9° WORKSHOP IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Bologna, Aula "G. Prodi", 19-20 maggio 2025

Tecnologie per l'analisi single-cell: dalla genomica alla multiomica

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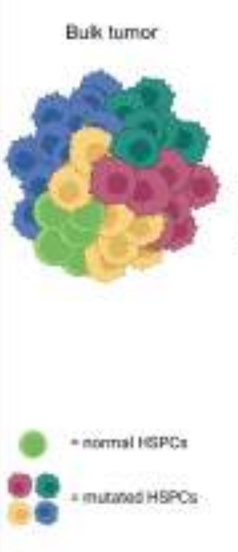
Disclosures of Sebastiano Rontauroli

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
No disclosures							

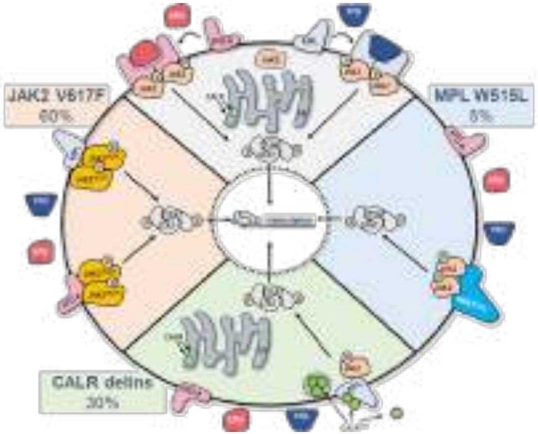
The power of single cell analysis



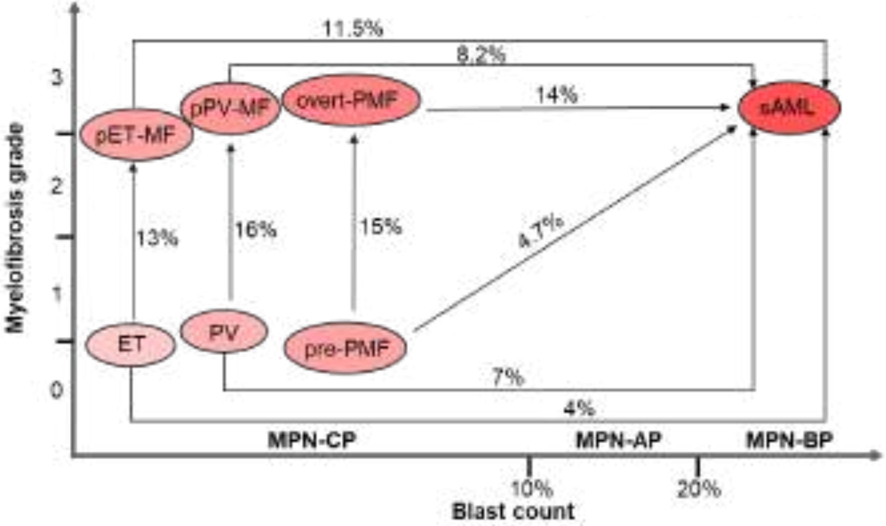
		VAF
●	**Mutation 1**	**25%**
■	**Mutation 2**	**65%**
▲	**Mutation 3**	**15%**
★	**Mutation 4**	**30%**

