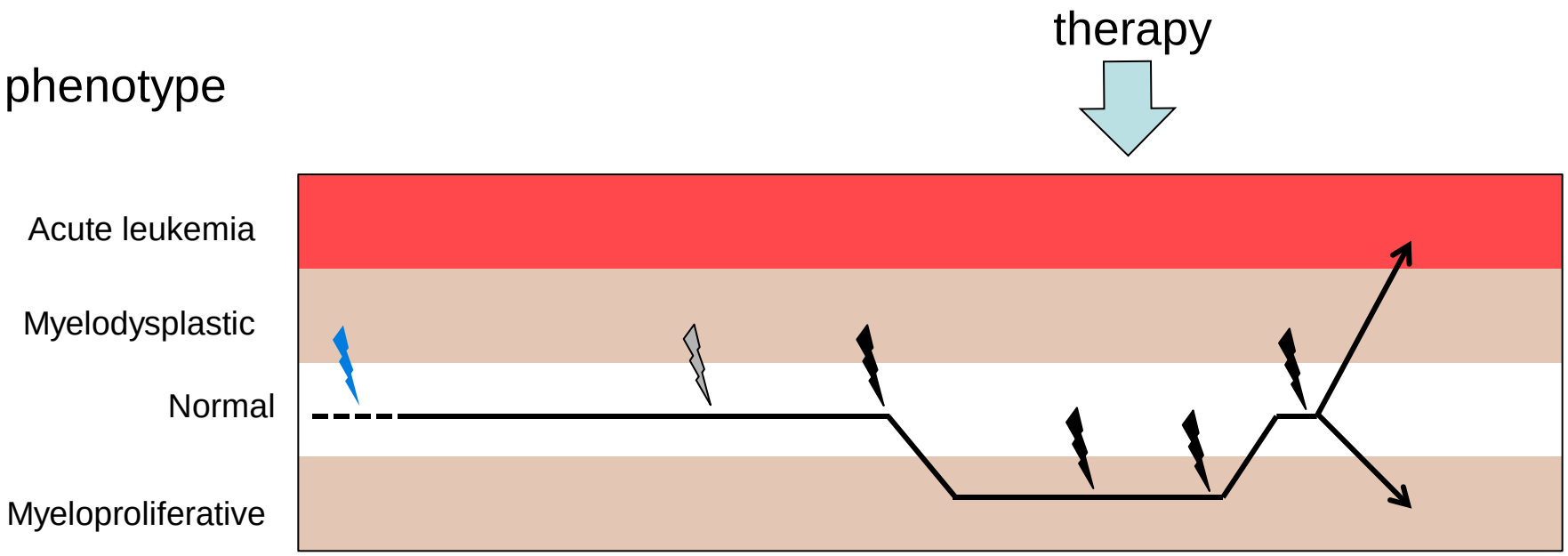
C



Risk of developing hematologic cancers



Genetic changes in MPN

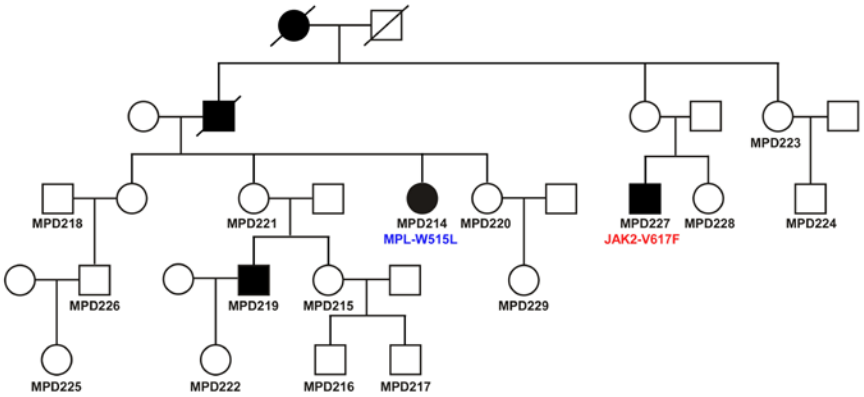


Germline factors

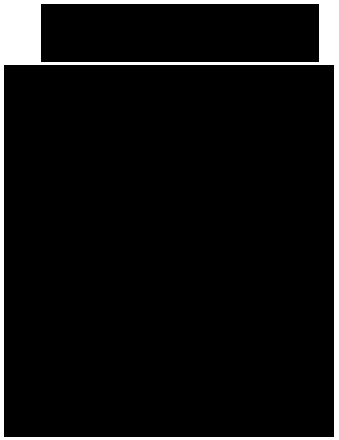
Clonal drivers
DNMT3A
TET2 mutations
del20q
del13q

