La Multiomica nelle Patologie Mieloproliferative: Nuovi Scenari Diagnostici e Terapeutici

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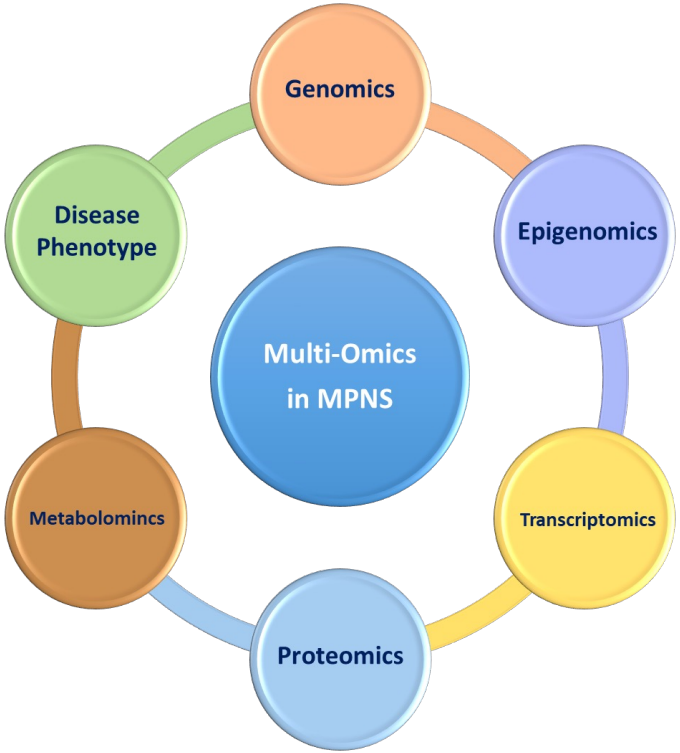
Disclosures of Name Surname

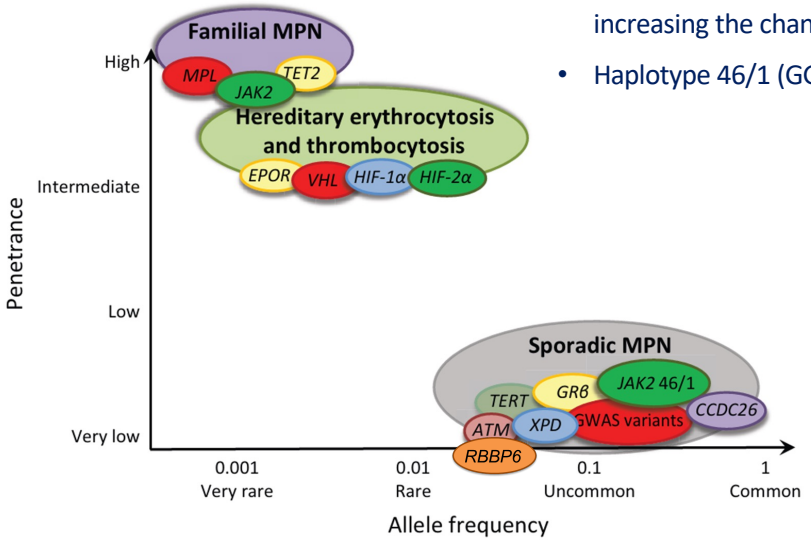
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
NOVARTIS					X	X	
GSK					X	X	
AOP					X		
Incyte						X	
Takeda						X	
Thermo Fisher						X	

Application of Omics in MPNs



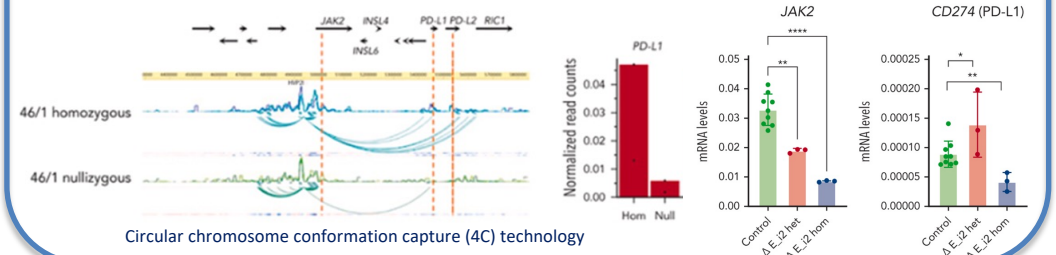
- Pathogenesis
- Diagnostic Criteria
- Disease Subgroups
- Prognostic Prediction
- Target Therapy

Germline Predisposition Variants in MPNs



- First-degree relatives of MPN patients have a markedly increased risk of PV (5/7 -fold) and ET (7/12-fold)
- MPN risk variants are enriched in accessible HSC chromatin and may facilitate expansion of the HSC pool, increasing the chance of acquiring somatic driver mutation
- Haplotype 46/1 (GGCC) is associated with MPNs, increasing familial MPN risk by 5-fold

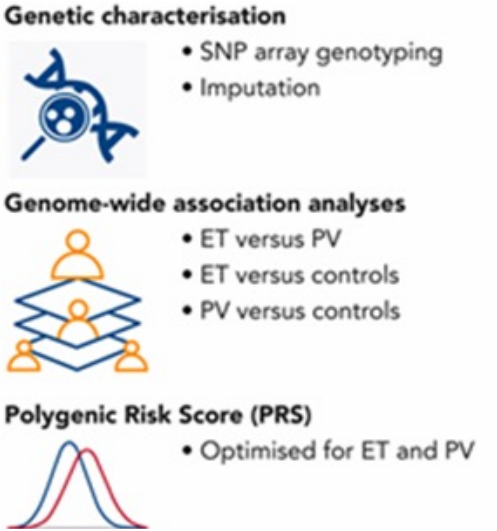
- PD-L1 expression is increased in 46/1 haplotype carriers.
- PD-L1 physically interact with JAK2 with different pattern between 46/1 and non-risk haplotypes
- A regulatory element located in the *JAK2* haplotype influences PD-L1 expression



Circular chromosome conformation capture (4C) technology

O. Landgren, et al. Blood. 2008 Sep 15;112(6):2199-204.; A.V. Jones, et al. Nat Genet. 2009 Apr;41(4):446-9.; D. Olcaydu, et al. Nat Genet. 2009 Apr;41(4):450-4.; D. Olcaydu, et al. Leukemia. 2009 Oct;23(10):1924-6. ; O. Kilpivaara, et al. Nat Genet. 2009 Apr;41(4):455-9.; A.V. Jones, et al. Blood. 2010 Jun 3;115(22):4517-23.; A.J. Mead et al. N Engl J Med. 2012 Mar 8;366(10):967-9; A. Oddsson, et al. Leukemia. 2014 Jun;28(6):1371-4; W. Tapper, et al. Nat Commun. 2015 Apr 7:6:6691.; A.S. Harutyunyan, et al. Blood. 2016 Jan 21;127(3):362-5; M. Germeshausen, et al. Blood Adv. 2018 Mar 27;2(6):586-596.; E.L. Bao, et al. Nature. 2020 Oct;586(7831):769-775.; Han EY et al. Br J Haematol. 2021 Sep;194(6):1098-1105; E.M. Braunstein, et al. Leukemia. 2023 Mar;37(3):627-635.; C. D.W. Brown, et al. Nat Commun. 2022 Sep 8;13(1):5284; Lim J et al. Leuk Res. 2024 Nov:146:107566. Carreno/Tarragona G et al. Blood. 2025 May 8;145(19):2196-2201.