Myeloproliferative neoplasms (MPN) molecular pathogenesis and disease evolution

Grinfeld J. et al. New Engl J Med. 2018

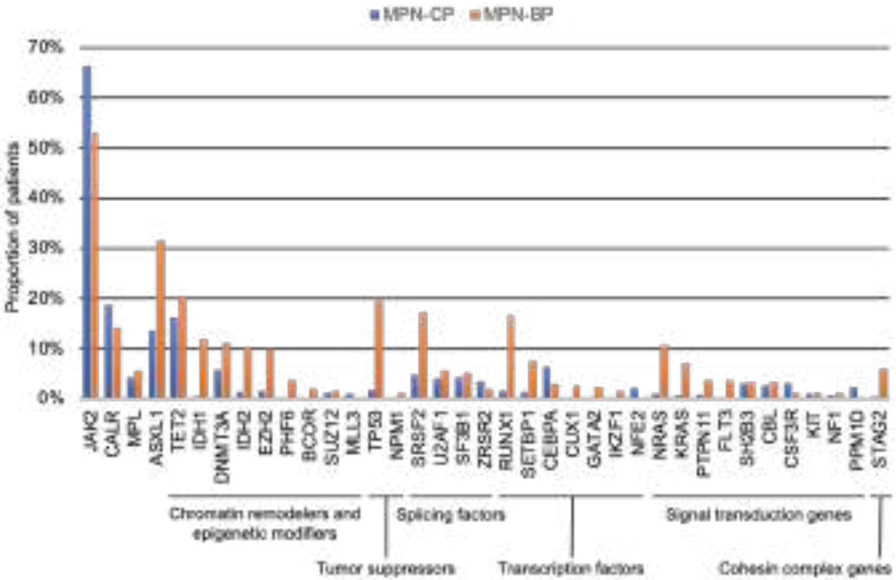


Adapted from Schieber, M. et al., Blood Cancer J. 2019

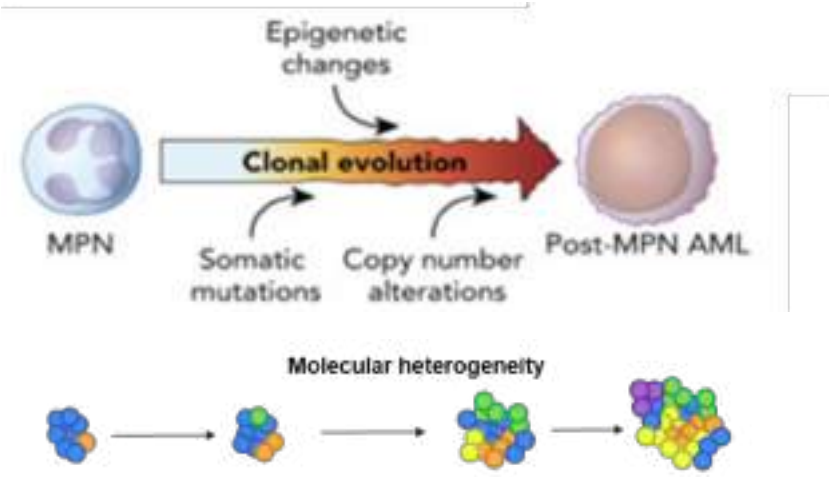


Rontauroli, S. et al., IJMS. 2022

MPNs disease evolution

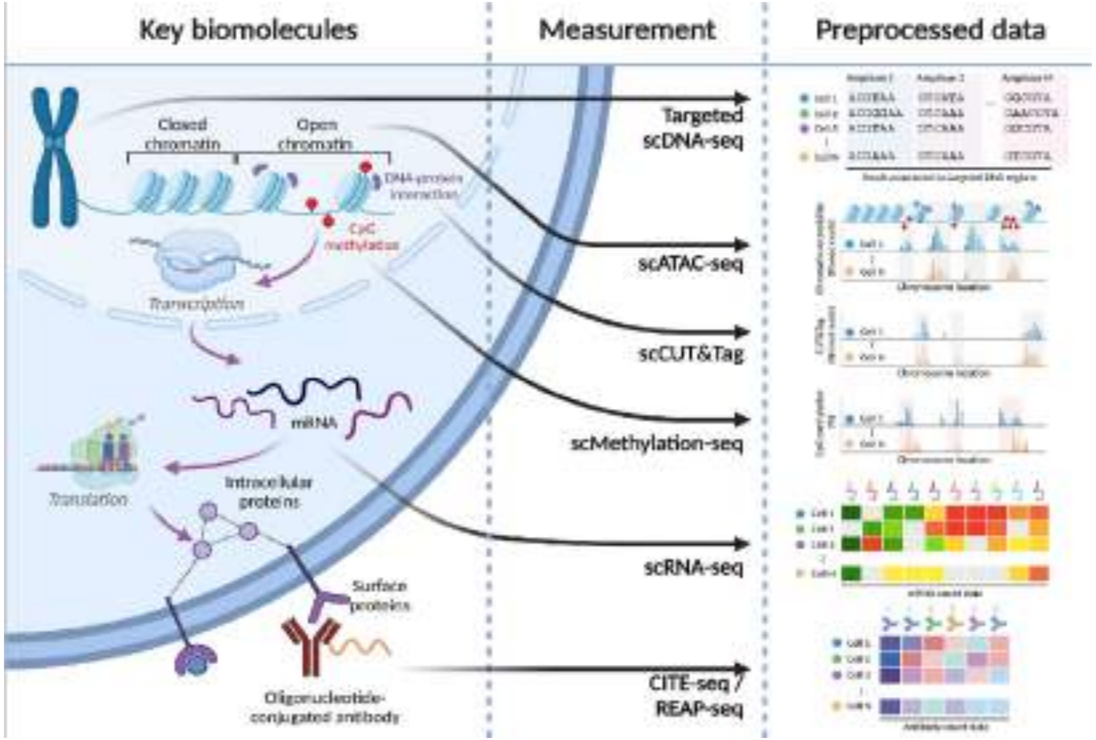


Rontauroli, S. et al., IJMS. 2022

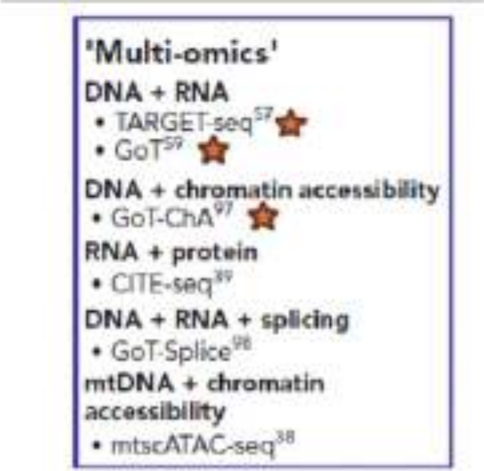


adapted from Dunbar, J. et al., Blood. 2020

Single cell omics opportunities



De Jonghe, J. et al., Cell genomics. 2024



'Multi-omics'

DNA + RNA
- TARGET-seq[57] ★
- GoT[59] ★

DNA + chromatin accessibility
- GoT-ChA[97] ★

RNA + protein
- CITE-seq[39]

DNA + RNA + splicing
- GoT-Splice[98]

mtDNA + chromatin accessibility
- mtscATAC-seq[38]

