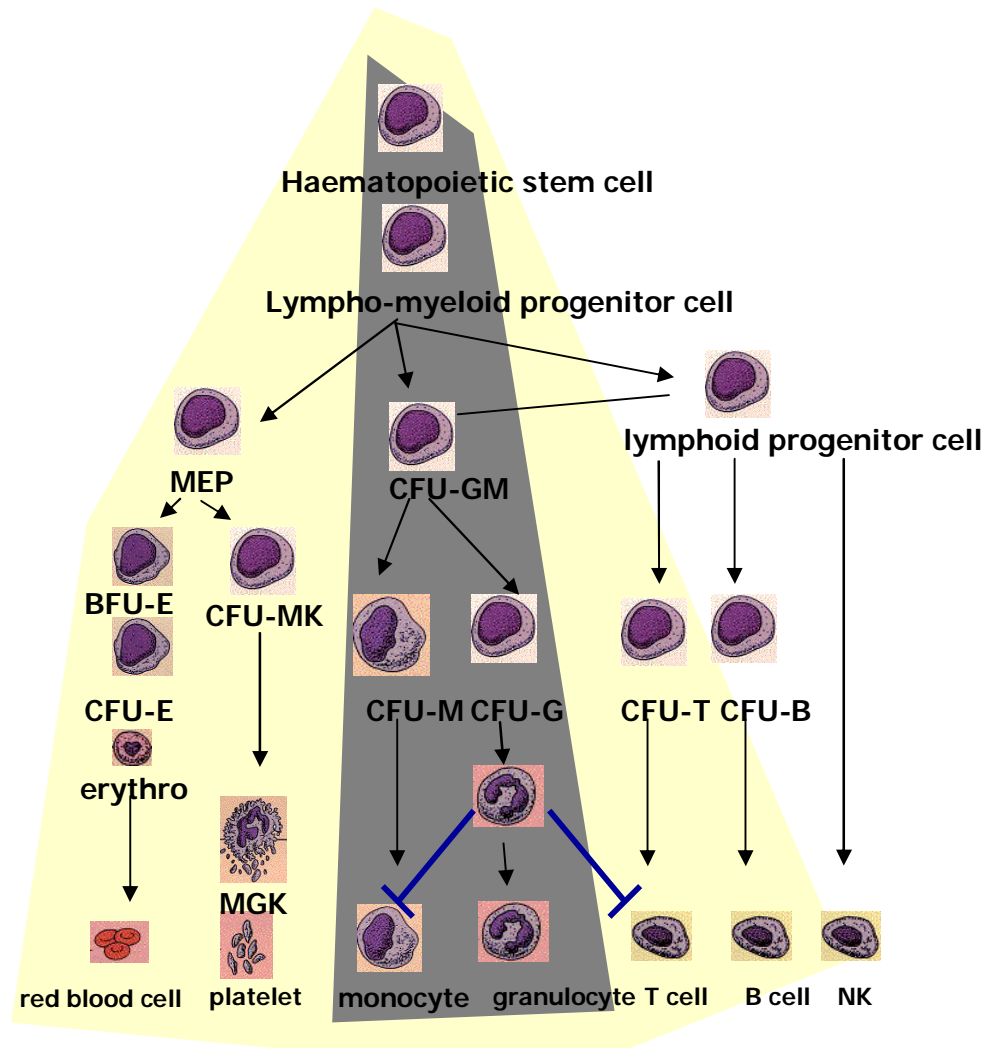
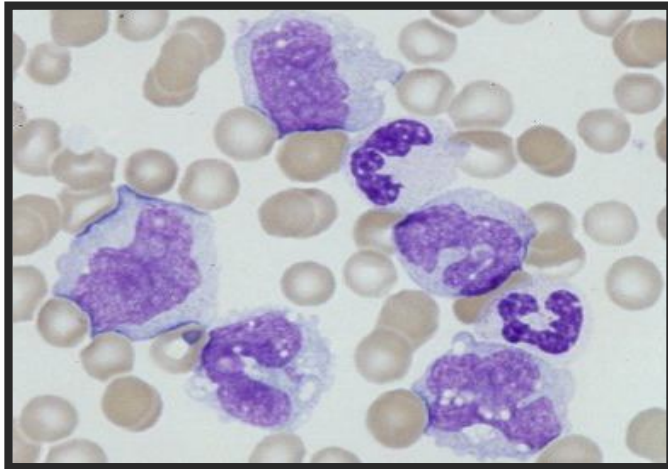
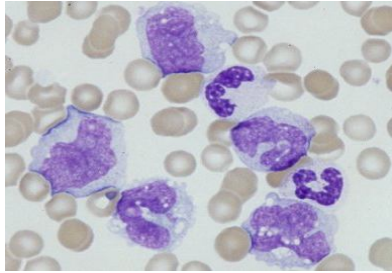


Juvenile and Chronic Myelo-Monocytic Leukemia





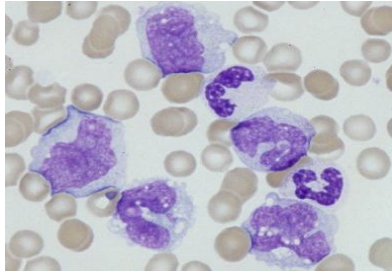
MDS/MPN of the WHO classification

Clonal diseases of the HSC

No BCR-ABL or Ph1
Bone marrow blast cells < 20%

1. Chronic myelomonocytic leukemia
2. Juvenile myelomonocytic leukemia
3. Atypical chronic myeloid leukemia,
4. Refractory anemia with ring sideroblasts and thrombocytosis
5. MDS/MPN unclassifiable

Excluded in 2009 : *PDGFBR* rearrangement and eosinophilia



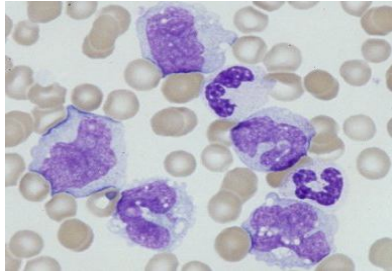
JMML

An aggressive myeloid malignancy of childhood

MPN / MDS in the WHO classification

- Infant or young child (0-6), male > female, with fever and pallor,
 - Circulating WBC count > 10G/L; Peripheral monocytosis > 1 G/L
 - A few circulating myeloid precursor cells
 - Splenomegaly, hepatomegaly

 - Sometimes : skin rash, cough, bloody stools
-
- Lack of BCR-ABL
 - Less than 20% blast cells in the bone marrow



JMML

An aggressive myeloid malignancy of childhood

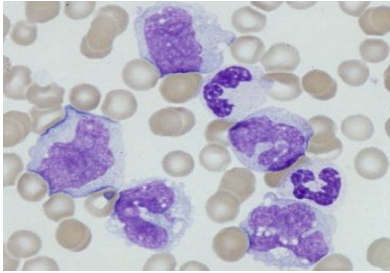
Increased HbF for age

Cytogenetic abnormality (monosomy 7, 25%)

Hypersensitivity of myeloid progenitor cells to GM-CSF (not to IL-3 or G-CSF)

AML4 / AML5 or spontaneous improvement

Treatment : ABMT (EFS 52%)