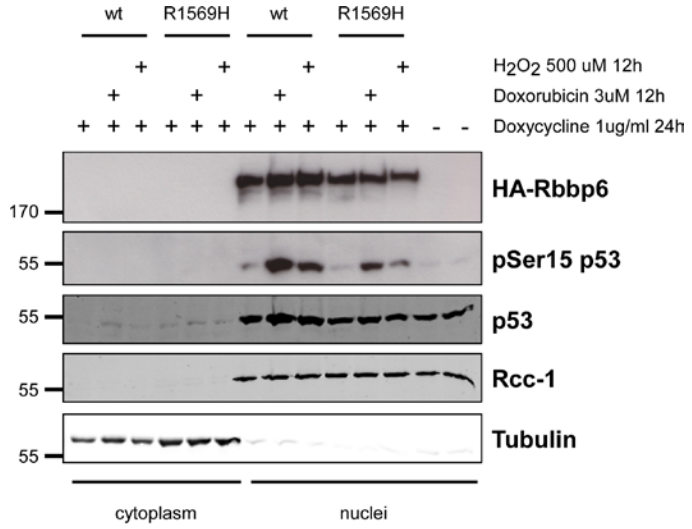
Disease causing
JAK2
MPL
CALR

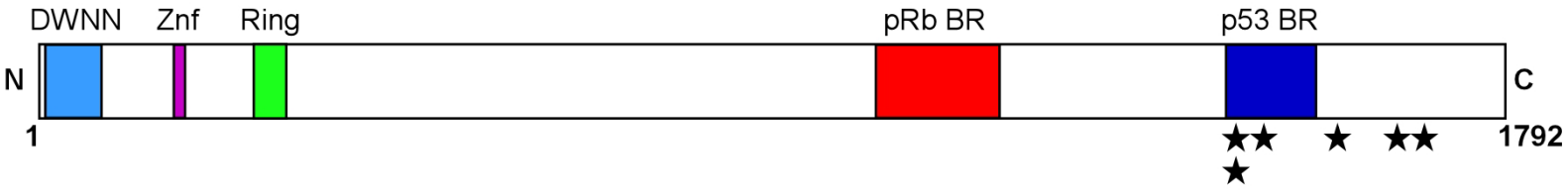
Familial MPN caused by gain-of-function mutations of RBBP6