O'Sullivan, JM. et al., Blood. 2023

The basics of single cell isolation and analysis approaches

Droplet based methods

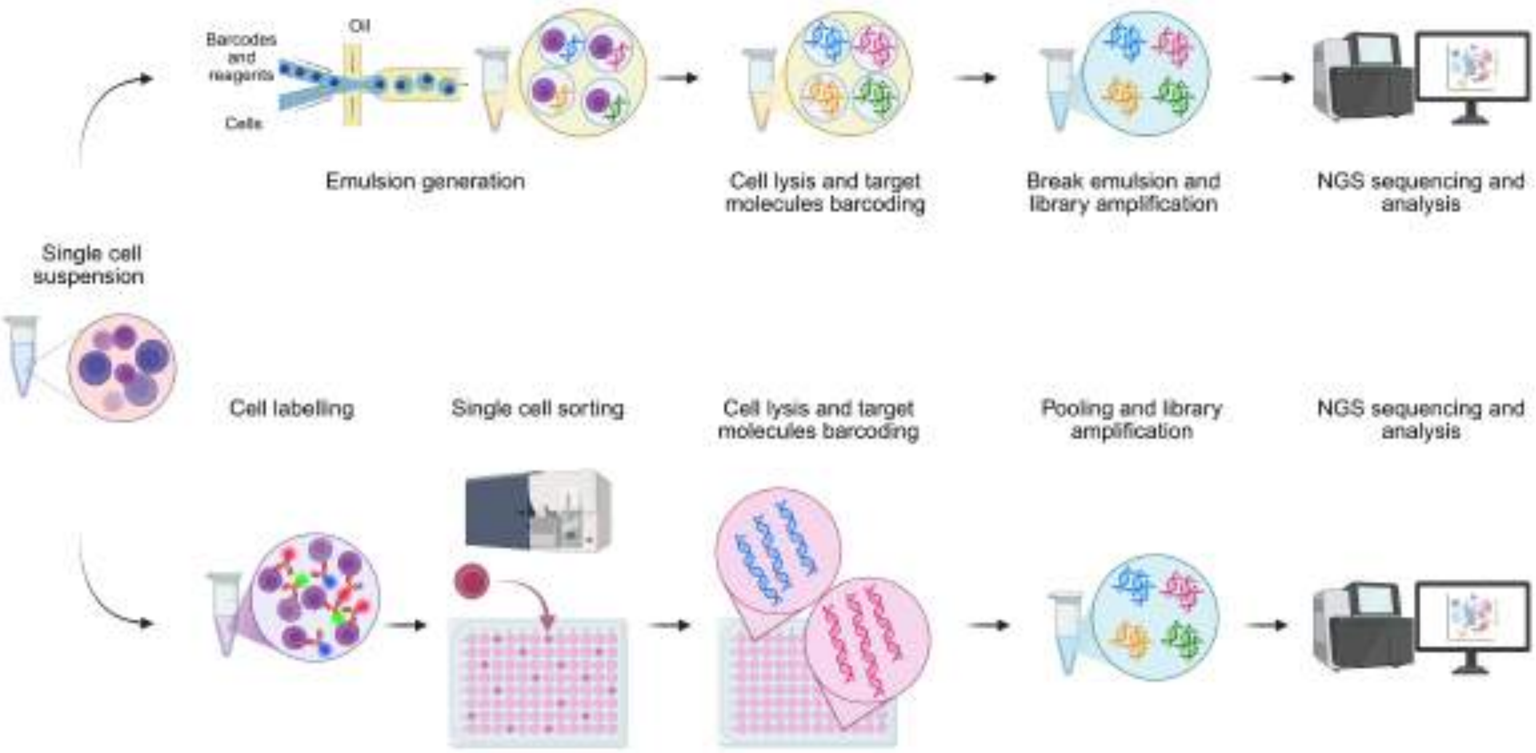
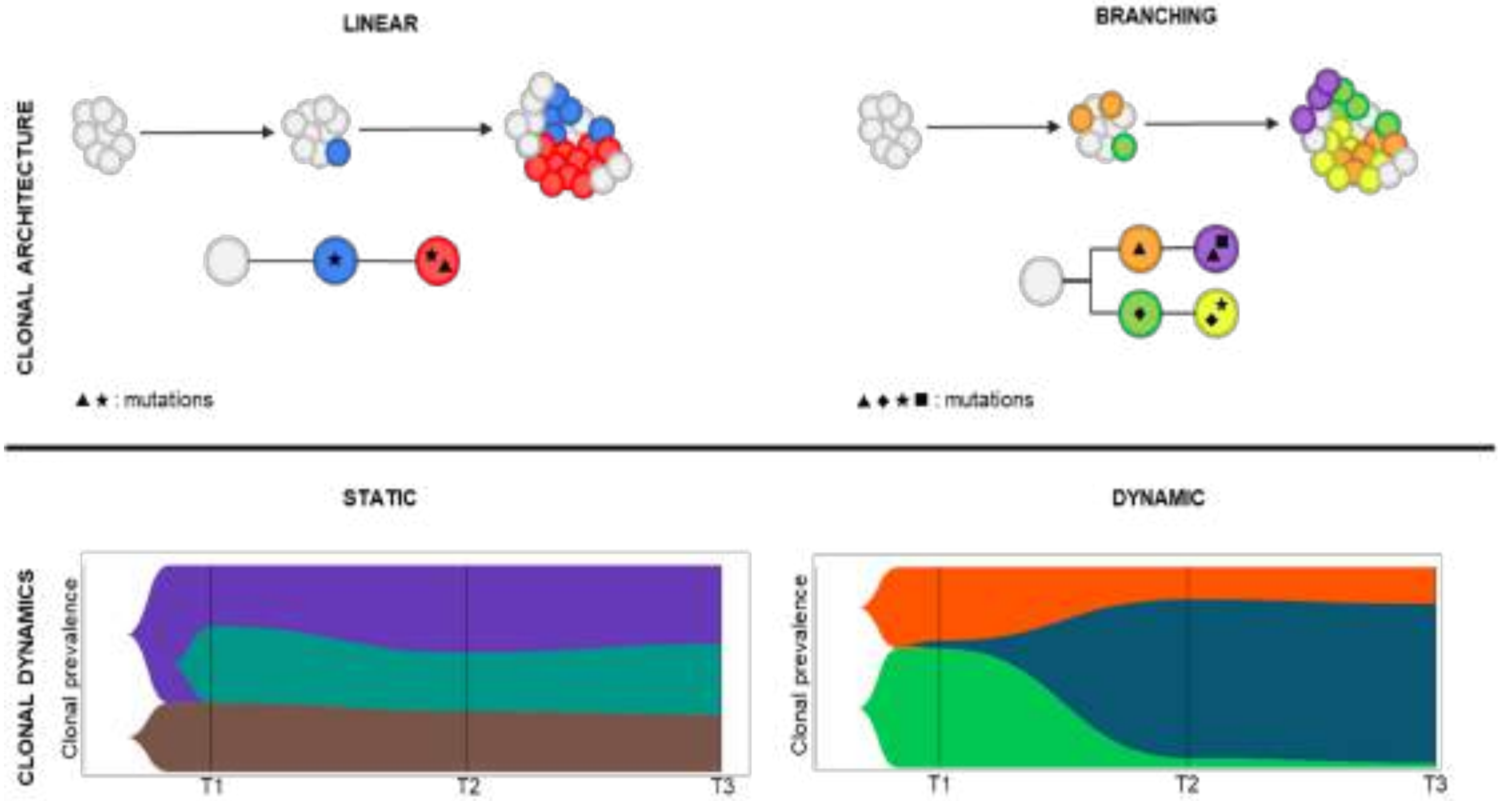