Multiple Germline Variants Influence MPN Phenotype

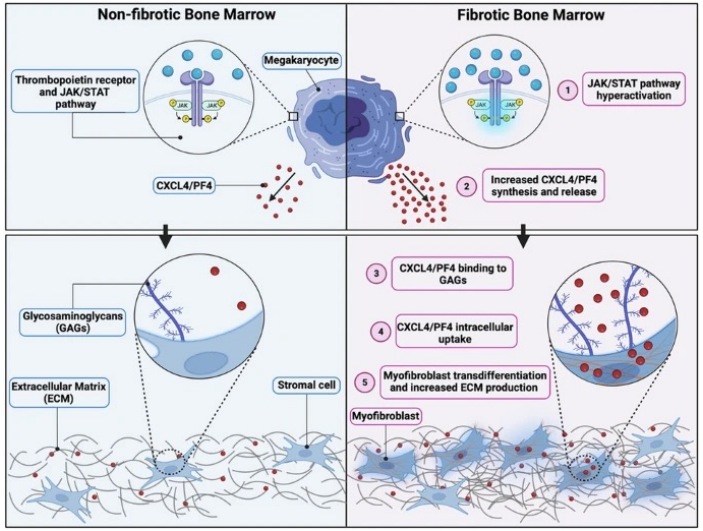


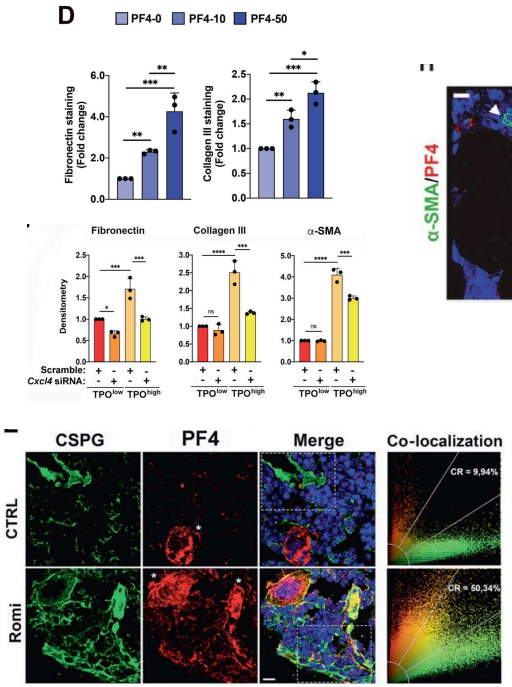
1069 patients (535 with ET and 534 with PV)

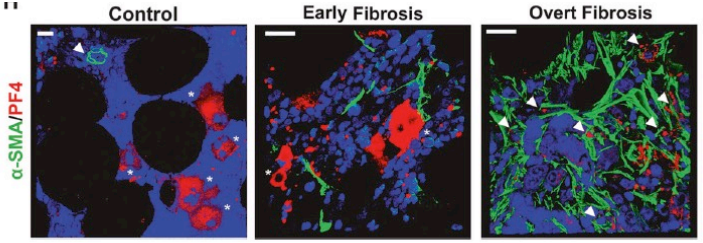
- ***HBS1L-MYB***
  >ET risk (OR = 1.28) and < PV risk (OR = 0.81)
- ***GFI1B-GTF3C5***
  predisposed to PV only (OR = 1.38)
- ***CDH22* (rs2425786 and rs2425788)/*CD40*** a novel female-specific germline variant associated with MPN phenotype (aberrant demethylation on X chr)
- A **polygenic risk scores** (48 SNPs) in 31 indipendent loci showed strong association with ET and PV

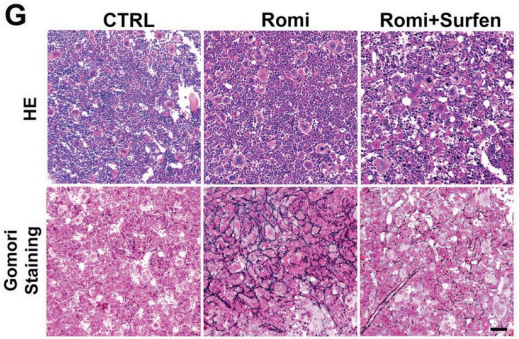
Tapper WJ et al. Blood. 2025 Dec 25;146(26):3228-3233.

PF4/Cxcl4 Axis as a Critical Driver of BM Fibrosis in PMF







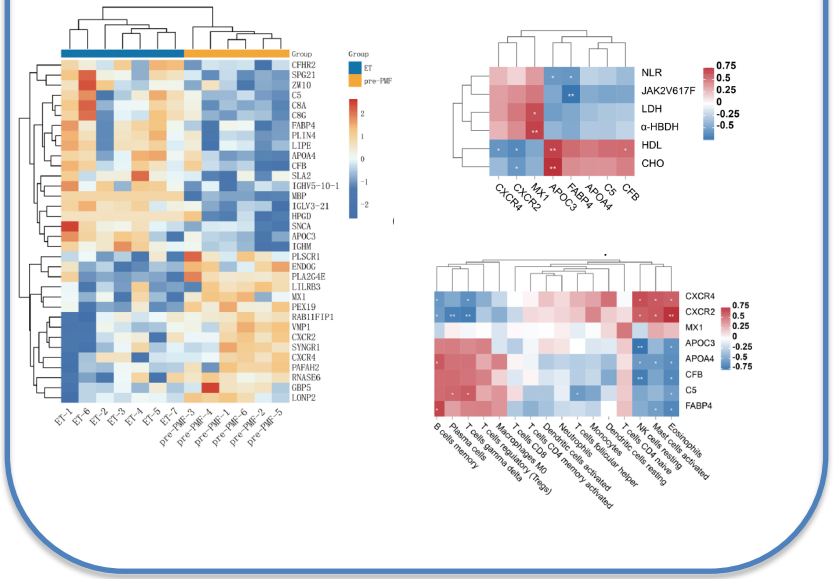


Capitanio D et al. Leukemia.2024 Sep;38(9):1971-1984.

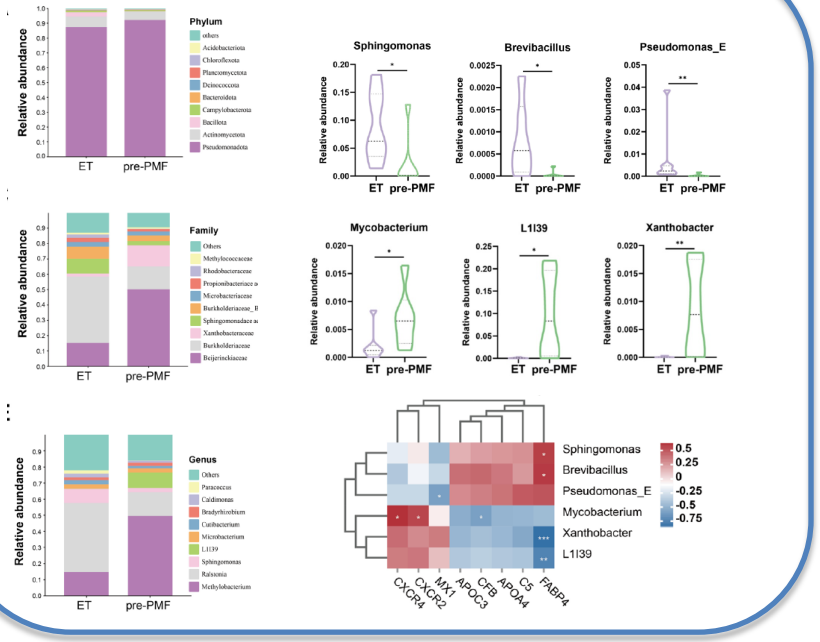
Differences in the Bone Marrow between ET and Prefibrotic PMF