Genetic syndromes that predispose to JMML

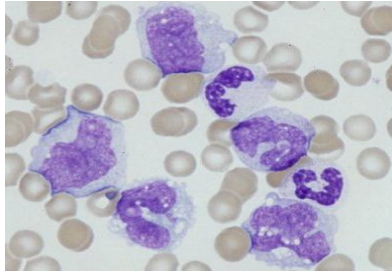
Neurofibromatosis, type I (NF1) : prone to JMML in the first decade

- Autosomal dominant disorder (or spontaneous)
- At least 6 café au lait macules,
- At least 2 neurofibromas or 1 plexiform neurofibroma,
- Lisch nodules (iris hamartomas), axillary or inguinal freckling, and/or optic gliomas

- NF1 encodes neurofibromin protein, a GTPase activating protein for Ras
- Enhances the hydrolysis of the active, GTP-bound conformation of Ras

- In the bone marrow of children with JMML: loss of the wild type allele

- Mouse models of *nf1* conditional deletion reproduce the disease



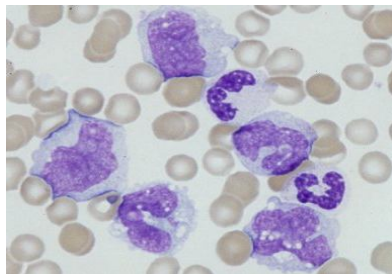
Genetic syndromes that predispose to JMML

Noonan syndrome (*NS*)

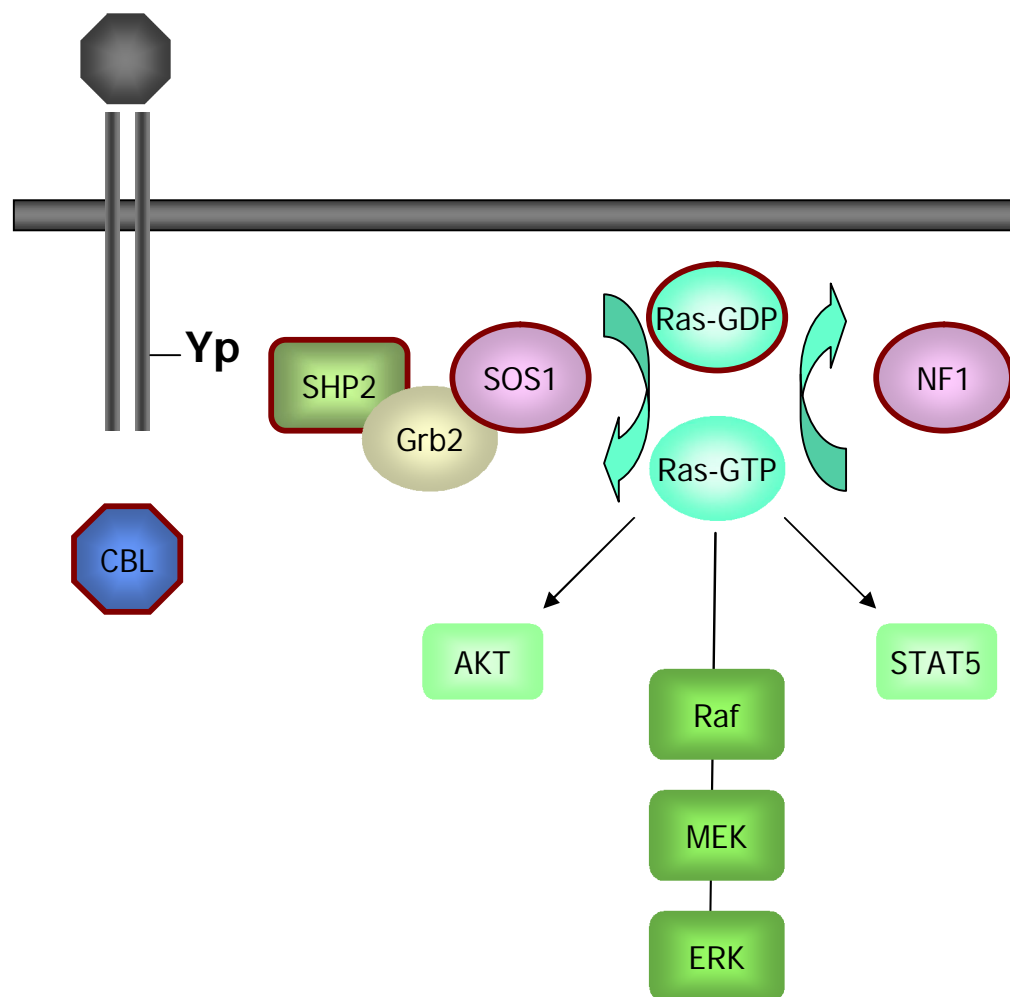
- Autosomal dominant (or spontaneous) disorder
- Facial dysmorphism, short stature, webbed neck, cardiac anomalies,
- Varying levels of impaired cognition,
- Self-resolving myeloproliferative disorder in infancy that resembles JMML

- **Germline mutations in *PTPN11*** (former *SHP2*) in 50% of cases
- Encodes a non-receptor protein tyrosine phosphatase (also *SHP2*)
- That connects tyrosine kinase receptors to Ras

- **Other Noonan** : germline mutations in
SOS1, a RAS-GEF (Guanine nucleotide-exchange factor)
KRAS



Gene mutations in JMML

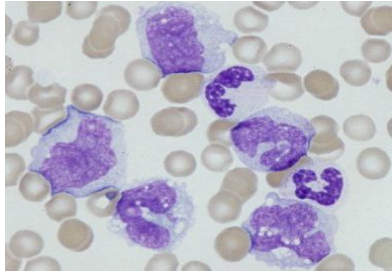


Mutually exclusive mutations in

<i>PTPN11</i>	35%
<i>NF1</i>	25%
<i>N/KRAS</i>	20%
<i>CBL</i>	10%

Other in *SOS1*, *FLT3*, *ASXL1*

No *TET2* mutation



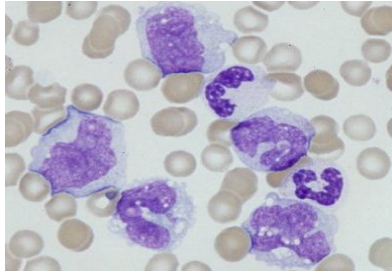
CMML

a disease of the elderly
defined by only one positive criteria

MPN / MDS in the WHO classification

Clonal disease of HSC with monocytosis

- Persistent monocytosis ($> 1 \text{ G/L}$)
- Lack of Ph1 or Bcr-Abl
- Blood and bone marrow blast cells $< 20\%$
- Cell dysplasia, at least one cell line



Molecular abnormalities identified in human CMML

1 – Cytogenetic abnormalities : 15-40%

2 – Uniparental disomy : ~50 %

3 – Mutations in

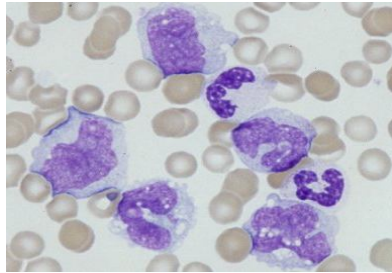
Epigenetic genes : TET2 ; ASXL1 ; AML1/RUNX1; IDH1/2; EZH2; UTX