Plate based methods

Describing clonal trajectories through single cell genomics



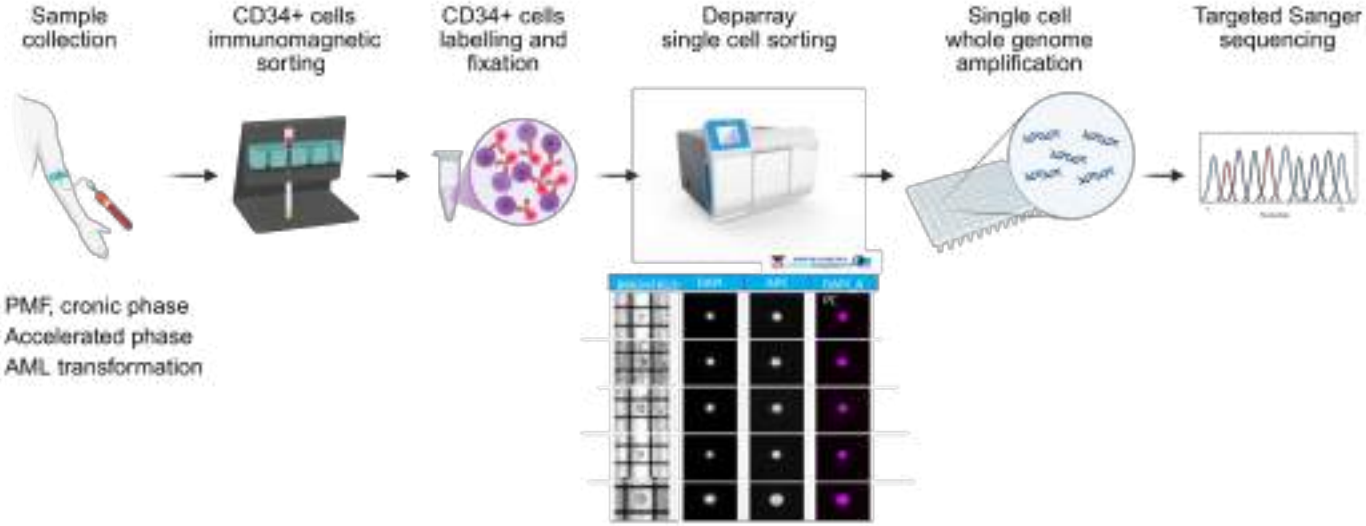
Rontauroli, S. et al., IJMS. 2022

Clonal hierarchy reconstruction in PMF leukemic transformation by single cells genomics

ARTICLE OPEN

Mutated clones driving leukemic transformation are already detectable at the single-cell level in CD34-positive cells in the chronic phase of primary myelofibrosis

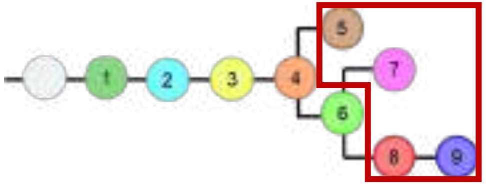
Sandra Parenti[1,7], Sebastiano Rontauroli[1,7], Chiara Carretta[1,7], Selene Mallia[1,7], Elena Genovese[1], Chiara Chiereghin[2], Clelia Peano[2,3], Lara Tavernari[1], Elisa Bianchi[1], Sebastian Fantini[1], Stefano Sartini[1], Oriana Romano[1], Silvio Bicciato[1], Enrico Tagliafico[4,5], Matteo Della Porta[2,6,8] and Rossella Manfredini[1,8]

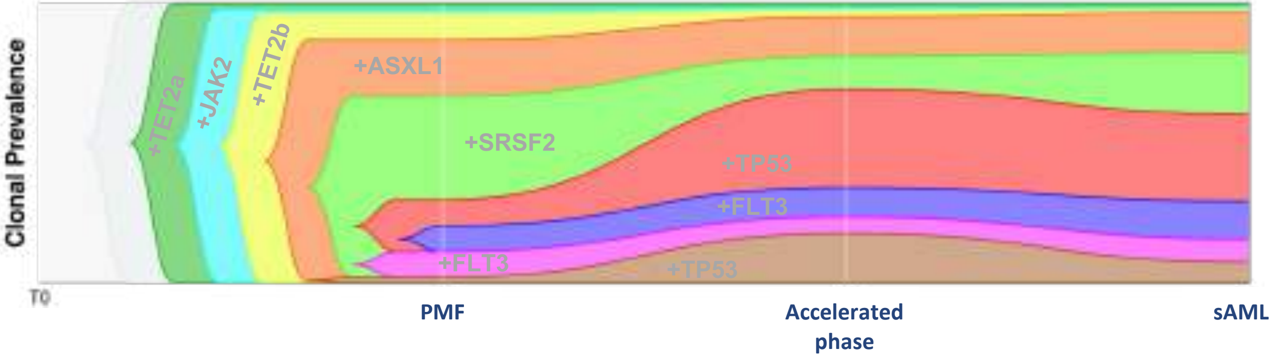


Time 1: PMF, cronic phase
Time 2: Accelerated phase
Time 3: AML transformation

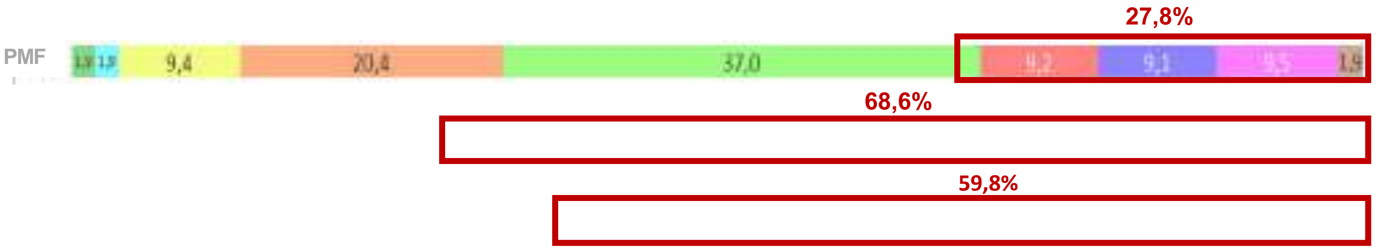
Clones harboring TP53 and FLT3 mutations drive PMF transformation and are already present in chronic phase

