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One Hundred Suspected Myeloproliferative Neoplasms with a JAK2 V617F Variant Allele Frequency <2%: Clinical, Genetic and Histopathological Correlates

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Disclosures of Giuseppe G. Loscocco

Company name	Research support	Employee	Consultant	Stockholder	Speakers' bureau	Advisory board	Other
GSK					x		
Novartis					x		
AOP					x		

Background (1)

- *JAK2*V617F mutation is detected in >95% of PV and 50-60% of ET and PMF cases. Mutations in *MPL* are found in 3–8% of ET and MF and consist in gain of function variants at tryptophan 515 (W515) in exon 10, whereas more than 50 different mutations in *CALR* exon 9 have been described in approximately 25% of ET and PMF cases.

- Early clinical studies focused on distinguishing heterozygous from homozygous mutations while more recent methods utilize more sensitive techniques, including quantitative real-time PCR, to measure variant allele frequency (VAF).

- Both the International Consensus Classification (ICC) and the World Health Organization (WHO) classification systems include *JAK2* mutation information in their diagnostic criteria for MPN; however, neither defines a diagnostic VAF cut-off level.

Background (2)

- *JAK2* V617F was also detected in healthy subjects showing a negative effect on cancer-free and overall survival. ***JAK2* V617F positive individuals had higher erythrocyte thrombocyte and leucocyte counts, and had higher risk of ischemic heart disease, and venous thromboembolism**.

- The presence of *JAK2* V617F was associated with a higher risk of coronary heart disease in the context of clonal hematopoiesis of indeterminate potential (CHIP).

- Individuals carrying mutations in *DNMT3A*, *TET2*, and *ASXL1* had a 1.7 to 2.0 times higher risk of developing coronary heart disease compared to those without mutations, while the ***JAK2* V617F mutation was associated with a 12.1-fold increased risk.**

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Background and aim

- To date, scanty comprehensive data on suspected MPN patients with a low JAK2V617F VAF, are reported. According to these studies, a VAF > 1 or 2% were necessary for a MPN diagnosis whereas the others had no hematologic diagnosis.

- Major limitations of the above studies comprise: i) the use of low-sensitivity methods to detect JAK2 V617F; ii) in some studies, criteria for MPN diagnosis were according 2008 WHO criteria; iii) BM biopsy was not performed in all the cases, particularly in patients with isolated erythrocytosis.

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The main objective of the current study was to analyze clinical, molecular and histopathological correlates in a series of **100 patients** with a clinical suspicion of a MPN and tested positive for ***JAK2*** **V617F with a VAF <2%.**

Methods

- After exclusion of secondary causes of erythrocytosis and thrombocytosis, we included 100 suspected MPN cases with JAK2 VAF <2% (2010–2025). All samples and data were collected **at diagnosis**.

- JAK2V617F mutation was detected by Droplet Digital PCR (ddPCR) method starting from peripheral blood (PB) granulocyte DNA with a variant allele frequency (VAF) limit of detection of 0.01%.

- Bone marrow samples were accurately reviewed by two expert pathologist (R.F, U.G.).

- The definition of these disorders was performed according to the 2022 ICC.

Clinical and laboratory variables

- According to clinical/laboratory presentation patients were grouped as follow: erythrocytosis (n=35), thrombocytosis (n=47), and miscellaneous (n=18).

- Miscellaneous group includes pts with splenomegaly (n=4), atypical venous thrombosis (n=7), leukocytosis (n=6), and erythrocytosis with suspected systemic mastocytosis (n=1).

Clinical variables	All patients n=100	Erythrocytosis n=35	Thrombocytosis n=47	Miscellaneous n=18	p value
Age in years; median (range)	62 (19-92)	62 (19-82)	65 (23-92)	58.7 (25-84)	p=0.8
Males; *n* (%)	64 (64)	32 (91)	20 (42)	12 (67)	**p<0.01**
Hemoglobin, g/dL; median (range)	14.6 (9-19.5)	17.1 (15.6-19.5)	13.6 (11.1-15)	14 (9-15.8)	**p<0.01**
Hematocrit, %; median (range)	43.5 (28-57)	51.9 (47-57)	41 (35-47)	42.7 (28-50)	**p<0.01**
Mean corpuscular volume (MCV), fL; median (range)	89 (63-100)	90 (78-96)	89 (63-100)	88 (73-99)	p=0.2
Platelets, x 10^9/L; median (range)	408 (98-1450)	253 (142-450)	748 (490-1450)	231 (98-574)	**p<0.01**
Leukocytes, x 10^9/L; median (range)	7.6 (5-30)	6.8 (5-12)	7.8 (5.1-16)	8.4 (4.5-29.5)	p=0.2
Lactate dehydrogenase (LDH), U/L; median (range)	228 (105-700)	203 (105-270)	253 (146-558)	210 (145-700)	**p<0.01**
Ferritin, ng/mL; median (range)	103(10-570)	100 (16-570)	100 (10-500)	112 (15-237)	p=0.9
Venous thrombosis at/prior to diagnosis; *n* (%)	10 (10)	3 (9)	0 (0)	7 (39)	**p<0.01**
Arterial thrombosis at/prior to diagnosis; *n* (%)	12 (12)	6 (17)	5 (11)	1 (6)	p=0.4