Proteomic

DEPs involved in immune response and lipid metabolism

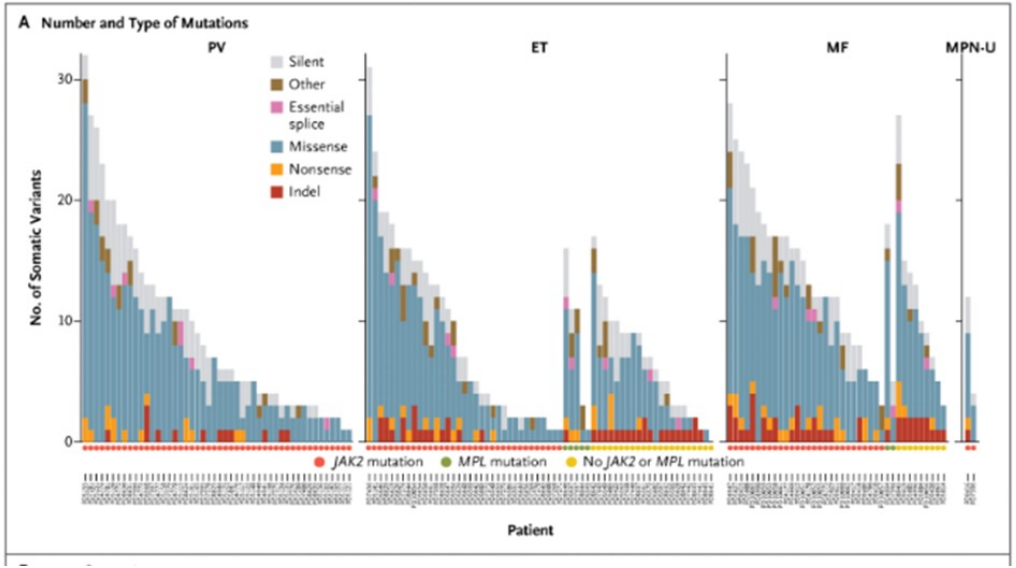


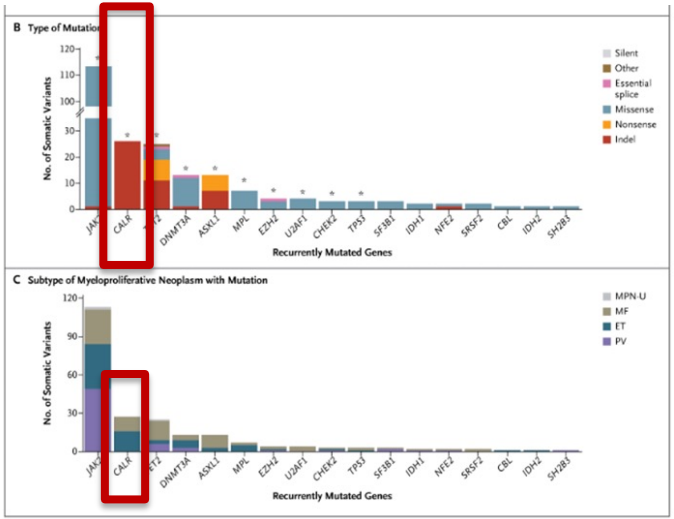
Microbiomics



Zhang A et al. Clin Exp Med. 2024 Jul 8;24(1):154.

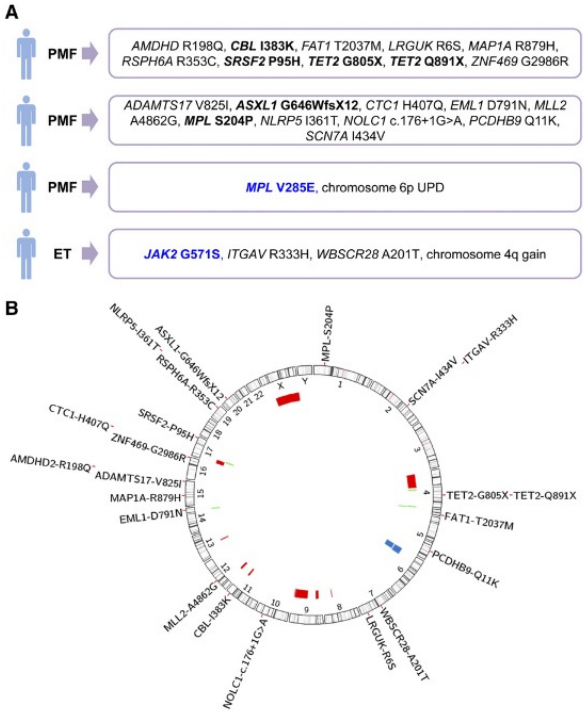
Identification of Novel Driver Variants in MPNs to Refine Diagnosis

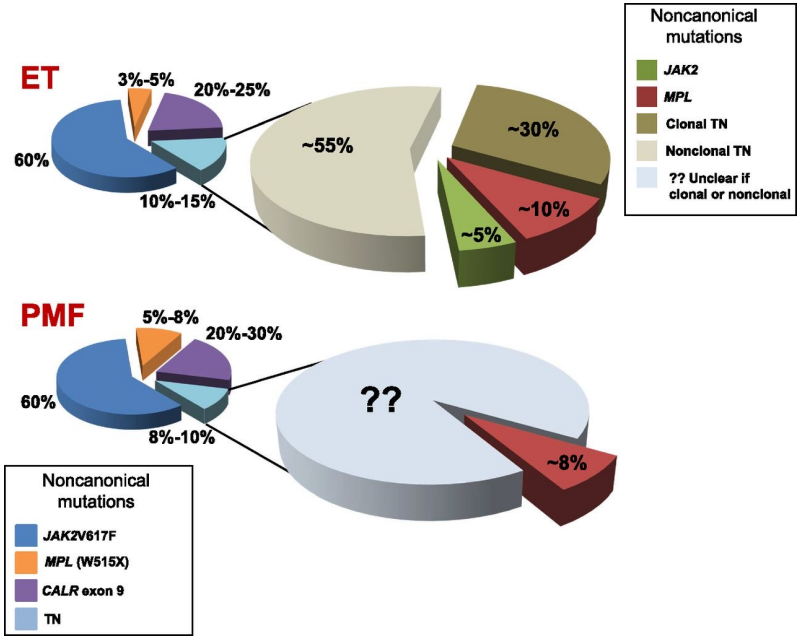




Klampfl T, et al. NEJM 2013;369(25):2379-90; Nangalia J, et al. NEJM 2013;369(25):2391-405; Grinfeld et al, NEJM 2018;

Diagnostic Implications of Non-canonical Mutations in Triple-negative PMF and ET





C.N. Harrison, A.M. Vannucchi Blood 2016;127:276-278; ; Milosevic JD et al. Blood 2016;127:325-332; Cabagnols J et al. Blood 333-342

CCDC6::JAK2 Fusion Gene as a Driver of Atypical *JAK2*-unmutated MPN

30 y

WBC 20x109/L,
Hb 19.4 g/dL
Hct 60.7%
PLT 107x109/L

- BM biopsy:
  - Hypercellularity (80%)
  - Panmyelosis
  - Reticulin fibrosis grade 1

- Cytogenetic Analysis:
  46,XY,t(9;10)(p24;q2?2)

- NGS analysis:
  MPN drivers: negative
  - 40 myeloid genes: negative
  - Germline mutations associated with Erythrocytosis: negative

Bartalucci N et al. Am J Hematol. 2026 Feb;101(2):379-383

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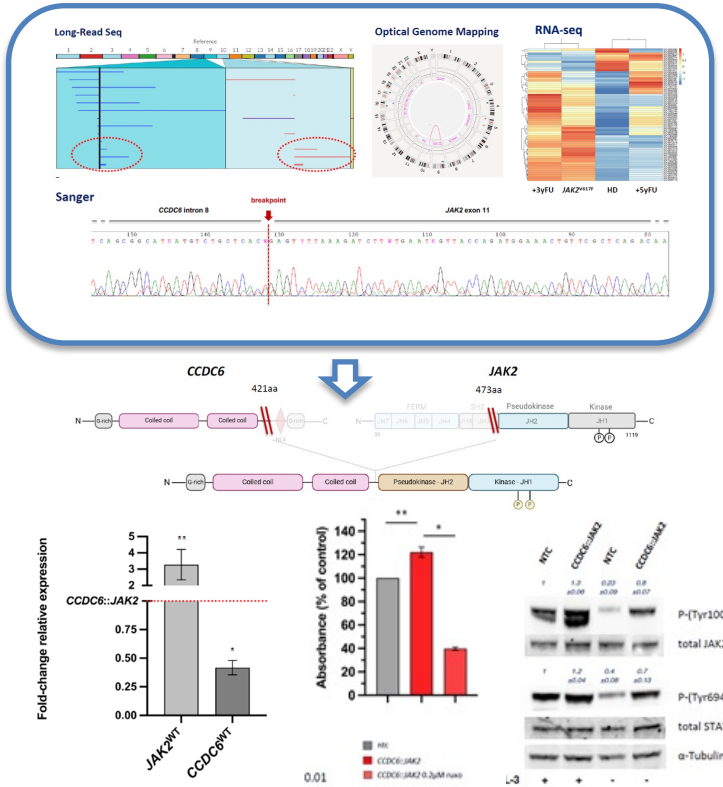