Signalling : N/KRAS; CBL; FLT3-ITD, JAK2; NOTCH2 /NCST / MALM

Splicing : SRSF2; ZRSR2; J2AF35; SF3B1

4 – Deregulated expression of

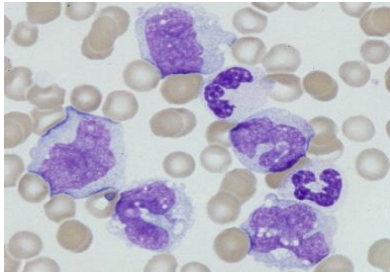
TIF1 γ , miRNA, CJUN, CFOS



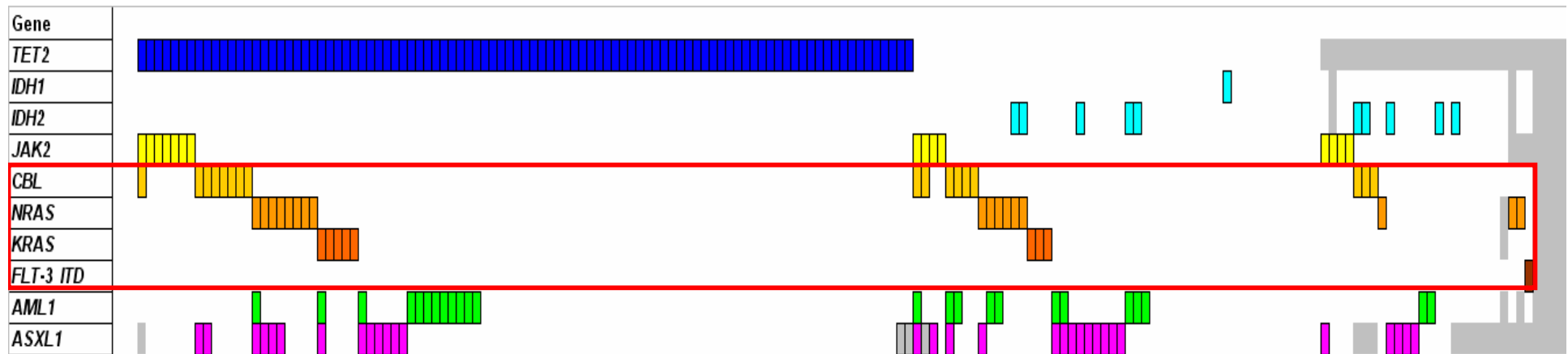
Molecular abnormalities identified in human CMML (GFM 175 patients)

Gene	Frequency
TET2	95/145 (65,5%)
IDH1	1/169 (0,6%)
IDH2	10/169 (5,9%)
JAK2_V617	15/168 (8,9%)
CBL	17/171 (9,9%)
NRAS	17/170 (10,0%)
KRAS	8/170 (4,7%)
FLT-3_ITD	1/171 (0,6%)
AML1	24/169 (14,2%)
ASXL1	31/154 (20,1%)
WT1_SNP	20/116 (17,2%)
CMML-2	28/171 (16,3%)
Abn K	19/124 (15,3%)

IDH/TET2 pathway: 72%
mutations



Molecular abnormalities identified in human CMML

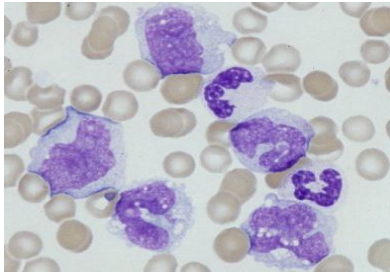


No specific mutation

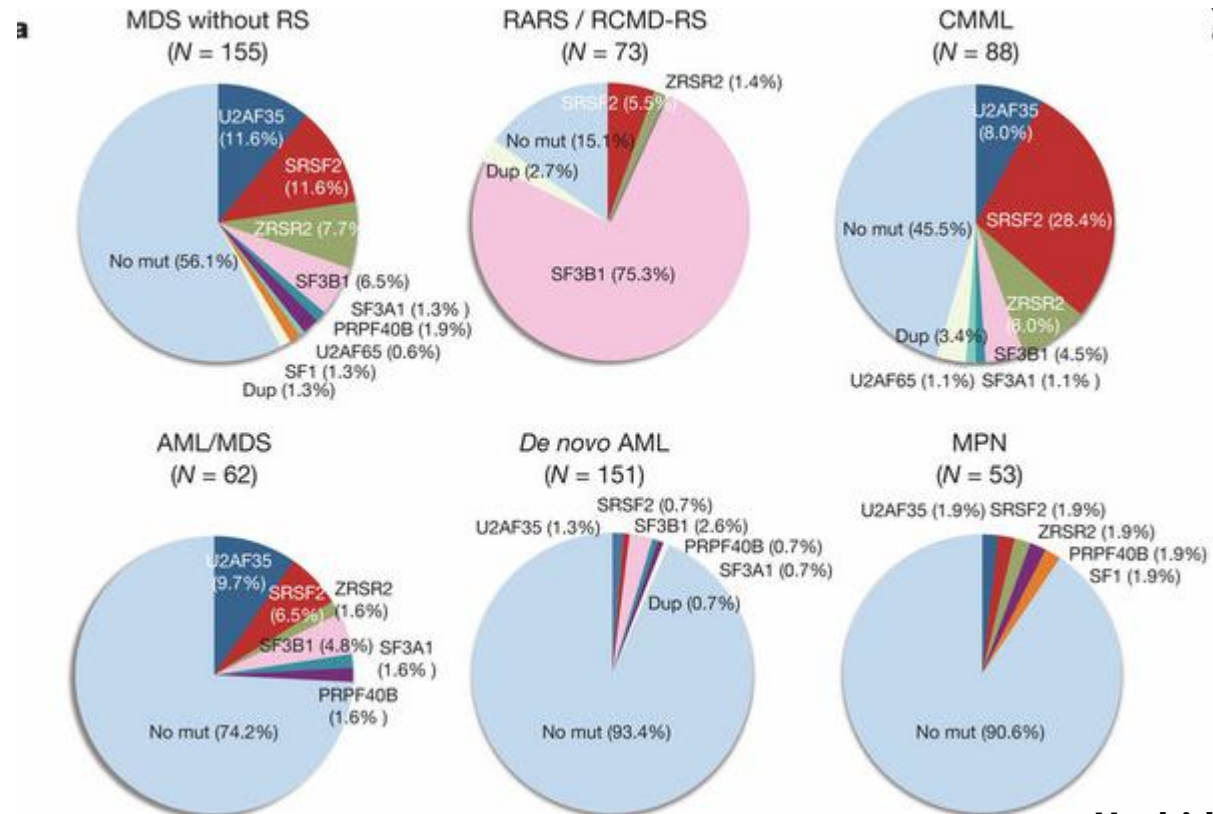
High frequency of TET2/IDH pathway mutations (> 70%)