Molecular variables

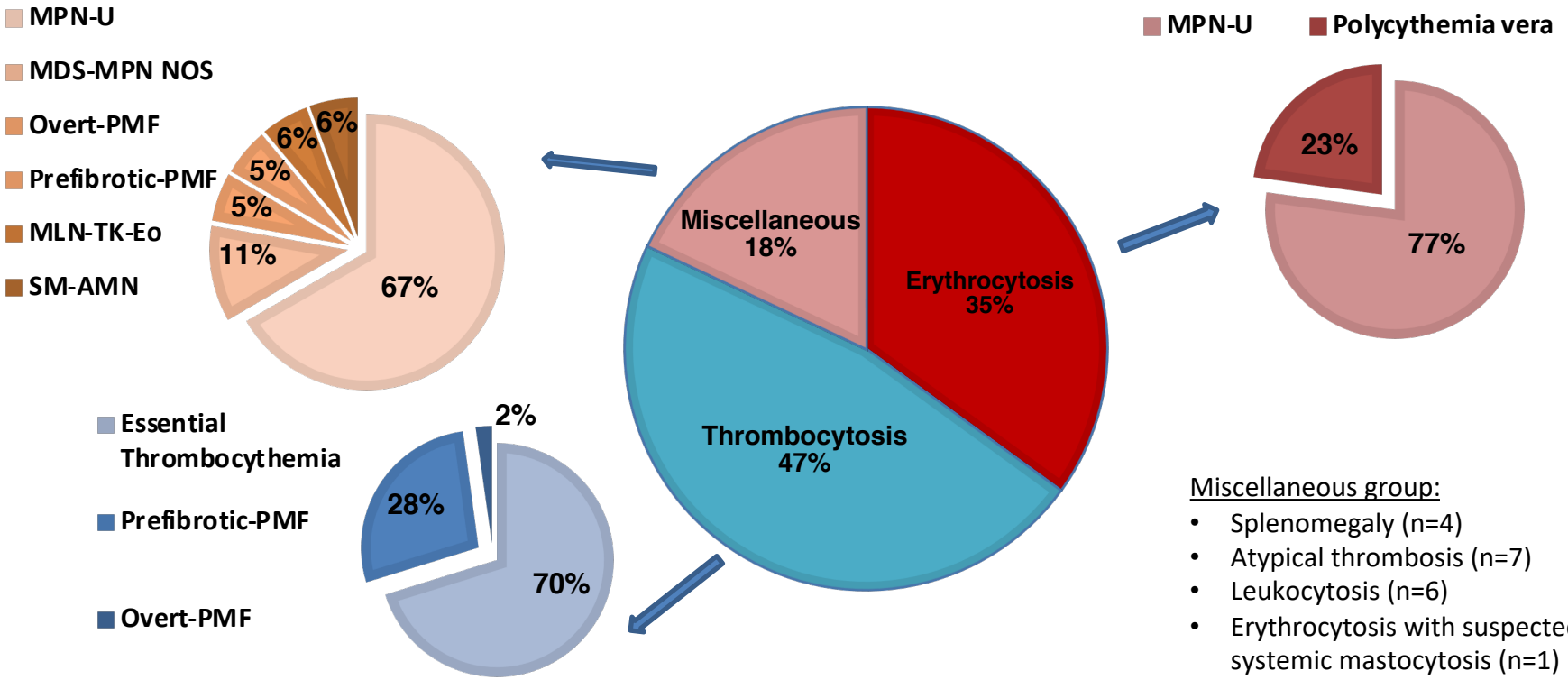
Laboratory variables	All patients n=100	Erythrocytosis n=35	Thrombocytosis n=47	Miscellaneous n=18	p value
***JAK2* V617F variant allele frequency (VAF)%; median (range)**	0.22 (0.01-1.95)	0.3 (0.01-1.95)	0.2 (0.01-1.9)	0.6 (0.1-1)	p=0.1
***MPL*, n (%)**	9 (9)	---	9 (19)	0 (0)	**p<0.01**
***CALR*, n (%)**	24 (24)	---	23 (49)	1 (6)	**p<0.01**
Double driver mutation	33 (33)	---	32 (68)	1 (6)	**p<0.01**
Abnormal karyotype *n* (%)	9 (9)	4 (11)	3 (6)	2 (11)	p=0.7
Patients with at least 1 additional NGS mutation; n (%); n. evaluable=46	24 (52)	8 (50)	6 (37)	10 (71)	p=0.2
ASXL1	6 (13)	1 (6)	2 (12)	3 (21)	p=0.5
TET2	3 (7)	2 (12)	1 (6)	0 (0)	p=0.4
SF3B1	4 (9)	1 (6)	2 (13)	1 (7)	p=0.8
SH2B3	3 (7)	2 (12)	1 (6)	0 (0)	p=0.4
KIT	3 (7)	1 (6)	0 (0)	2 (14)	p=0.3
DNMT3A	3 (7)	1 (6)	0 (0)	2 (14)	p=0.3
NF1	3 (7)	0 (0)	1 (6)	2 (14)	p=0.3
NFE2	2 (4)	0 (0)	0 (0)	2 (14)	p=0.09
SETBP1	1 (2)	0 (0)	0 (0)	1 (7)	p=0.3
SRSF2	1 (2)	0 (0)	0 (0)	1 (7)	p=0.3
CBL	1 (2)	0 (0)	0 (0)	1 (7)	p=0.3
EZH2	1 (2)	0 (0)	0 (0)	1 (7)	p=0.3
ZRSR2	1 (2)	0 (0)	0 (0)	1 (7)	p=0.3