Clonal phylogeny



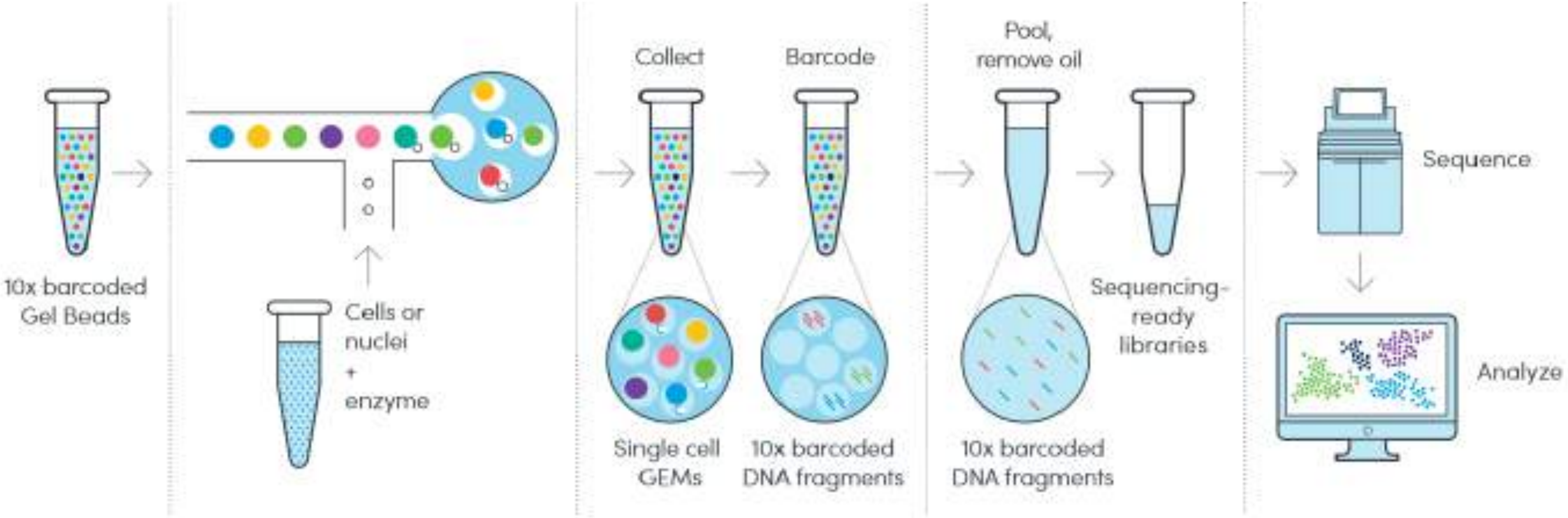


Pathogenic Variants	PMF NGS (VAF %)
TET2 (a) p.L1248P	48.1
JAK2 p.V617F	75.8
TET2 (b) p.G1137Pfs*5	37.7
ASXL1 p.G646Wfs*12	43.6
SRSF2 p.P95H	46.1
TP53 p.C238S	5.7
FLT3 TKD (p.D835Y)	**ND**