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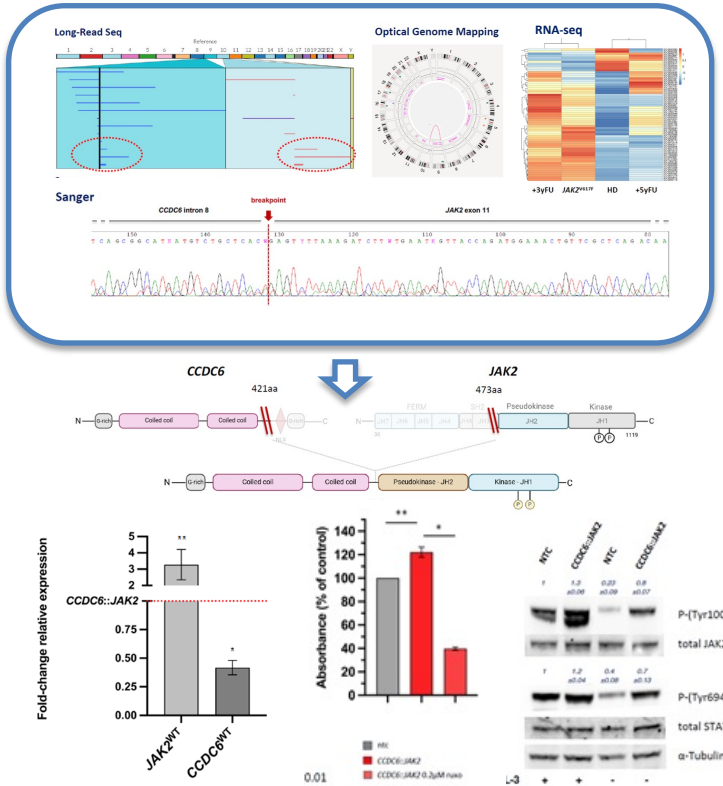
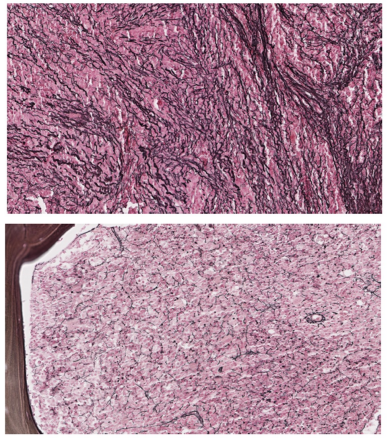
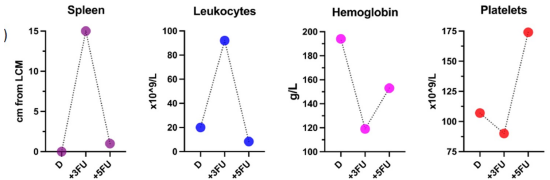


Ruxolitinib 10mg BID





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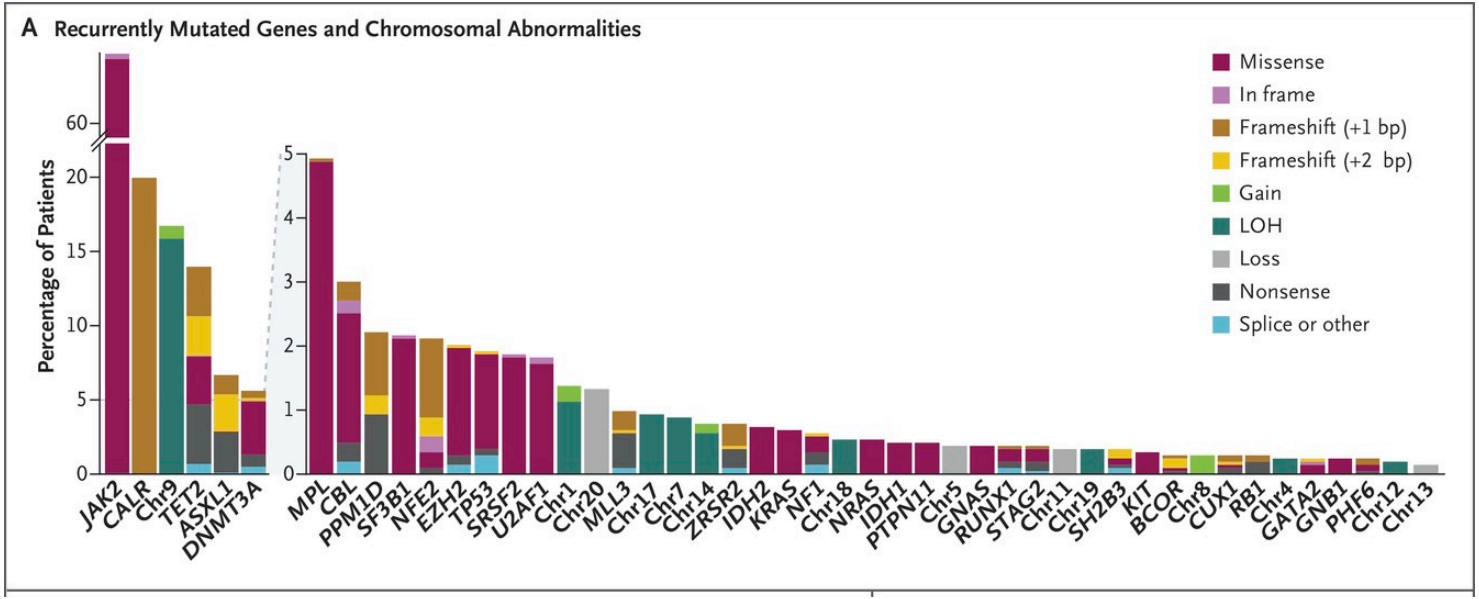
Target Sequencing to Uncover Molecular Heterogeneity