Combinations of mutations in signaling, epigenetic, splicing

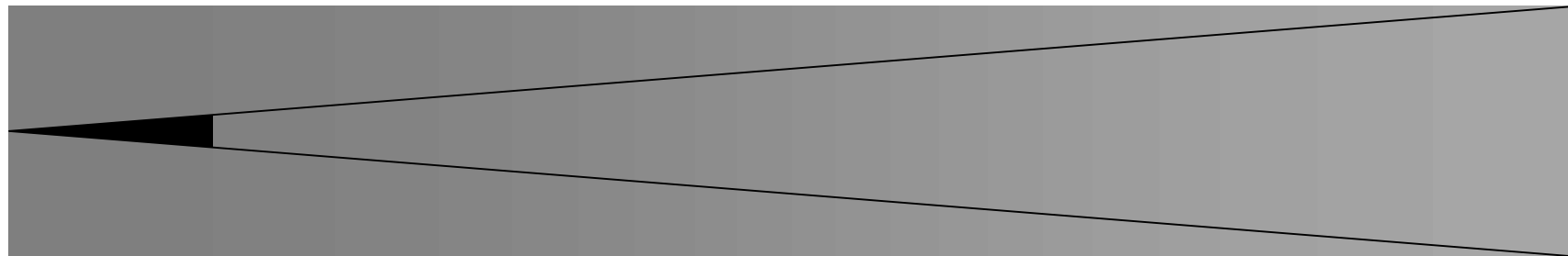
More mutations to identify (NGS)



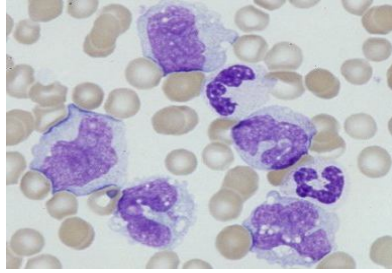
Molecular abnormalities identified in human CMML



Increasing complexity
***from* myeloproliferative neoplasms**
***to* myeloproliferative / myelodysplastic diseases**

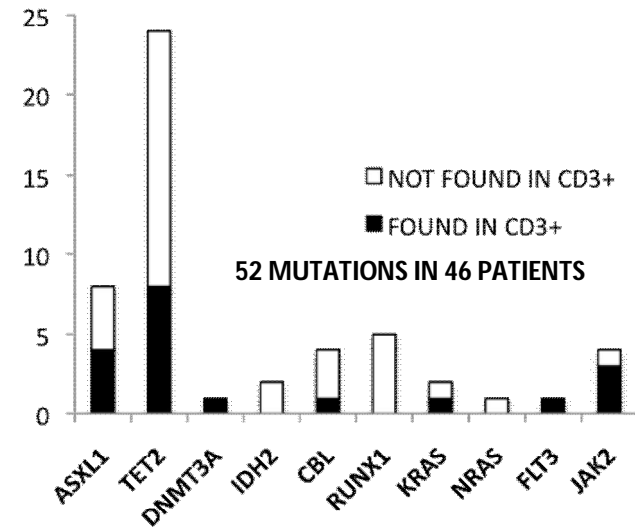
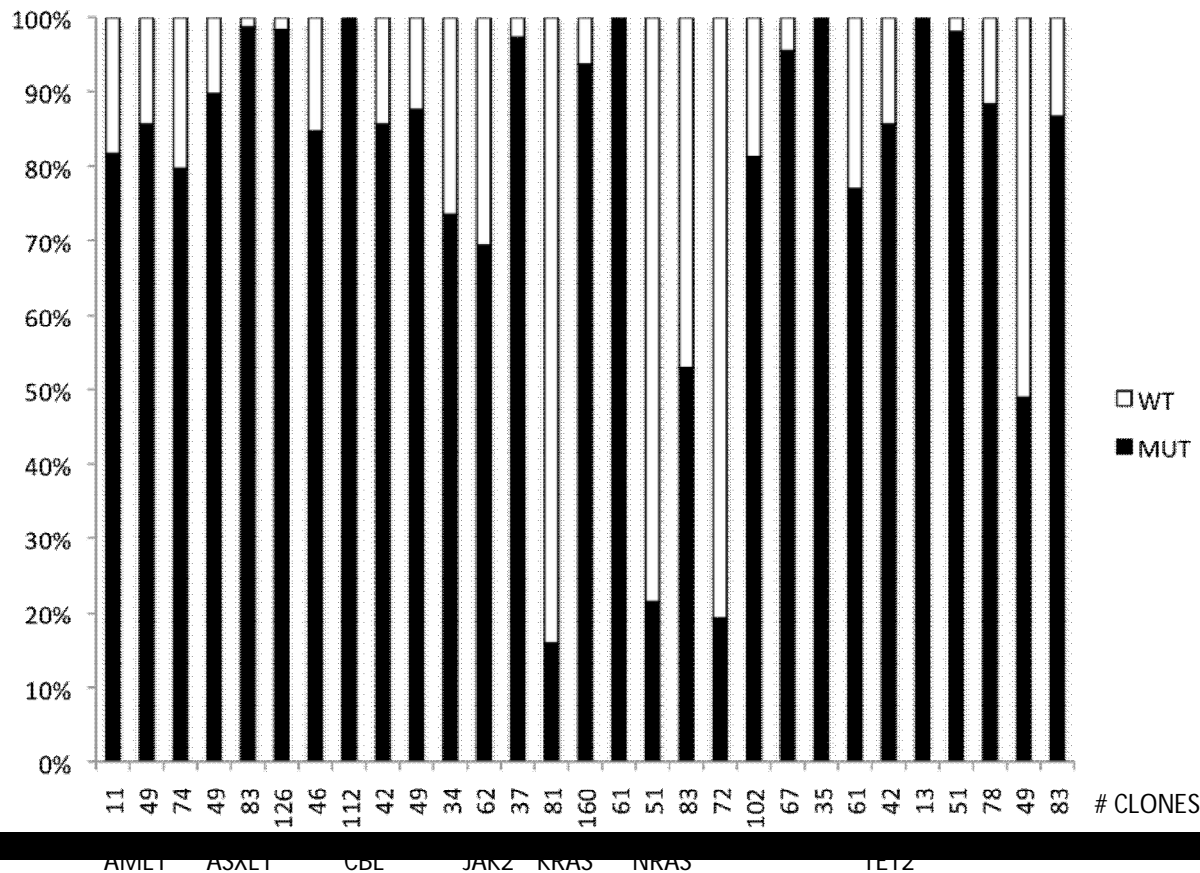


CML	TE	PV	MF	CMML
<i>BCR-ABL</i>	<i>JAK2* hZ</i>	<i>JAK2* HZ</i>	<i>JAK2*</i> <i>other</i>	<i>TET2* - IDH1/2*</i> <i>other</i>
	<hr style="width: 60%; margin: 0 auto;"/> <i>TET2 rare</i>			<i>JAK2 rare</i>