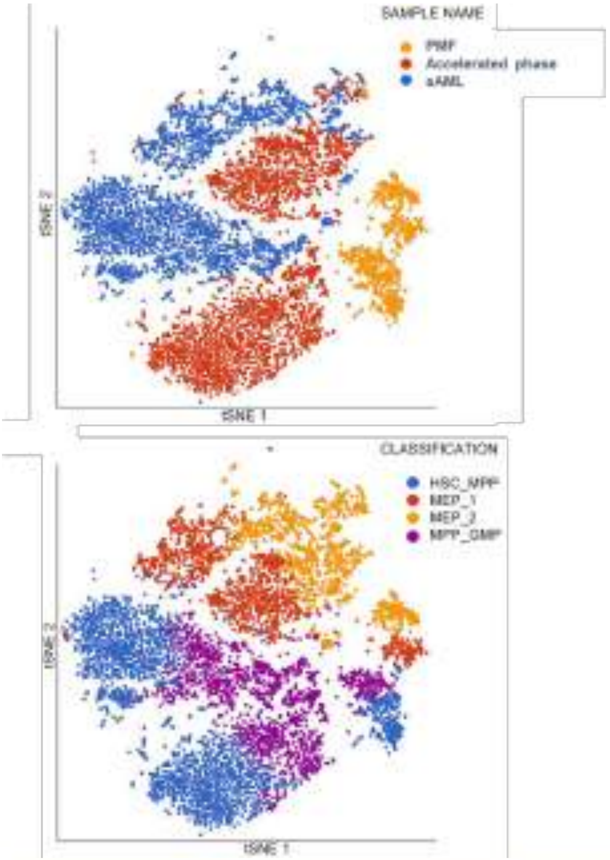


S.Parenti et al. Npj Precision Oncology, 2021

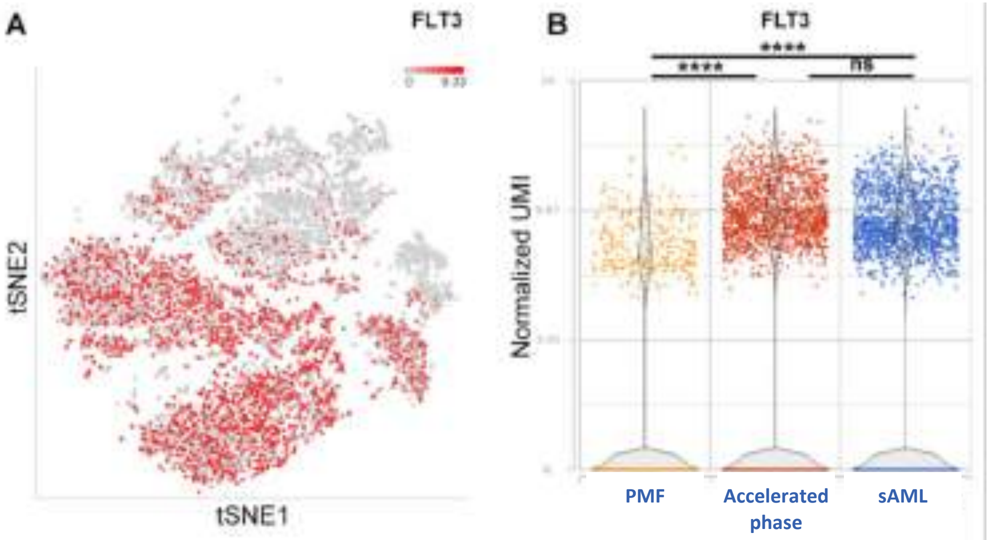
Single cell transcriptomics workflow



FLT3 expressing cells are expanded in sAML



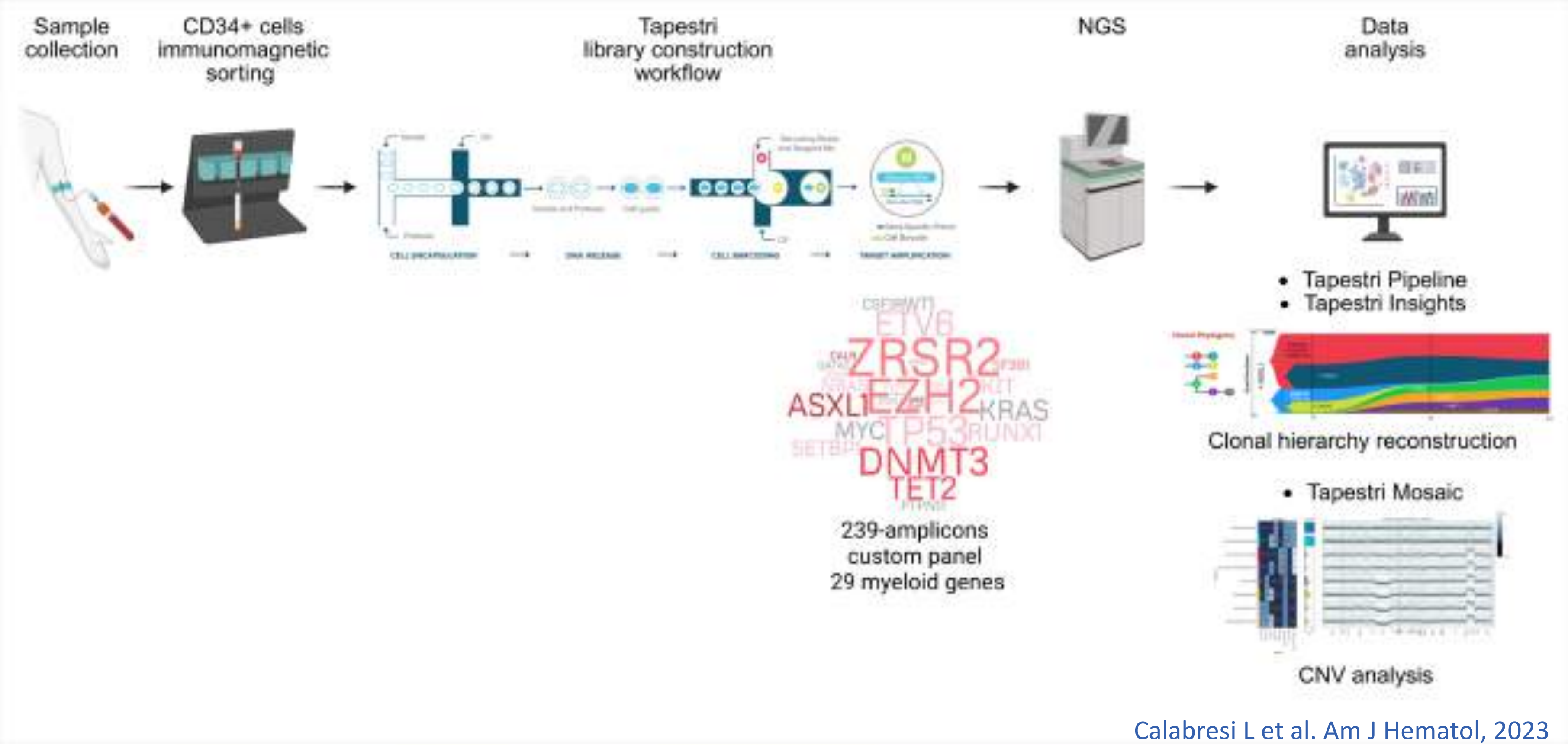
FLT3 expression



PMF	Accelerated phase	sAML
280/972 **28.8%**	1383/3296 **41.9%**	1402/3285 **42.7%**

S.Parenti et al. Npj Precision Oncology, 2021

Droplet based single cell genomics



Calabresi L et al. Am J Hematol, 2023

Single cell genomics allows the precocious identification of leukemic clones

Clonal dynamics and copy number variants by single-cell analysis in leukemic evolution of myeloproliferative neoplasms

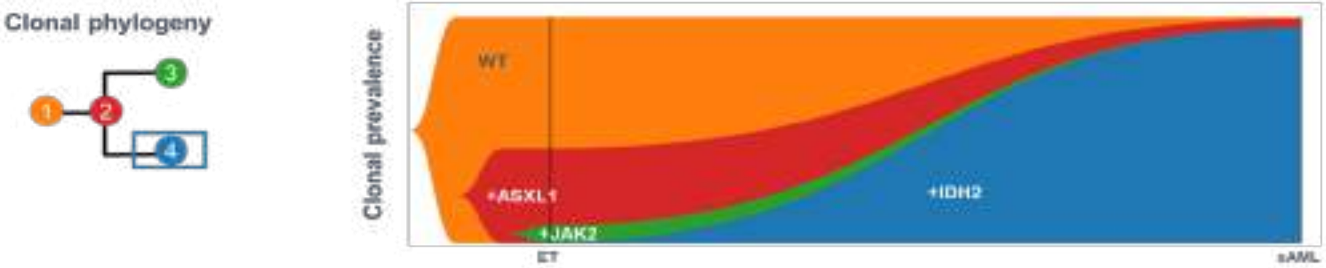
Laura Calabresi, Chiara Carretta, Simone Romagnoli, Giada Rotunno, Sandra Parenti, Matteo Bertesi, Niccolò Bartalucci, Sebastiano Rontauroli, Chiara Chiereghin, Sara Castellano ... See all authors