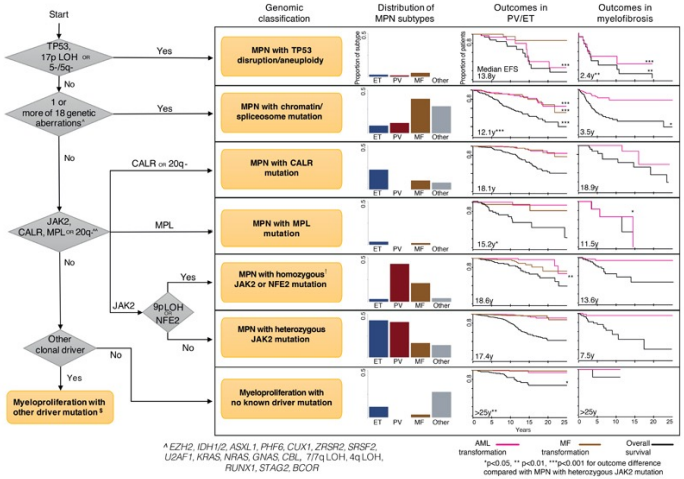


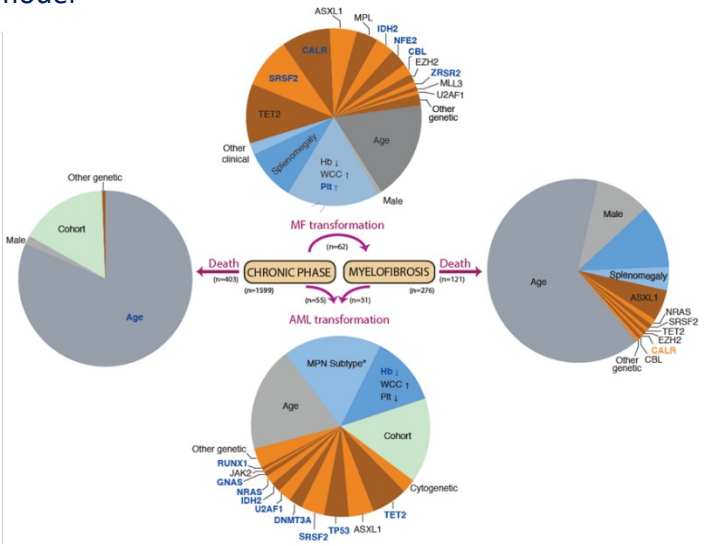
Development of prognostic models integrating molecular information
(MF: MIPSS70, MIPSS70+v2.0,GIPSS; PV: MIPSS-PV; ET: MIPSS-ET)

Grinfeld J et al. N Engl J Med. 2018 Oct 11;379(15):1416-1430

Personalized Prognostic Predictions Through Integration of NGS and Clinical Information

- ≃70 genes in 2035 MPNs samples established myeloid drivers, other putative drivers (other malignancies, recurrent in WES)
- >1700 SNPs, including ~90 associated with variation in hematological parameters or MPN risk
- 63 clinical and genomic variables have been integrated to create a prognostic model



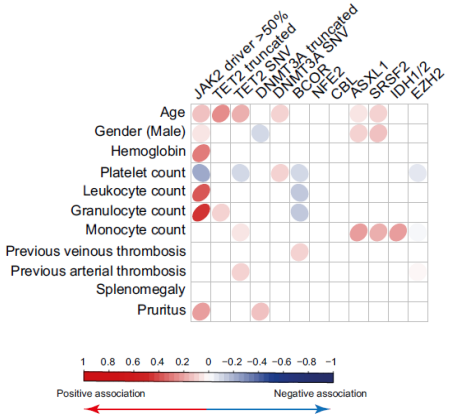


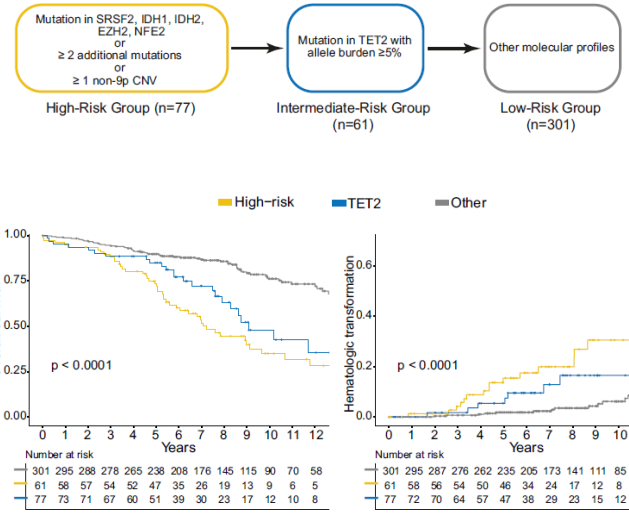
(https://cancer .sanger.ac.uk/mpn-multistage/)

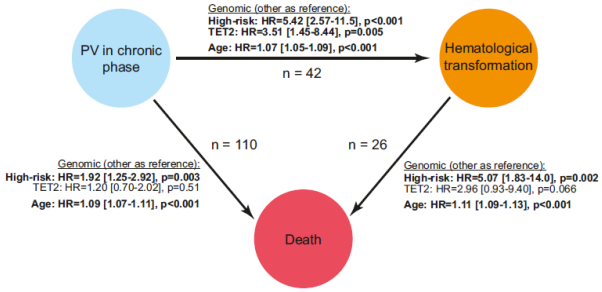
Grinfeld J et al. N Engl J Med. 2018 Oct 11;379(15):1416-1430

A Molecular Signature Predicts Hematologic Evolution in PV

- In PV, *ASXL1/SRSF2/IDH2* mutations confer adverse prognosis[1], with *SRSF2* informing MIPSS-PV[2]
- 234/439 (53.3%) had ≥1 additional mutation: 13.4% had 2, 9.3% had ≥3[3]



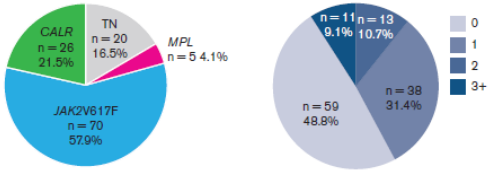


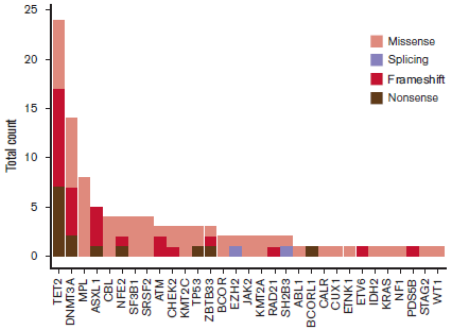


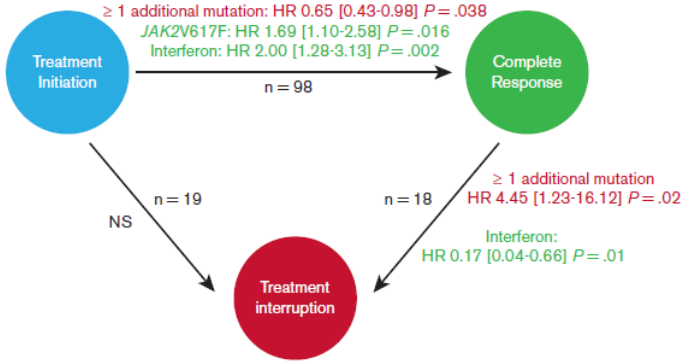
[1]Tefferi et al. Blood Adv. 2016;1:21–30; [2]Tefferi et al. Br J Haematol. 2020;189:291–302; [3]Mansier O et al. Leukemia. 2025 Aug;39(8):1937-1947

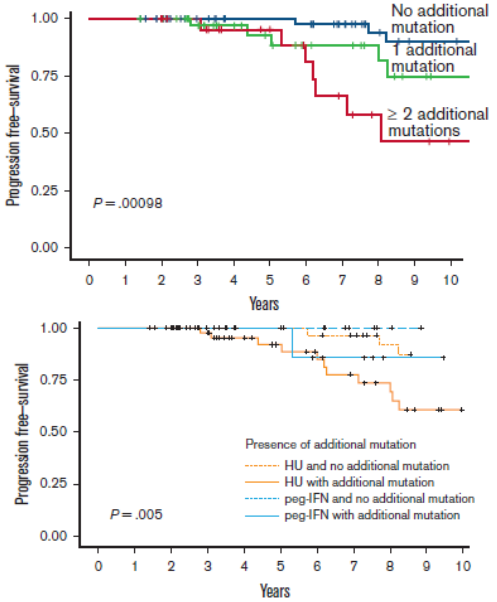
Impact of Additional Mutations on Response in ET

- In ET, ~45% of patients carry additional somatic mutations[1]; some (*TP53*/spliceosome) linked to MF/AML progression[2] or incorporated (*SRSF2, SF3B1, U2AF1, TP53*) into mutation-enhanced prognostic scores[3]
- At diagnosis, 62/121 (51%) had ≥1 additional mutation[4]
- IFN treatment at 12 months : CR 75 (62%), PR 37 (31%), No response 7 (6%)[4]