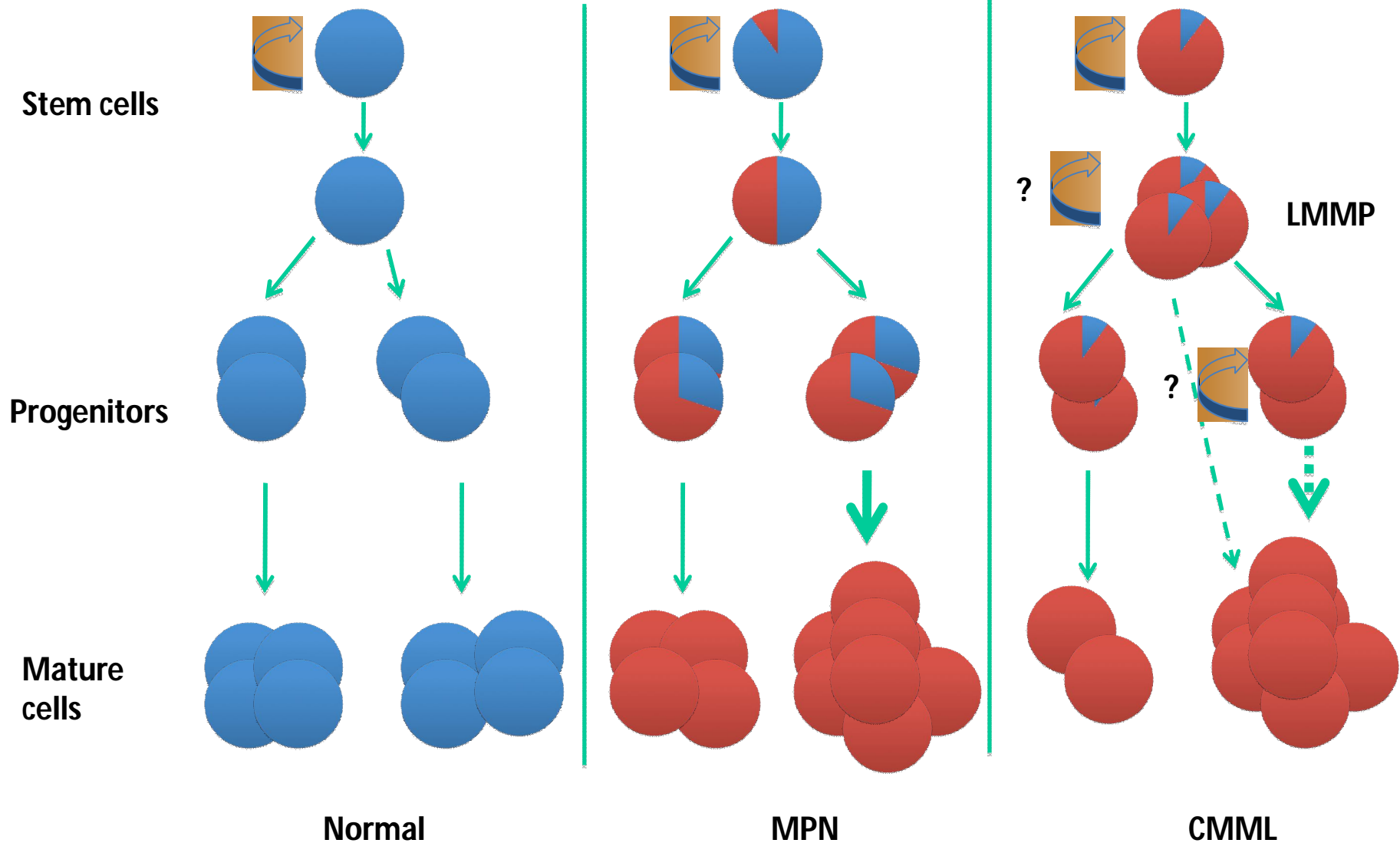


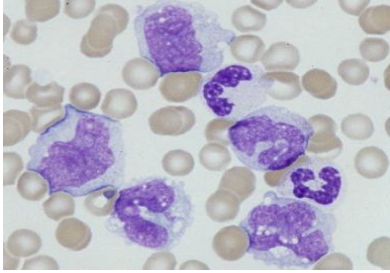
Clonal expansion occurs in the CD34⁺/CD38⁻ compartment

29 MUTATIONS IN 17 PATIENTS



CMML : clonal amplification at the stem cell level

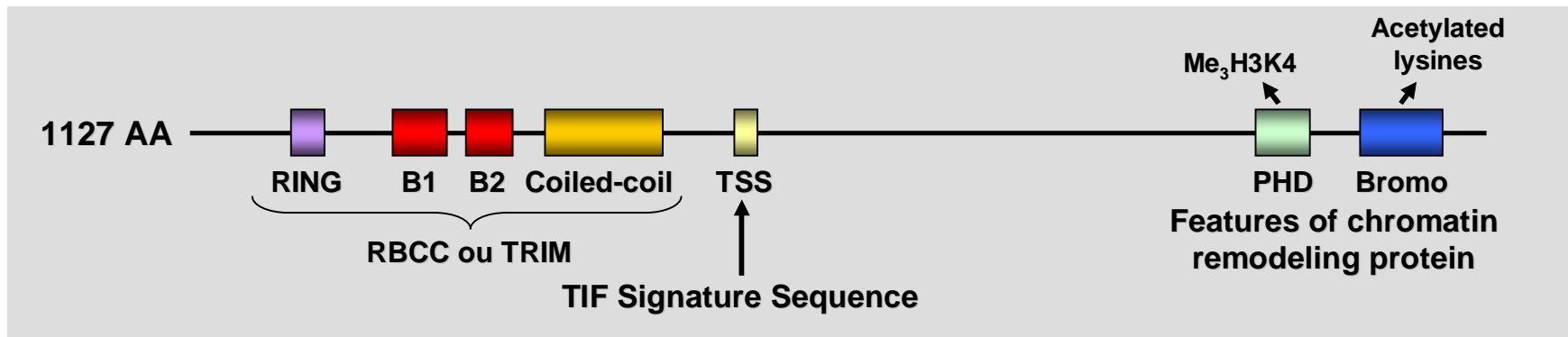




TIF1 γ (Transcription Intermediary Factor 1 γ)

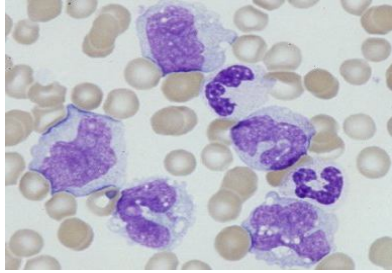
TIF1 γ involved in zebrafish erythropoiesis (severe anemia in « moonshine » mutant)
 human erythropoiesis (*ex vivo* differentiation of CD34+ cells)

TIF1 γ knock-out embryonic lethal in mice



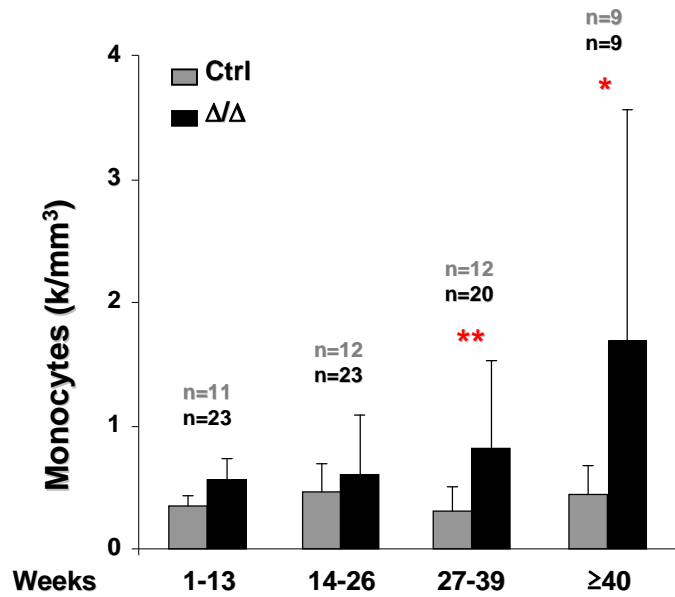
TIF1 γ

- belongs to a group of four proteins (TIF1 α , β , γ , δ)
- modulates the TGF- β signaling pathway
- is a transcriptional co-regulator (TAL1/PU1)