Linkage analysis
Exome sequencing

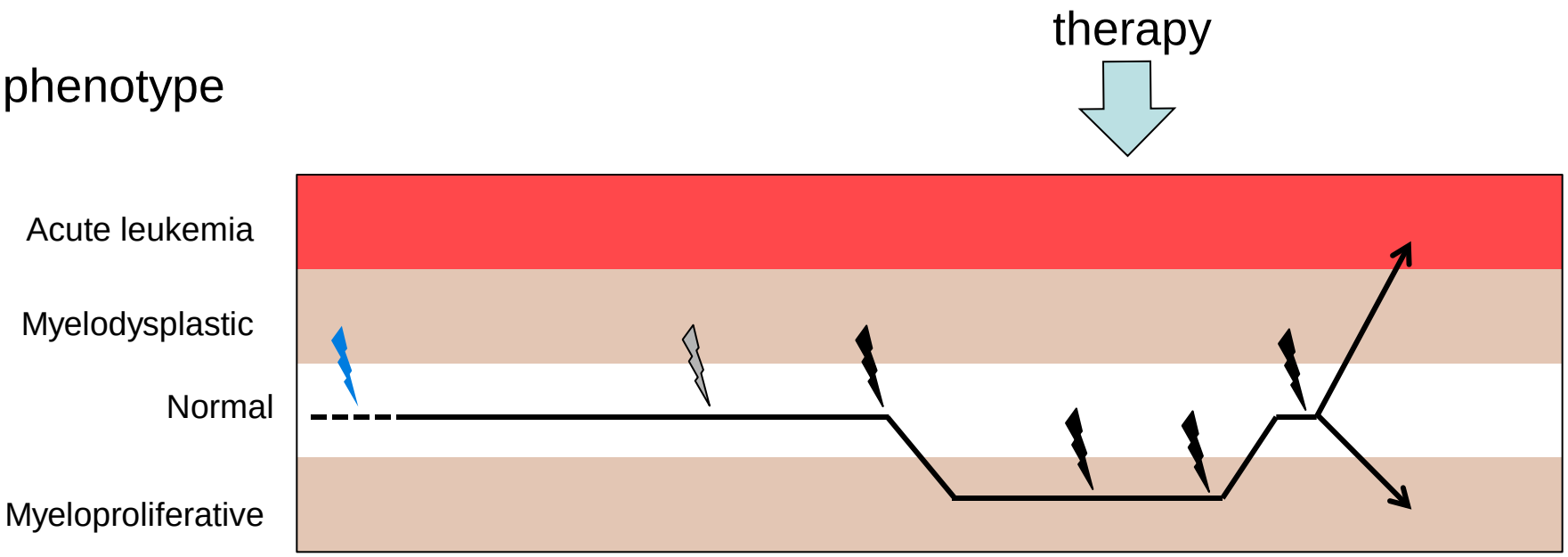






Harutyunyan et al., in preparation

Genetic changes in MPN



Germline factors
JAK2-GGCC
TERT
RBBP6

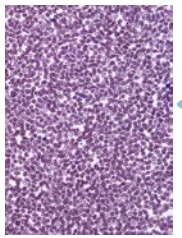
Clonal drivers
del20q
del13q
TET2 mutations

Disease causing
JAK2
MPL
CALR

Progression associated
TP53/MDM4
PRC2
DNMT3A
ASXL1
CUX1
IKZF1

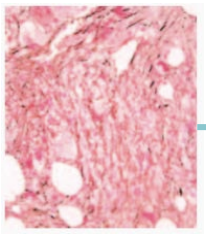
Adaptation to therapy

How we do it?

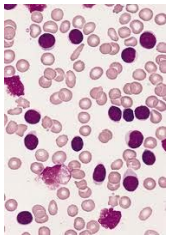


Chronic phase MPN

14 x



Accelerated phase MPN



Secondary AML

T cells as the control tissue

Chronic phase sample

Accelerated phase sample

Leukemic sample

Mutations causing MPN

Mutations causing disease progression

Mutations causing leukemic transformation

Exome sequencing

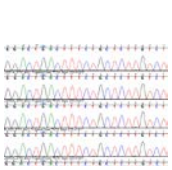
RNA sequencing for fusion detection

Project outline II

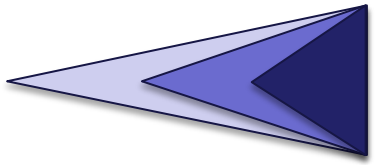
Affymetrix genome wide human SNP array 6.0 analysis