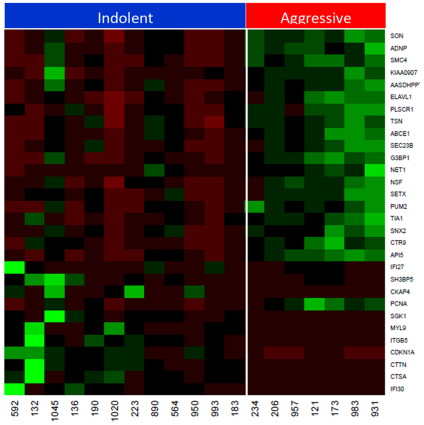
[1]Grinfeld J et al. NEJM. 2018; 379 (15) (2018: 1416-1430; [2]PLuque Paz DL et al. Haematologica. 2019 Apr;104(4):e134-e137; [3]Tefferi A et al. Br J Haematol. 2020 Apr;189(2):291-302; [4]Mansier O et al. Blood Adv. 2025 Mar 25;9(6):1303-1311

Transcriptomic Signature Identfies More Aggressive Disease and Poorer Outcomes in MPNs

Several studies identified transcriptomic signatures linked to clinical phenotypes and more aggressive MPN disease

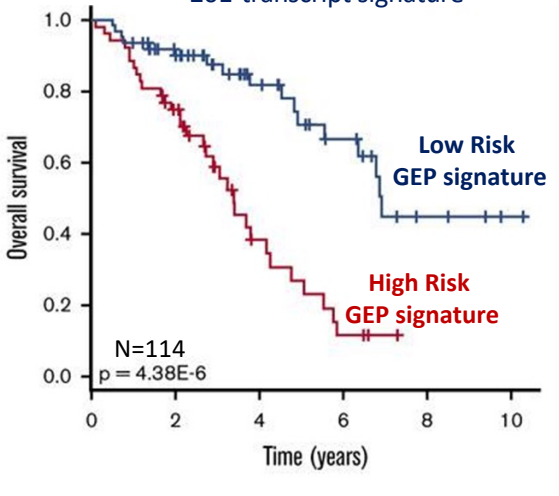
Polycythemia Vera[1]

N=19



Primary Myelofibrosis[2,7]

201-transcript signature



All MPNs

Platelet RNA-seq studies reported:

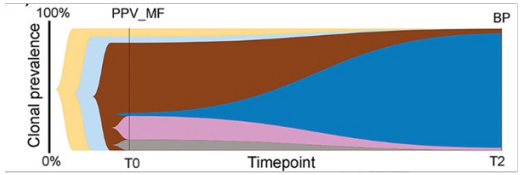
- PV : thrombo-inflammatory gene signatures (e.g., *BCL2, CXCL1, MMP7, Rap1)*[3,4]
- ET: Dysregulated calcium signaling, impaired receptor recycling, and altered apical junctions may drive sustained activation and vascular dysfunction[4].
- MF: fibrosis-associated signatures in MF (e.g., *CCND1, H2AFX, CEP55)*[5] and ER stress/UPR markers (e.g., *CREB3L1, CALR*)[6] suggesting impaired PLT proteostasis.

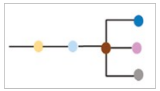
[1]Spivak JL, et al. N Engl J Med. 2014; [2]Rontauroli S et al. Blood Adv. 2021 Mar 5;5(5):1452–1462; [3]Gangaraju R et al. Blood Adv. 2020 Mar 24;4(6):1115-1130; [4]Bassan VL et al Thromb Res. 2026 Jan:257:109559. [5]Guo BB et al.c Br J Haematol. 2020 Jan;188(2):272-282. [6]Shen Z et al. Cell RepMed. 2021 Oct 19;2(10):100425; [7]CaluraE et al. Blood Cancer J. 2016 Jun 24;6(6):e439

Branching Evolution Is a Major Mechanism Leading to BP Evolution in MPNs

- SCS identified three BP clonal patterns and trajectories revealing the clone(s) that ultimately dominated the BP.
- Almost half of all mutations at BP involved epigenetic genes (*ASXL1, TET2, EZH2, IDH1, DNMT3A*).
- VAF of mutated transcription factor genes (*ETV6, PHF6, RUNX1, WT1, SETBP1*) was remarkably increased in BP compared to CP

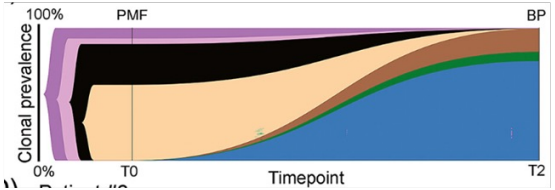
1st driver genes mutated clone





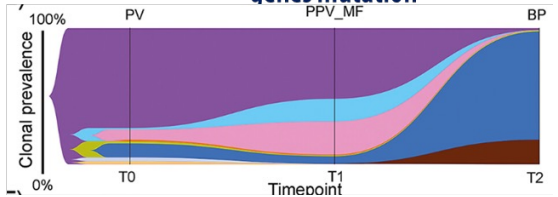
Subclones	T0 (%)	T2 (%)
WT	6.79	0
JAK2_Hom	4.88	0.21
JAK2_Hom/TET2_Het	57.4	3.7
JAK2_Hom/TET2_Het/SF3B1_Het	2.7	96.9
JAK2_Hom/TET2_Het/ASXL1_Het (a)	19.33	1.96
JAK2_Hom/TET2_Het/ASXL1_Het (b)	8.9	0.3

1st myeloid genes mutated clone



Subclones	T0 (%)	T2 (%)
WT	8	0.6
EZH2_Hom	4.1	0
EZH2_Hom/JAK2_Het	30.8	0
EZH2_Hom/JAK2_Het/DNMT3A_Het	57	0
EZH2_Hom/JAK2_Het/DNMT3A_Het/CBL_Hom	0	17.4
EZH2_Hom/JAK2_Het/DNMT3A_Het/CBL_Hom/ETV6_Het	0	7
EZH2_Hom/JAK2_Het/DNMT3A_Het/CBL_Hom/ETV6_Het/SETBP1_Het	0.1	75.1

relative to driver genes mutation

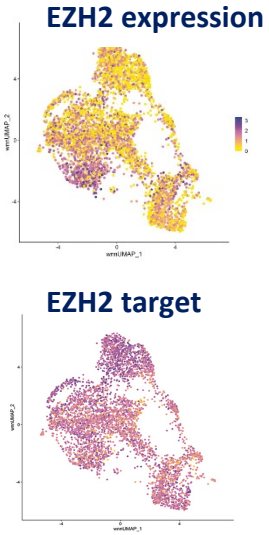


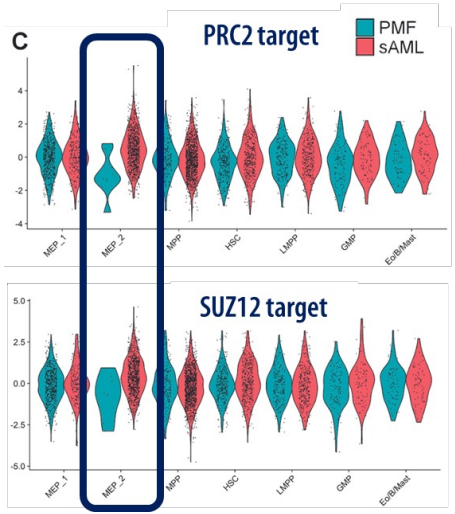
Subclones	T0 (%)	T1 (%)	T2 (%)
WT	73.5	51.9	1.5
JAK2_Het	1.2	16.7	0
TP53_Het (a)	1.65	0.53	0
KRAS_Het	2.77	0.15	0
TP53_Hom (b)	2.12	16.7	0
JAK2_Hom	7.4	24.3	0.16
TP53_Hom (a)	10.3	5.4	79.6
JAK2_Hom/IDH2_Het	1.1	0.23	0.03
TP53_Hom (a)/EZH2_Het	0	0.08	17.9