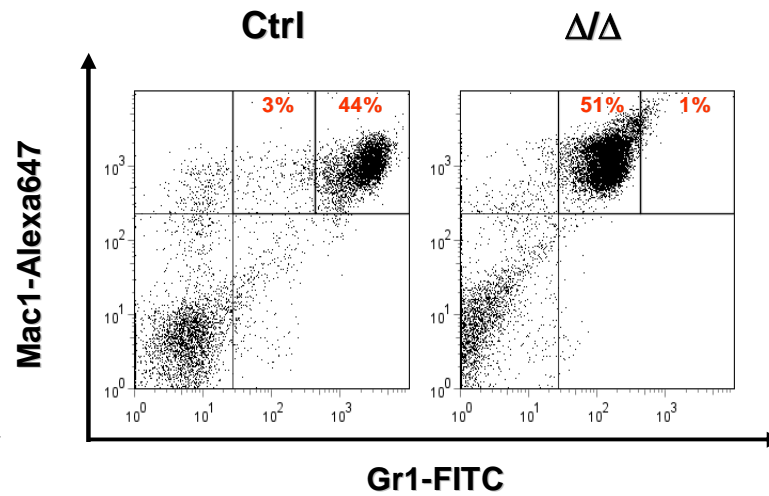


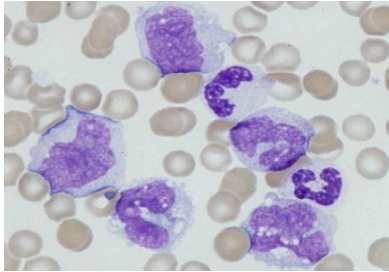
Ageing *Tif1γ*^{Δ/Δ} mice develop a CMML-like disease

Peripheral blood

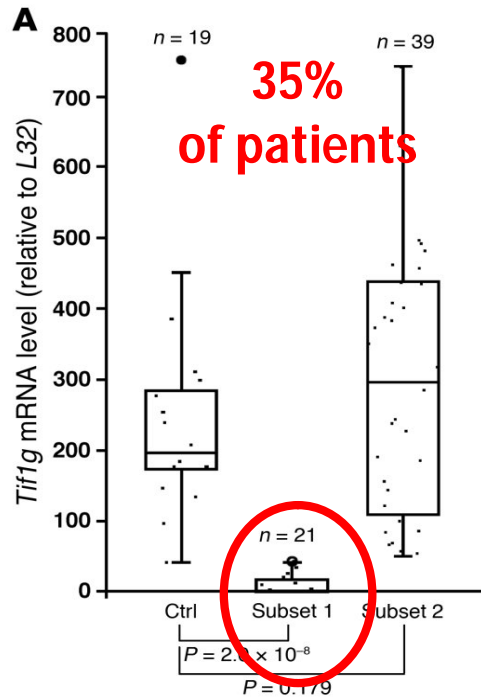


Spleen

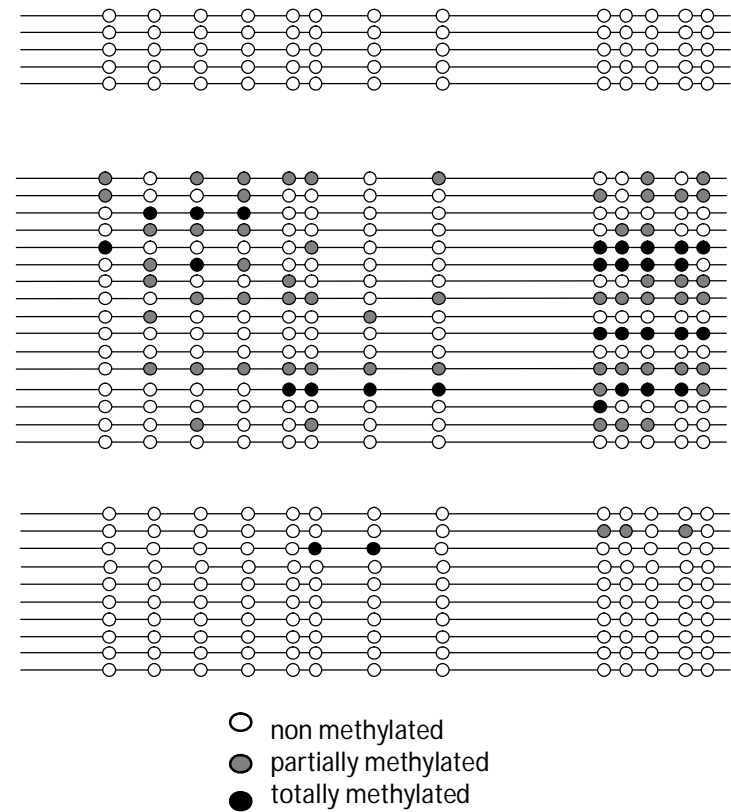


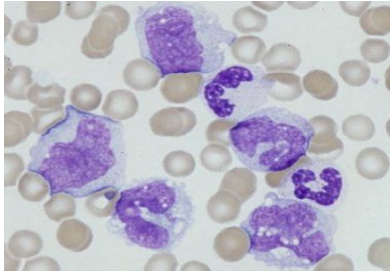


TIF1 γ gene promoter is methylated in 35-50% of CMML

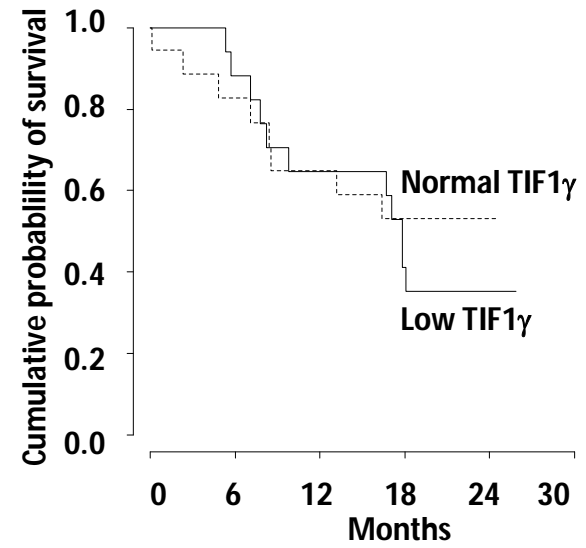
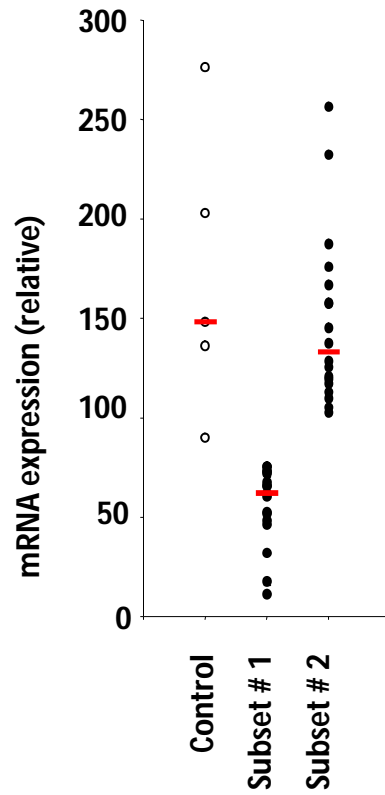