&

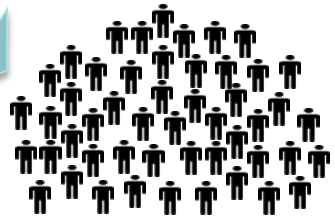
Exome & RNA sequencing



Validation of somatic mutations and gene fusions by Sanger sequencing



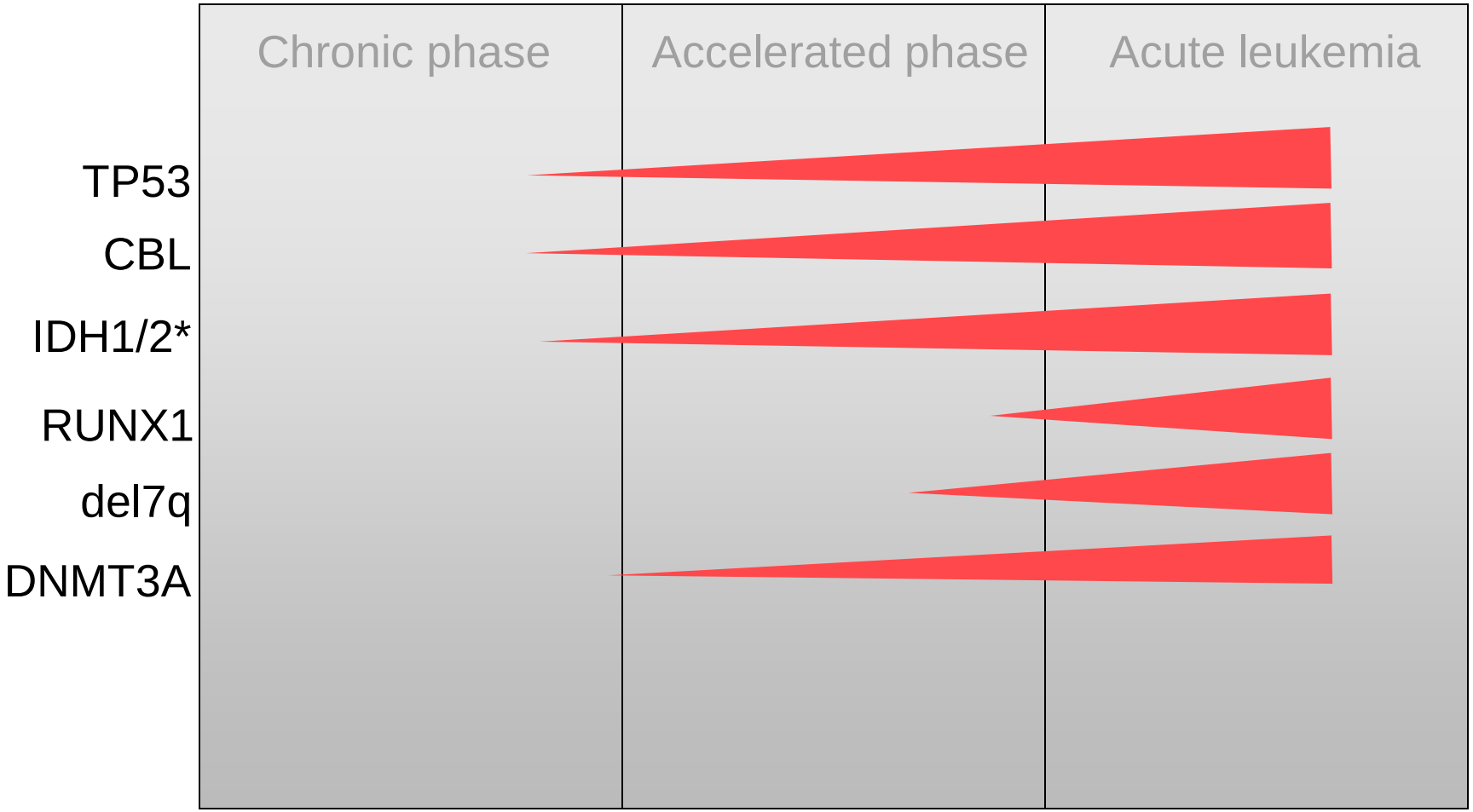
Clonal evolution paths analysis



Testing for recurrence in a larger patient cohort