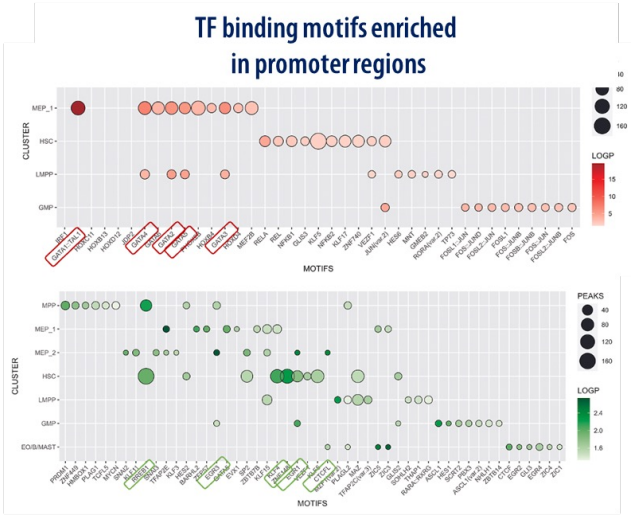
Parenti S et al. NPJ Precis Oncol. 2021 Feb 4;5(1):4; Calabresi L et al Am J Hematol. 2023 Oct;98(10):1520-1531c

Branching Evolution Is a Major Mechanism Leading to BP Evolution in MPNs

- The median number of CNVs per patient in BP clones was higher compared to CP (p =0 .04).
- *EZH2* was the gene most frequently affected by CNVs in the leukemic clones
- >7-fold more regions affected by CN gain or loss in BP compared to CP samples



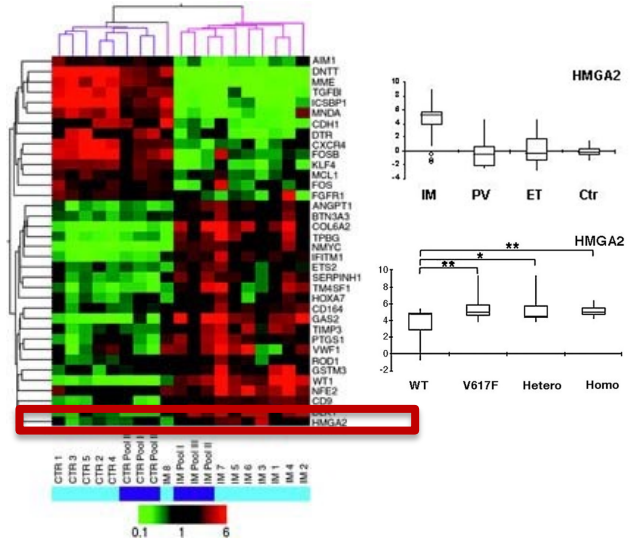


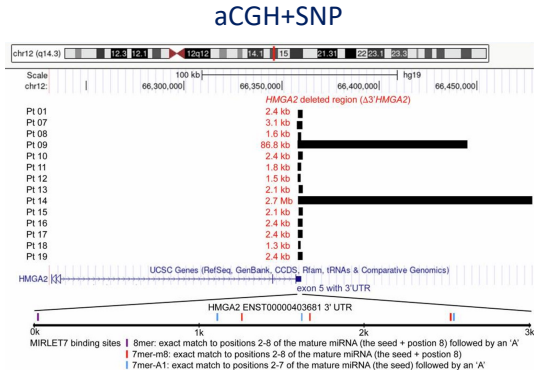


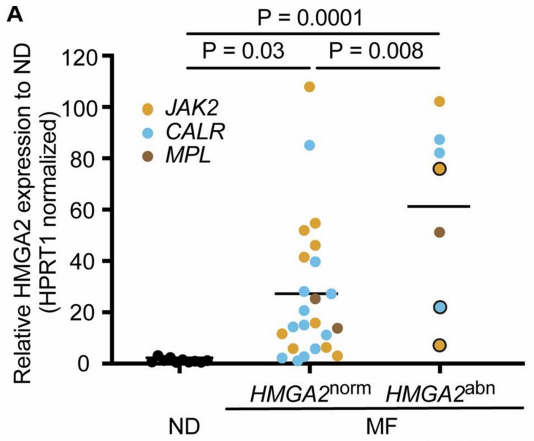
Calabresi L et al Am J Hematol. 2023 Oct;98(10):1520-1531c

HMGA2 Overexpression Driven by Specific Genomic Alteration Contributes to MPNs progression

- HMGA2 promotes a growth advantage in stem/progenitor cells and supports self-renewal/differentiation.
- Mir-LET7 microRNAs negatively regulate HMGA2 expression.
- *HMGA2* + *JAK2*V617F confers a competitive advantage to MPN stem cells, accelerating an MF-like phenotype.
- *HMGA2* overexpression is seen in nearly all MF cases and ~10–30% of ET/PV vs normal donors.







12q14.3 lesions (Δ3'HMGA2) preferentially co-occurred with ASXL1mut (70%), and were linked to progression to MPN-AP/BP

Guglielmelli P et al. Stem Cells. 2007 Jan;25(1):165-73. Handa S et al. Leukemia . 2025 Mar;39(3):663-674. Bartalucci N et al. Leukemia. 2025 Aug;39(8):2042-2045

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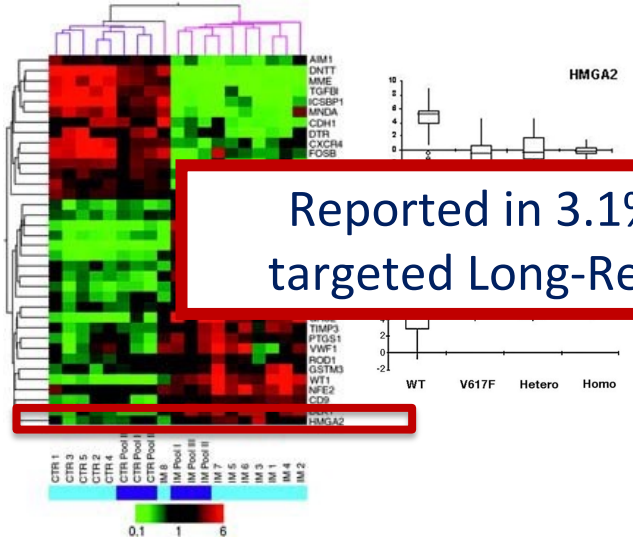

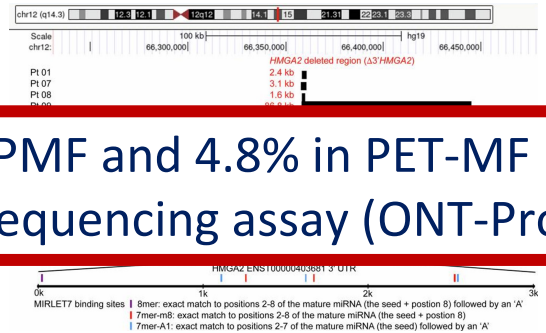

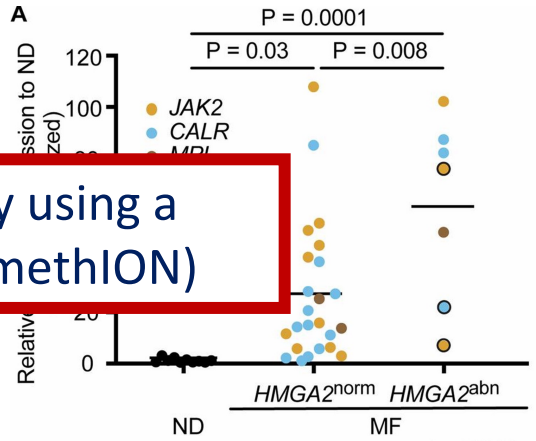

Reported in 3.1% of PMF and 4.8% in PET-MF by using a targeted Long-Read Sequencing assay (ONT-PromethION)