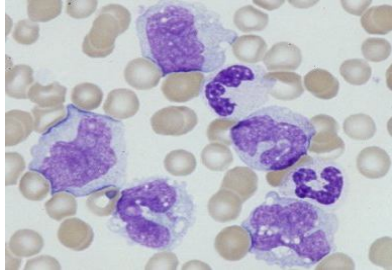
-139GGGAGGACGTCCTCCGTGCGTACGTGCGCGTGCCGCAACCGCCCTCCTTCAAACGCGGACGCGG **unconverted**
 -139GGGAGGAVGT TYGTGYGTA YGTGYGYGTGT YGTAAT YGTTT TT TTTTAAA YGYGYGA YGYG



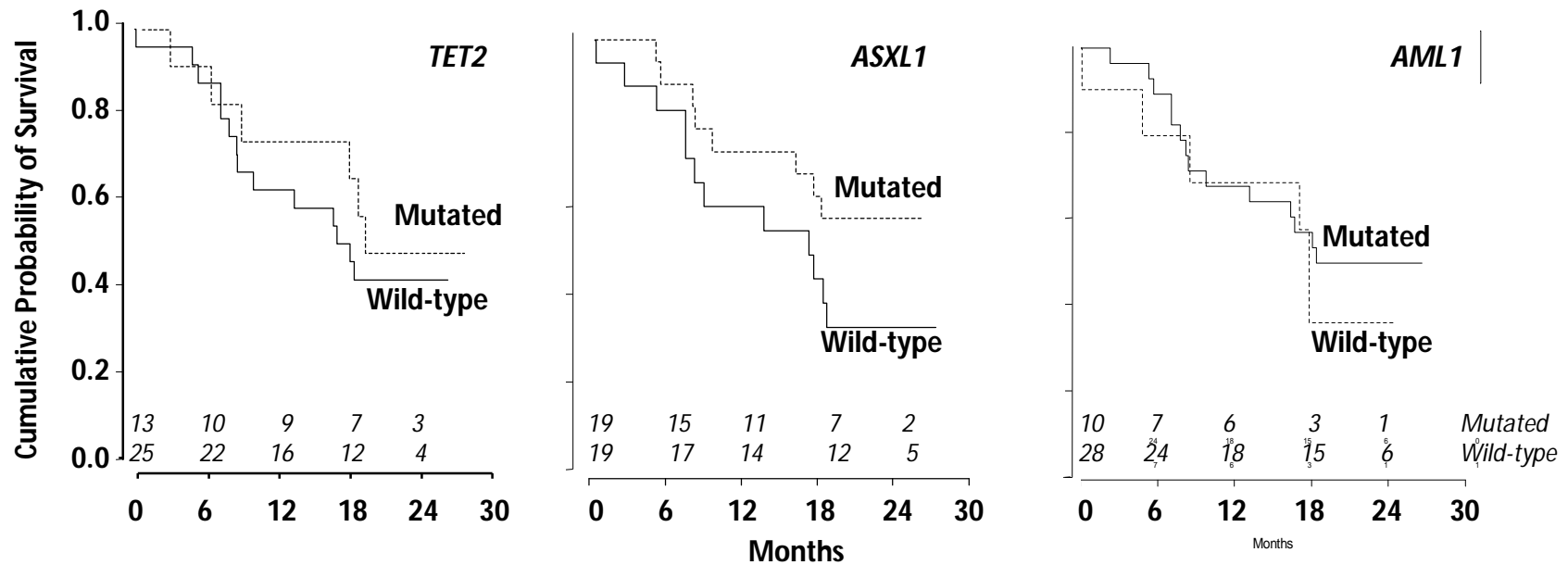


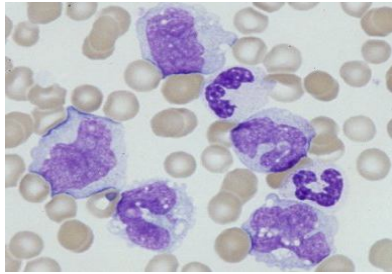
TIF1 γ expression level
does not predict decitabine efficacy



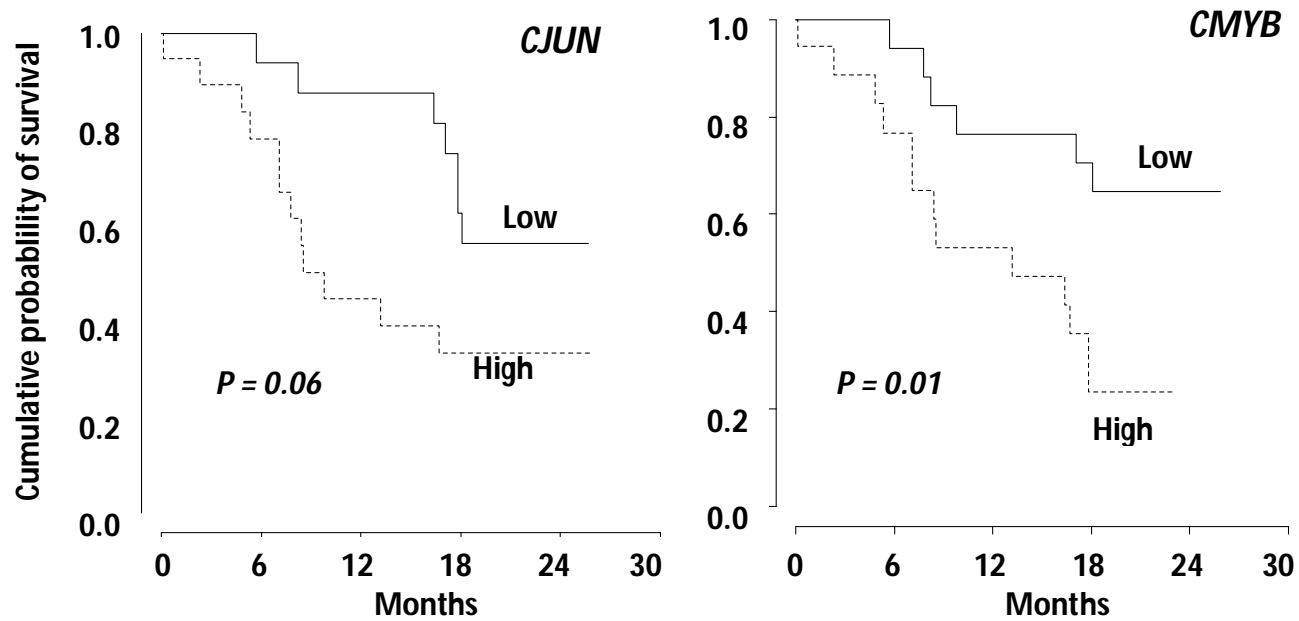


Gene mutations in *TET2*, *ASXL1*, *AML1* do not affect survival

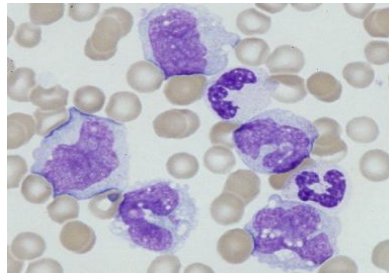




CJUN level correlates with response
CMYB level correlates with survival

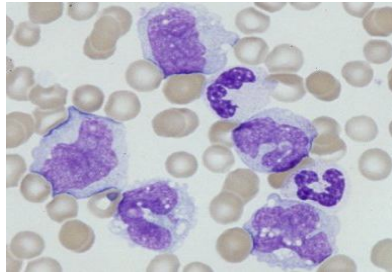


Chronic myelomonocytic leukemia



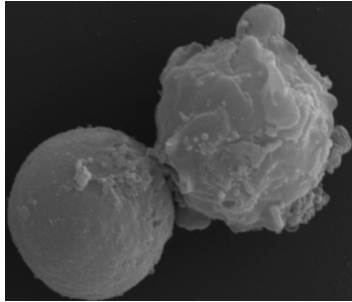
Persistent monocytosis ($> 1000/\mu\text{L}$)

Why do these patients die?