First published: 03 July 2023 | https://doi.org/10.1002/ajh.27013 | Citations: 2

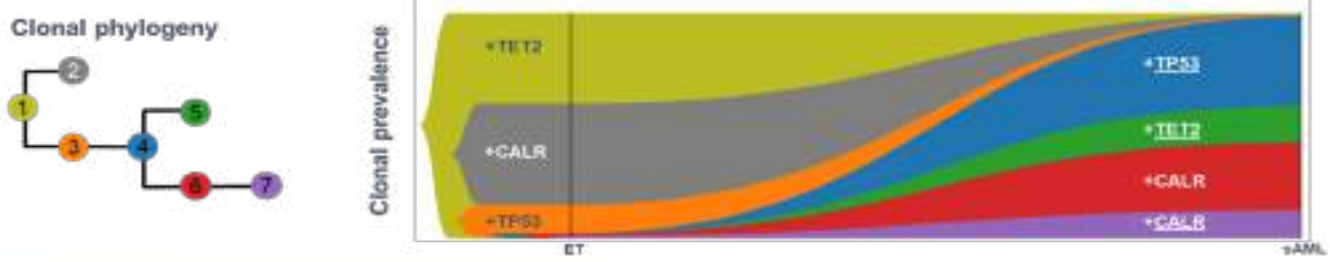
American Journal of Hematology AJH

RESEARCH ARTICLE | Open Access

Patient #1

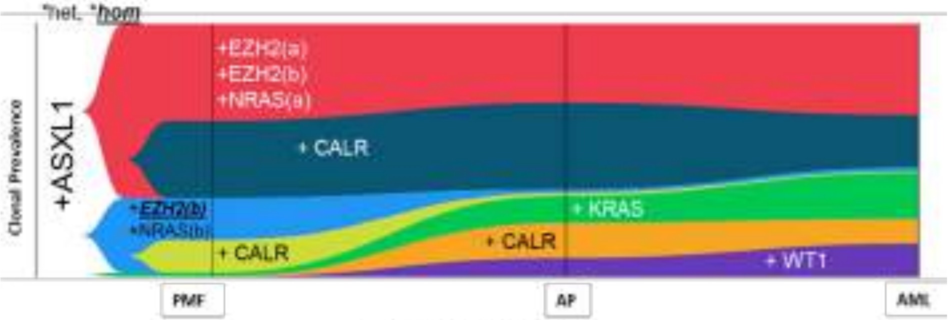


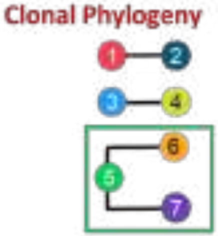
Patient #2



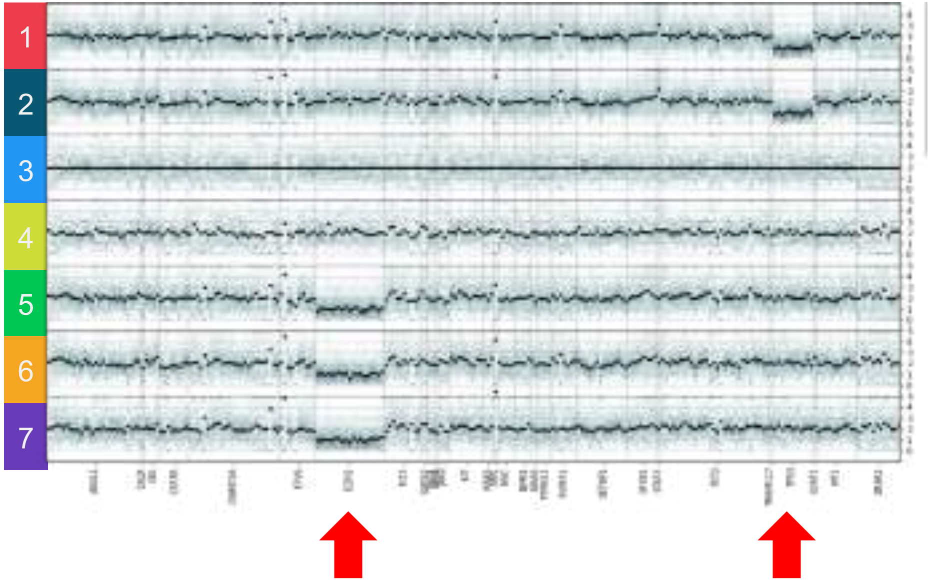
Leukemic clones differ from those driving chronic phase in terms of gene mutations and copy number alterations.

Patient #3





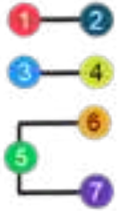
CNV analysis

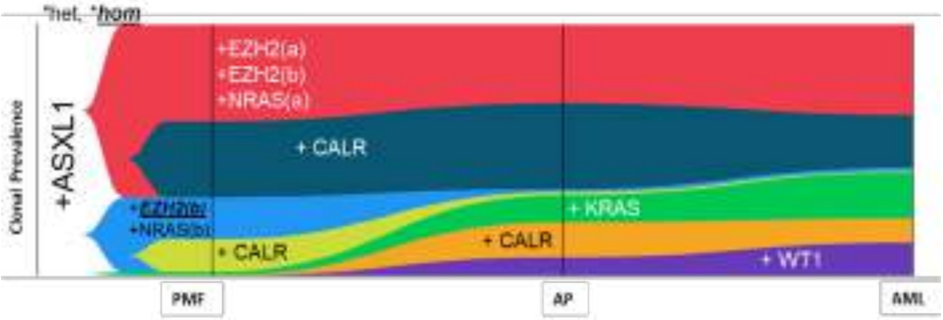


Calabresi L et al. Am J Hematol, 2023

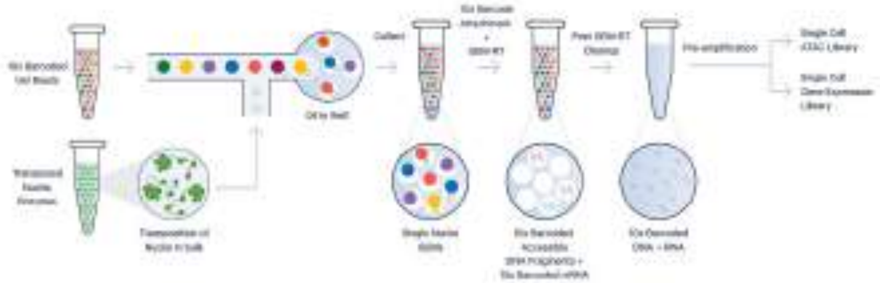
Acquisition of epigenetic modifier gene mutations before MPN driver variant impact disease evolution

Clonal phylogeny



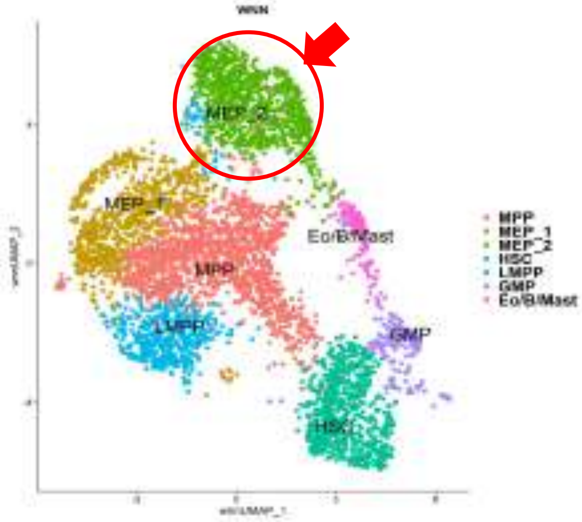


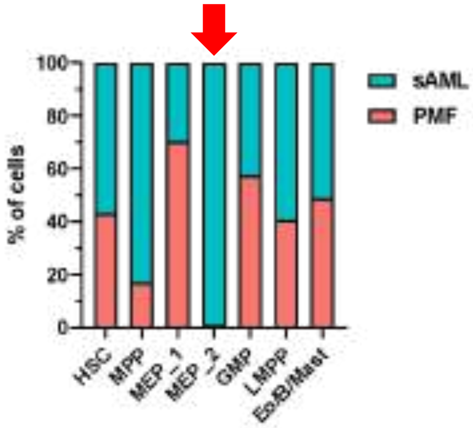
Single cell transcriptomics and assay for transposase accessible chromatin (ATAC) seq:
10X Genomics™ platform workflow