Functional validations

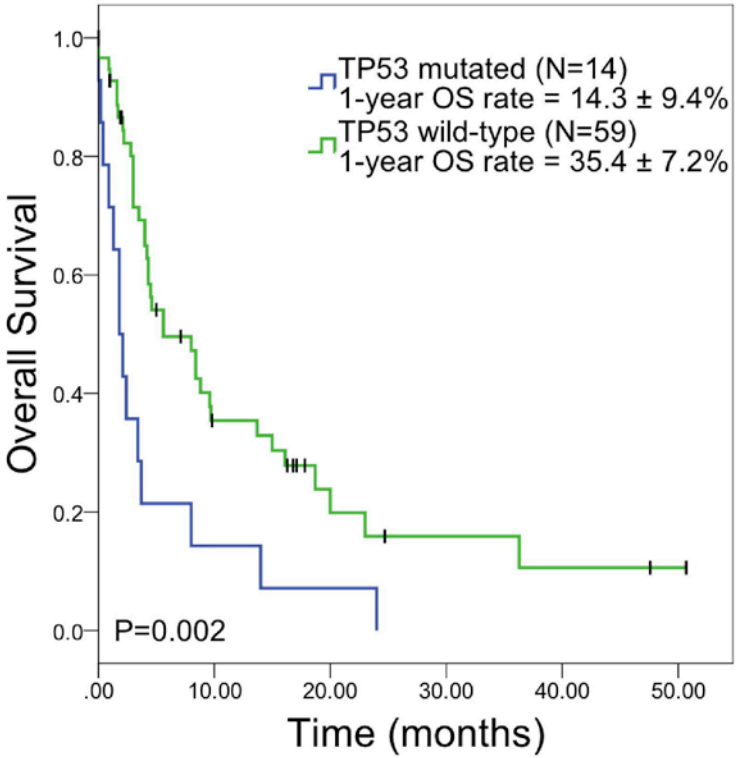
Prognostic markers for leukemic transformation in MPN ?



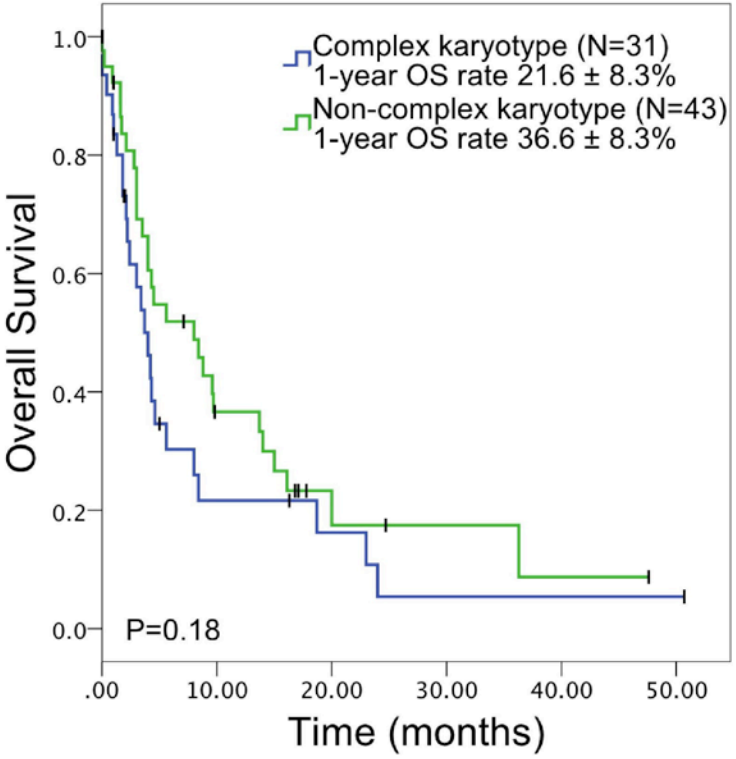
**Pardanani et al., Leukemia 2010; Tefferi et al., Leukemia 2010; Zhang et al., Blood 2012*