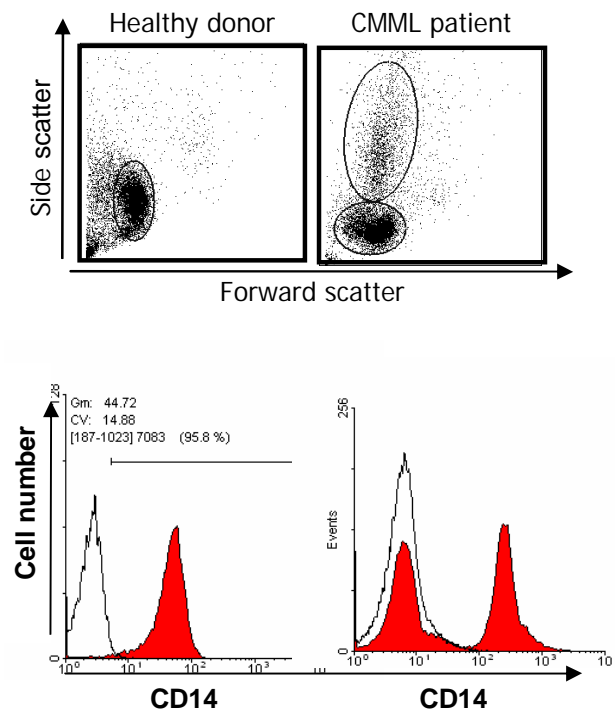


The best recognized prognostic factor is blast cell count

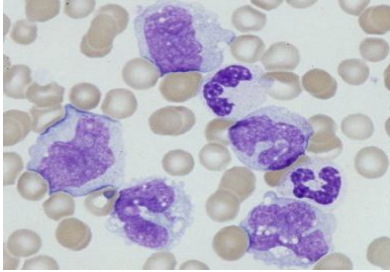
Diagnostic feature	CMML1	CMML2
Peripheral blood monocytes	> 1 G/L	> 1 G/L
Left shift and myelocytes	< 10%	> 10%
Peripheral blood blasts	< 5%	5-19%
Bone marrow blasts	< 10%	10-19%
Dysplasia	0, one, more	0, one, more
t(9;22) or BCR-ABL	No	No
PDGFRA/B	No	No



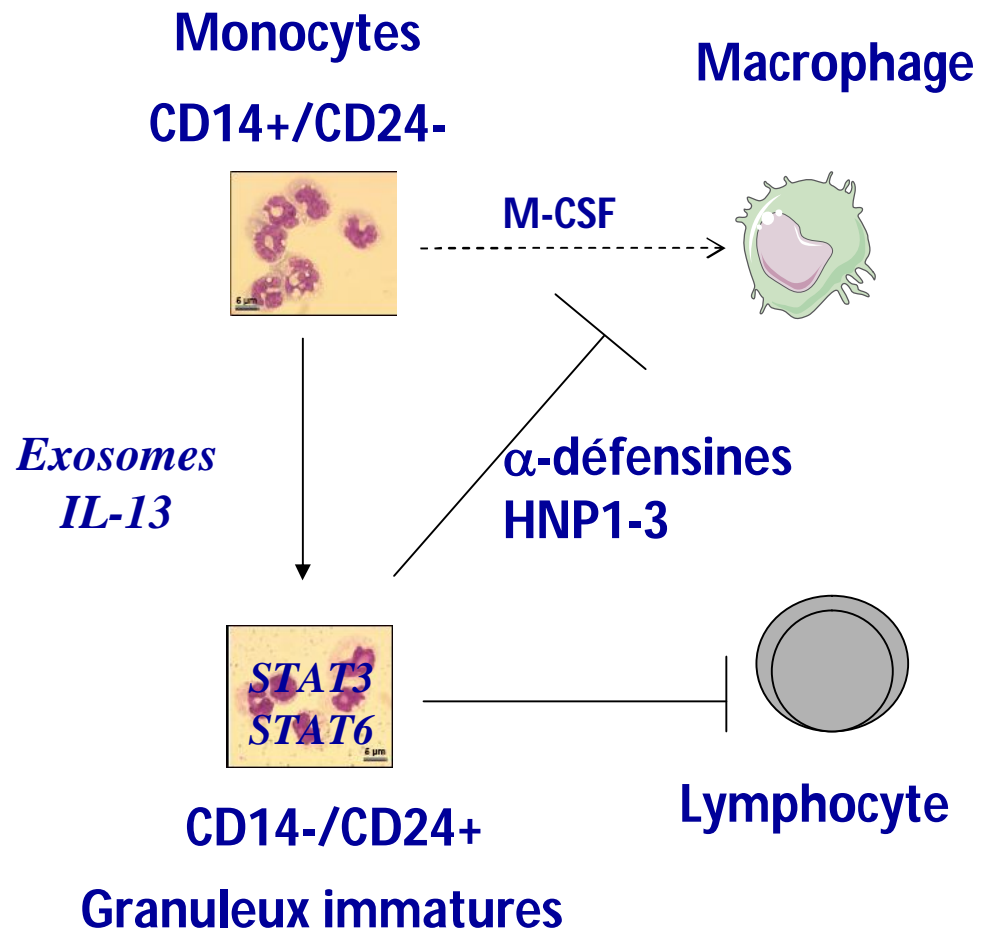
Cells counted as « monocytes »
in the peripheral blood
include 2 populations

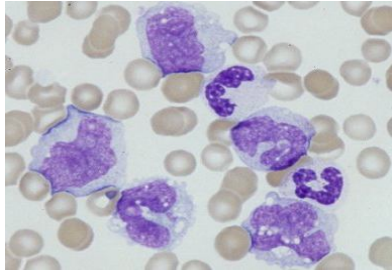


UPN	<i>TET2</i> mutation(s) in the two cell populations
47	None
54	R1214W; L1420FS
71	Q652X
72	Mutation splice donor site exon 6 + L1855FS
79	R544X ; E1492FS
81	None
85	E320X ; R1359H
96	K450X ; I1163FS
100	H1219Y
136	F1104FS , D1384V
153	None
107	T1883FS
171	None
174	Q1501FS ; G1861R
180	Q591X
194	None
207	L615FS ; K1422FS
211	K1005FS ; S1324FS
216	S354FS ; L615FS



The immunosuppressive properties of the CMML immature granulocytes





Take home messages

- 1 – JMML / CMML are distinct diseases
- 2 – JMML is a RAS pathway disease with hypersensitivity to GM-CSF
- 3 – CMML is a TET2 disease with many different mutations
- 4 – Epigenetic changes in addition to mutations (TIF1 γ , miRNA)
- 5 – Immunosuppressive dysplastic granulocytes

To further explore the disease pathogenesis : eric.solary@igr.fr