Calabresi L et al. Am J Hematol, 2023

Chronic phase and leukemic phase cells are differentially distributed in hematopoietic stem and progenitor cell clusters

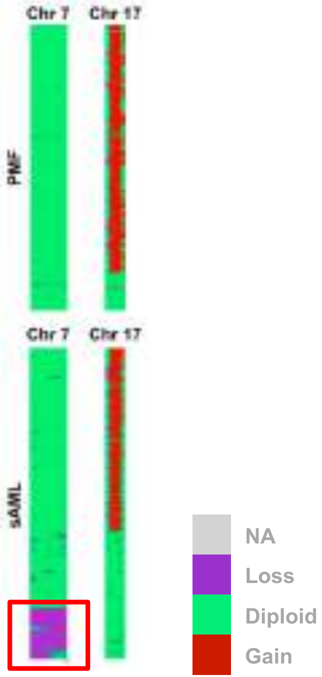




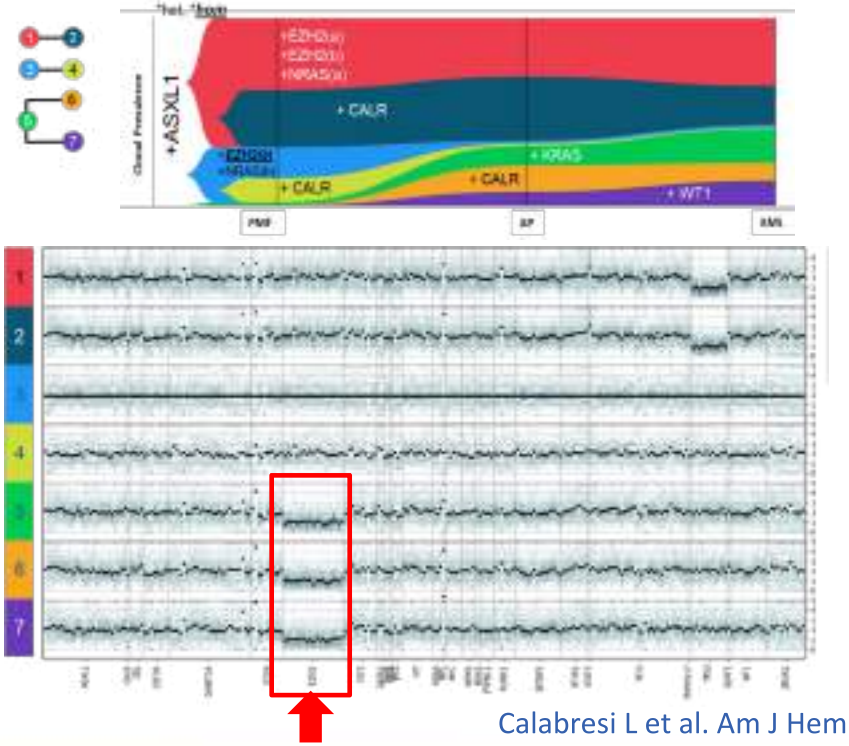
Calabresi L et al. Am J Hematol, 2023

CNV assessment from scATAC-seq data recapitulates single cell genomics findings

Karyogram



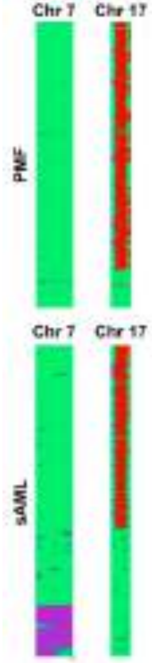
Sc genomics



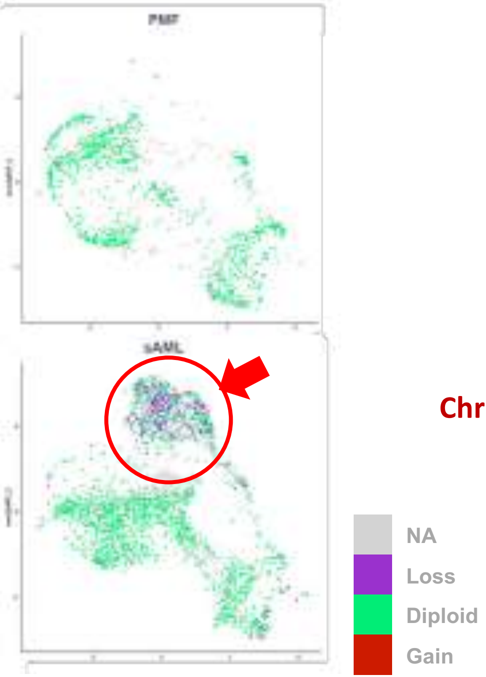
Calabresi L et al. Am J Hematol, 2023

Leukemic cells display EZH2 deletion leading to its reduced function

Karyogram

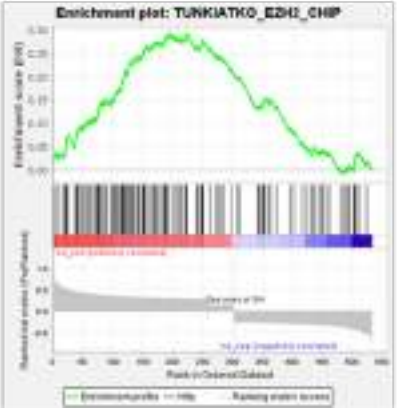


EZH2 Ploidy



GSEA

EZH2 targets accessibility



NES 2,4030285
FDR 0,014763195

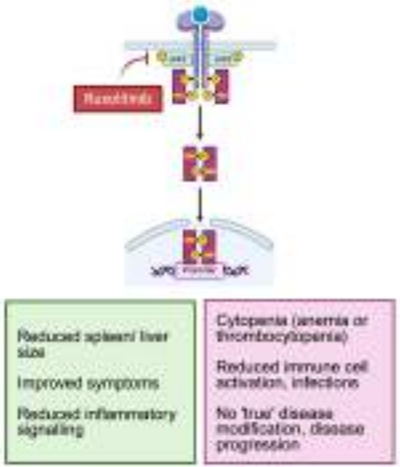
Chromatin module activation

EZH2 targets

Calabresi L et al. Am J Hematol, 2023

Study of molecular determinants of Ruxolitinib response by single cell multiomic

Ruxolitinib treatment



Reduced spleen/ liver size

Improved symptoms

Reduced inflammatory signalling

Cytopenia (anemia or thrombocytopenia)

Reduced immune cell activation, infections

No 'true' disease modification, disease progression

adapted from Verstovsek, S. et al.,
J Hematol Oncol 2023