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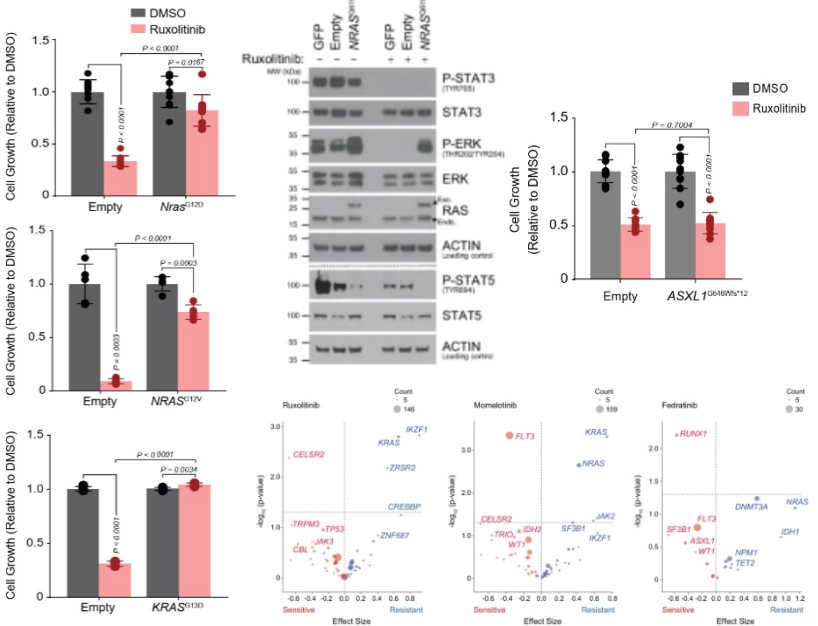
Mutational Profile Strongly Influences Response to Therapy in MPNs

Mutation Profile	Diseqase Type	Drug	Clinical Implication	Ref
HMR category (*ASXL1, IDH1, IDH2, SRSF2, EZH2, U2AF1*)	Myelofibrosis	Ruxolitinib	spleen response 9-fold lower. Higher rate of discontinuation	1,2,3
Clonal Evolution (any myeloid genes)	Myelofibrosis	pegIFNα-2	43% progressed vs 0% in pts w/o clonal progression	4
Clonal Evolution (any myeloid genes)	Myelofibrosis	Ruxolitinib	Loss of clinical response 7-fold increased Reduced OS after discontinuation	1,5,6
Ras-Pathway	Myelofibrosis	Ruxolitinib Pacriinib	Primary resistence	7,8
ASXL1	Polycythemia Vera	Ruxolitinib	Shorter EFS (HR 3.0) Lower Molecular response	9,10
DNMT3A	Polycythemia Vera	Ropeginterferon alfa-2b pegIFNα-2	controversial CMR 30% vs 56% NMR No response correlation	11,12,13
IFNL4 **diplotype *SNPs***	Polycythemia Vera	Ropeginterferon alfa-2b	controversial Incresed MR response	14
AS-NFKB1	Polycythemia Vera Essential Thrombocythemia	Ropeginterferon alfa-2b	Increased CHR	15



[1]Pacilli et al BCJ.2018; 8:122; [2]Patel K.P et al. Blood. 2015;126:790–7; [3]Palandri F et al. Cancer. 2024 Dec 15;130(24):4257-4266. [4]R.T. Silver, et al. 2017. Cancer, 123 (14), pp. 2680-2687; [5]Newberry KJ et al. Blood. 2017; 31;130:1125-1131; [6]Ortmann C.A et al NEJM 2015;372:601–612; [7]Coltro G et al. Blood Adv. 2020 Aug 11;4(15):3677-3687.[8]O'Sullivan JM et al. Leukemia. 2023 Dec;37(12):2497-2501; [9]Guglielmelli P et al, AJH, 2024 Aug;99(8):1550-1559; [10]Harrison CN, et al. J Clin Oncol. 2023 Jul 1;41(19):3534-3544; [11]Usart M et al. Blood 2024, 143 (24), pp. 2490-2503; [12]Knudsen TA et al. Blood Adv, 6 (7) (2022), pp. 2107-2119; [13]Abu-Zeinah G. et al Blood. 2025 Jul 3;146(1):123-126; [14]Jager R et al. Blood. 2021;137(3):387; [15]Song J et al: ASH 2024. *Blood*. 2024;144(suppl 2); 16Maslah N, etal, Nat Commun. 2025 Jul 8;16(1):6270.

Mutational Landscape of the Transcriptome Offers Putative Targets for Immunotherapy of MPNs

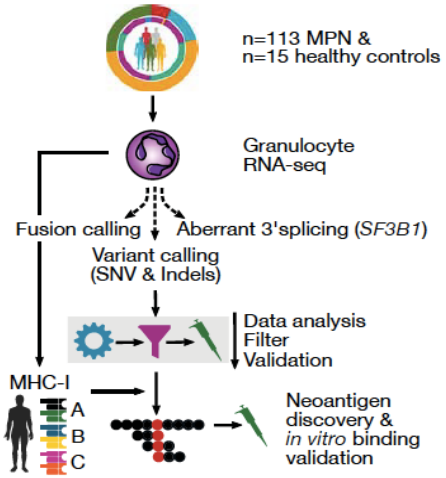
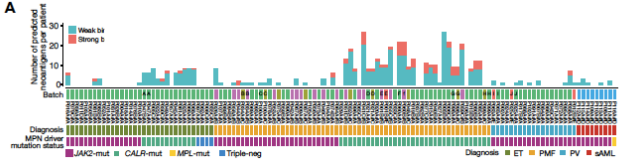
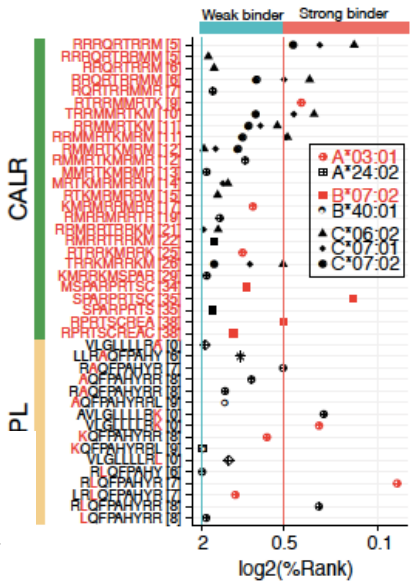
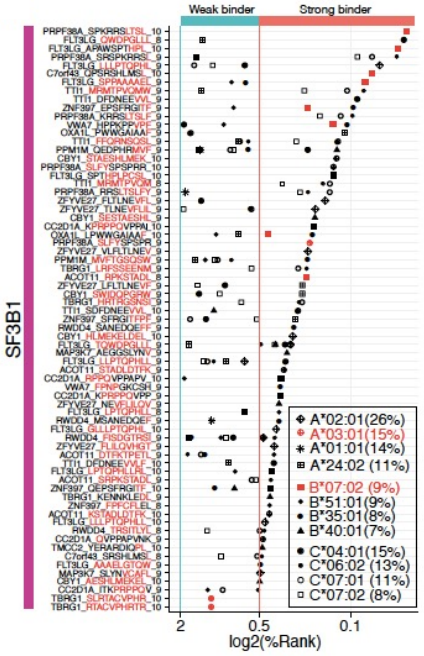


Figure 5

A







Schischlik F, et al. Blood. 2019 May 7. pii: blood.2019000519

Take Home Messages

- Current diagnostic criteria in MPNs are based on identification of gene mutations
- Multi-omics data improve MPNs diagnostic approach and risk stratification by enabling the discovery of prognosis-related biomarkers
- Recent studies leveraging high-throughput sequencing, mass spectrometry, and advanced computational tools have uncovered clonal evolution dynamics, and mechanisms that drive resistance to therapy, helping address key unmet needs.
- There are still obstacles and challenges in applying omics methods in mechanistic researches as well as for translational purposes (data integration complexity, cost barriers, and ethical considerations)

CRIMM-Center Research and Innovation of Myeloproliferative Neoplasms Florence

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