Patient survival in leukemic phase of MPN

TP53 mutation



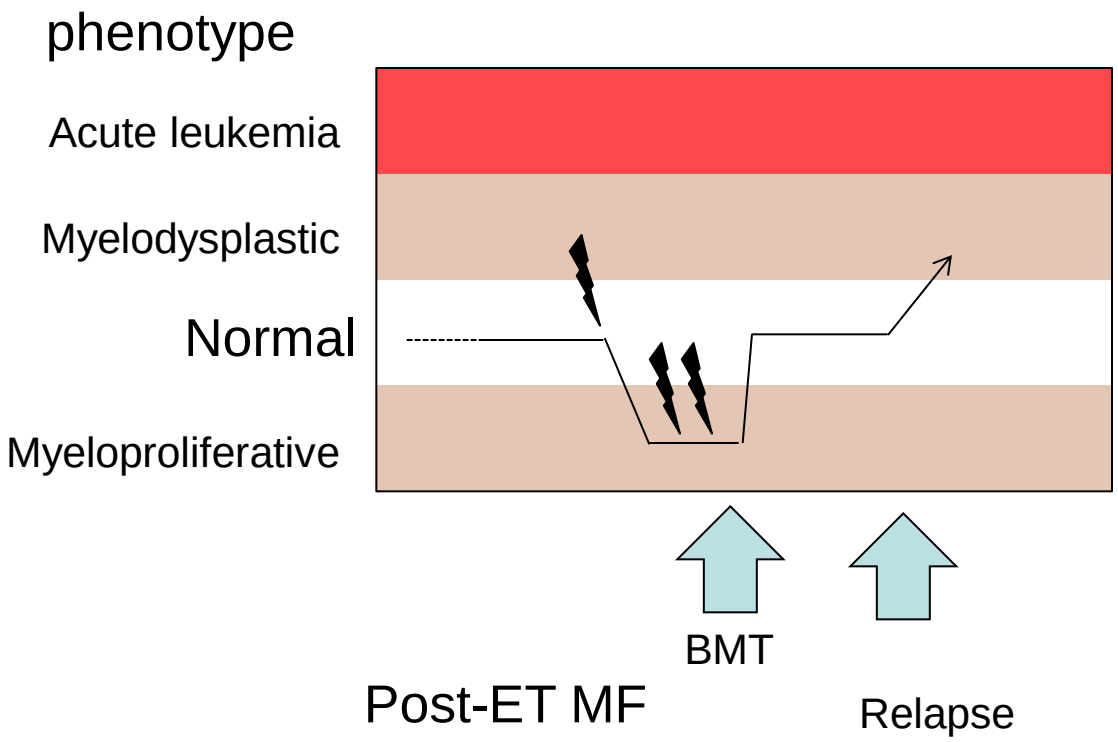
Complex karyotype



- no survival difference for del5q, del7p(IKZF1), del7q(CUX1), RUNX1 mutation

Milosevic et al., Am J Hematol, 2012

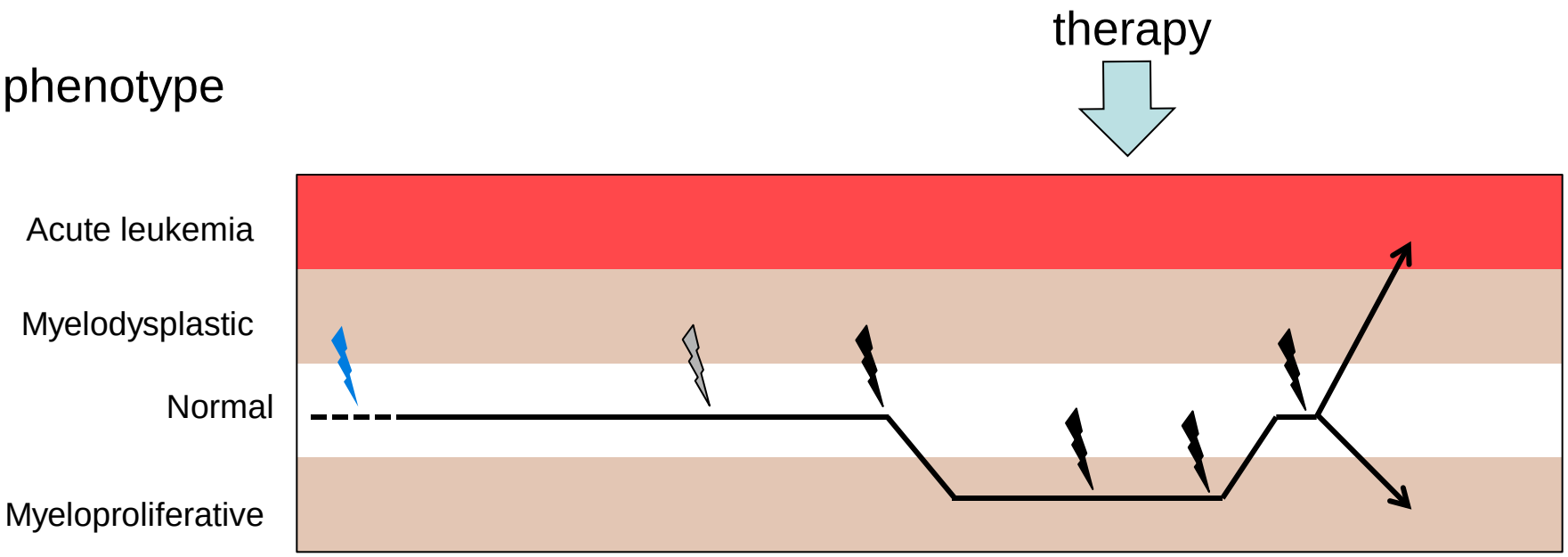
Genomic adaptation to therapy



Exome sequencing

Gene	Chr	Amino acid change	Validated
C15orf42	chr15	D1170G	SOMATIC
DNMT3A	chr2	P511L	SOMATIC
HEXIM1	chr17	R86G	SOMATIC
MPL	chr1	W515A	SOMATIC
OR4K17	chr14	P261L	SOMATIC
PDE4DIP	chr1	D84G	SOMATIC
RAD50	chr5	R352H	SOMATIC
SIRT2	chr19	R137X	SOMATIC
U2AF1	chr21	Q157P	SOMATIC
WDR81	chr17	S558R	SOMATIC
PLA2G4F	chr15	M369I	SOMATIC
C17orf56	chr17	A266V	SOMATIC
CDYL	chr6	G332V	SOMATIC
CRHR1	chr17	C102F	SOMATIC
HCAR2	chr12	R142W	SOMATIC
KIF20A	chr5	G115C	SOMATIC
MYO18B	chr22	S898A	SOMATIC
PSMD2	chr3	A368S	SOMATIC
TP53	chr17	R156G	SOMATIC
ZNF592	chr15		SOMATIC

Genetic changes in MPN



Germline factors
JAK2-GGCC
TERT
RBBP6

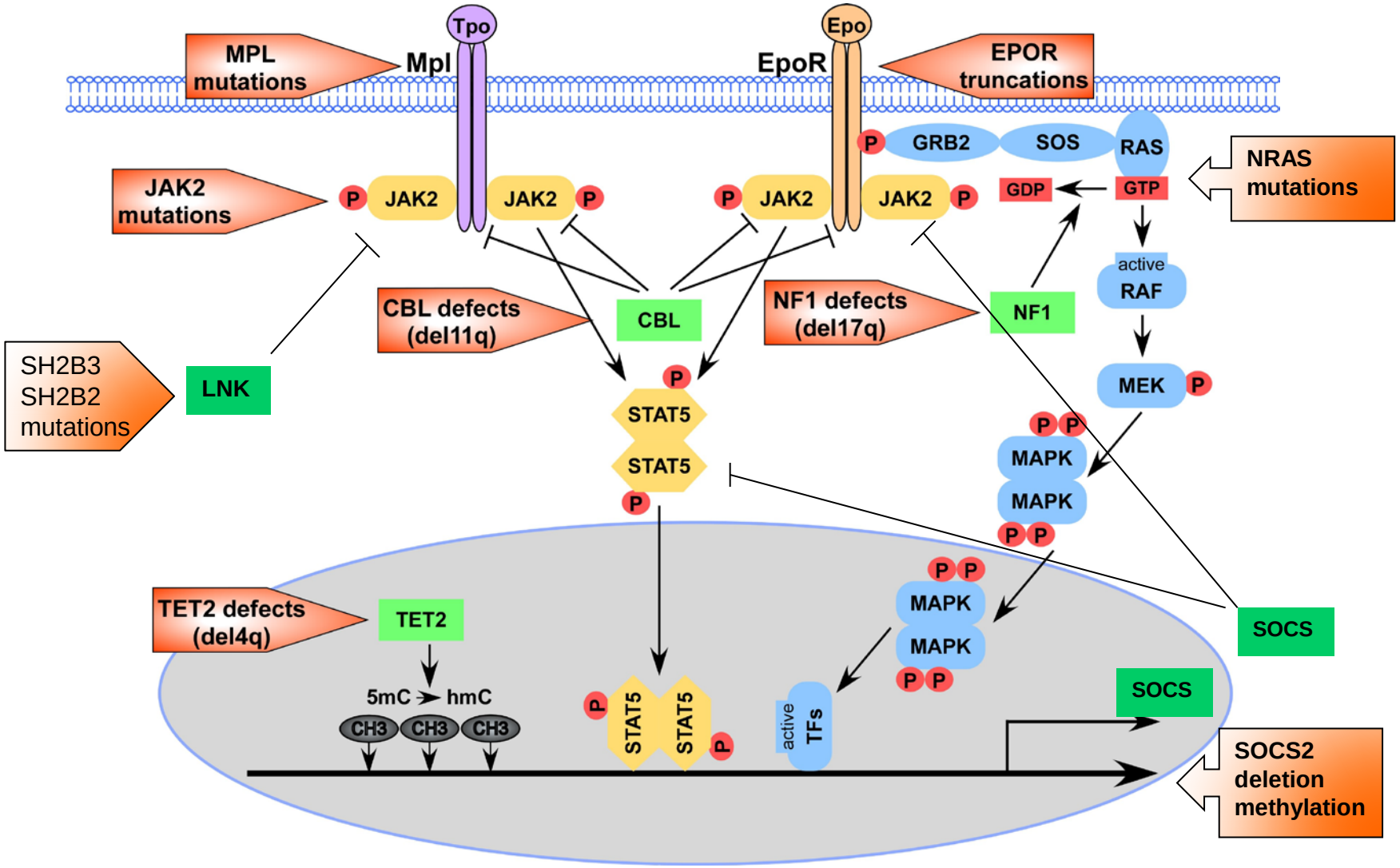
Clonal drivers
del20q
del13q
TET2 mutations

Disease causing
JAK2
MPL
CALR

Progression associated
TP53/MDM4
PRC2
DNMT3A
ASXL1
CUX1
IKZF1

Adaptation to therapy

Mechanisms and pathways targeted by mutations in MPN



Sources of genetic variability

- In average two individuals differ at 10^6 genomic positions
- 10^4 variants in protein coding sequences
- 500-1000 “private” germline variants in protein coding sequences

Acknowledgements

Ce–M–M– Research Center for Molecular Medicine of the Austrian Academy of Sciences

CeMM RK lab
Tiina Berg
Ashot Harutyunyan
Thorsten Klampfl
Ana Puda
Jelena Milosevic
Doris Chen
Klaudia Bagienski
Ciara Cleary
Nicole Them
Fiorella Schischlik
Roland Jäger
Damla Olcaydu

CeMM
Denise Barlow
Sebastian Nijman
Giulio Superti-Furga
Jacques Colinge
Keiryn Bennett
Christoph Bock

IMP
Johannes Zuber
Meinrad Busslinger

Med.Univ. Vienna
Heinz Gisslinger
Ulrich Jäger
Peter Valent

University of Pavia
Mario Cazzola
Francesco Passamonti
Daniela Pietra
Elisa Rumi

University of Florence
Alessandro Vannucchi
Lorenzo Tozzi

Masaryk University Brno
Miroslav Penka
Michael Doubek
Sarka Pospisilova
Zdenek Racil

SFB28
Veronika Sexl, VetMedUni
Richard Moriggl, LBI-CR
Mathias Mueller, VetMedUni

INSERM
Sylvie Hermouet, Nantes
Jean-Jacques Kiladjian, Paris
Christine Bellanne-Chantelot, Paris
William Vainchenker, Paris
Jean-Luc Villeval, Paris

University of Birmingham
Jon Frampton
Paloma Garcia

University of Southampton
Nick Cross

University of Belgrade
Sonja Pavlovic
Natasa Tosic

University Hospital Innsbruck
Michael Steurer

FWF

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