Driver mutations other than *JAK2* were documented in 33 patients: 24 *CALR* comprising type-1 (n=17), type-2 (n=5), atypical (n=2) and 9 *MPL* (7 at p.W515 and 2 at p.S505). Of note, **double driver mutated cases constituted 68% of all those with thrombocytosis. Respective median VAF for *CALR* /*MPL* mutations were 34%/21%.**

Clinical and molecular variables in patients with thrombocytosis

Clinical and laboratory variables at diagnosis	*JAK2* only n=15	*JAK2* + *CALR* n=23	*JAK2* + *MPL* n=9	p values
Age in years; median (range)	68 (23-92)	62 (29-82)	72 (42-81)	p=0.6
Males; *n* (%)	5 (33)	11 (48)	4 (44)	p=0.7
Hemoglobin, g/dL; median (range)	13.6 (11.1-15)	13.6 (11.8-15)	13.5 (11.2-14.6)	p=0.8
Hematocrit, %; median (range)	42 (36-46)	41 (36-47)	43 (35-44)	p=0.4
Mean corpuscular volume (MCV), fL; median (range)	90 (69-100)	89 (62-100)	88 (63-94)	p=0.9
Platelets, x 10^9/L; median (range)	546 (490-848)	876 (575-1450)	759 (557-1253)	**p<0.01**
Leukocytes, x 10^9/L; median (range)	7.6 (6.2-12.8)	7.5 (5.1-16)	9.4 (6.4-11.3)	p=0.2
Lactate dehydrogenase (LDH), U/L; median (range)	221 (146-292)	290 (205-558)	253 (234-460)	**p<0.01**
Ferritin, ng/mL; median (range)	99 (10-500)	103 (11-460)	92 (24-213)	p=0.7
Venous thrombosis at/prior to diagnosis; *n* (%)	0 (0)	0 (0)	0 (0)	**---**
Arterial thrombosis at/prior to diagnosis; *n* (%)	2 (13)	3 (13)	0 (0)	p=0.5
ET versus PMF; n(%)	14 (93)	14 (61)	5 (55)	**p=0.03**
***JAK2* V617F variant allele frequency (VAF)%; median (range)**	0.5 (0.05-1.9)	0.18 (0.05-0.8)	0.1 (0.01-0.5)	**p=0.04**
Abnormal karyotype *n* (%)	1 (7)	1 (4)	1 (11)	p=0.8
Patients with at least 1 additional NGS mutation; n evaluable=14; n (%)	2 (40)	1 (25)	4 (43)	p=0.8

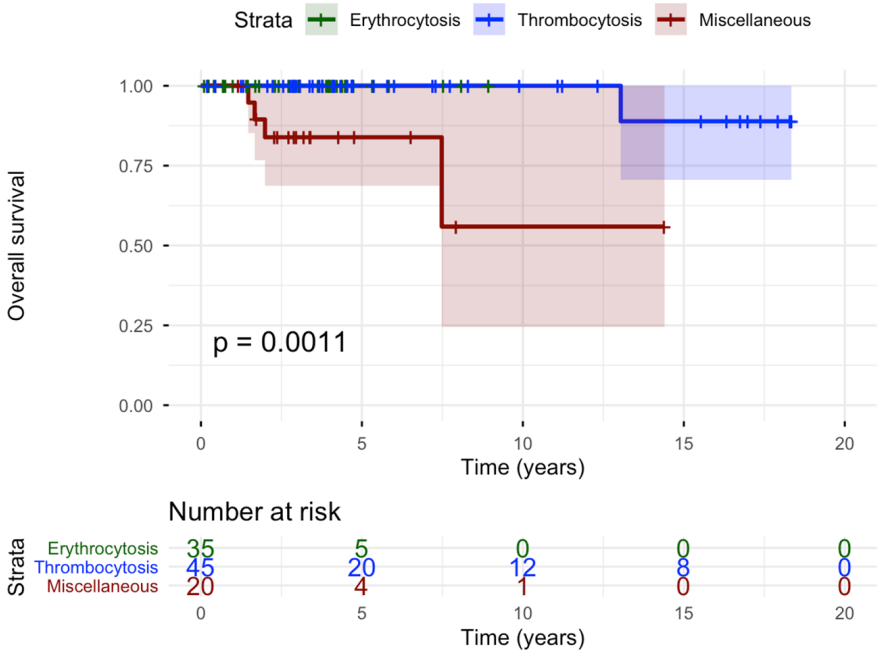
Patients' disposition and final diagnosis

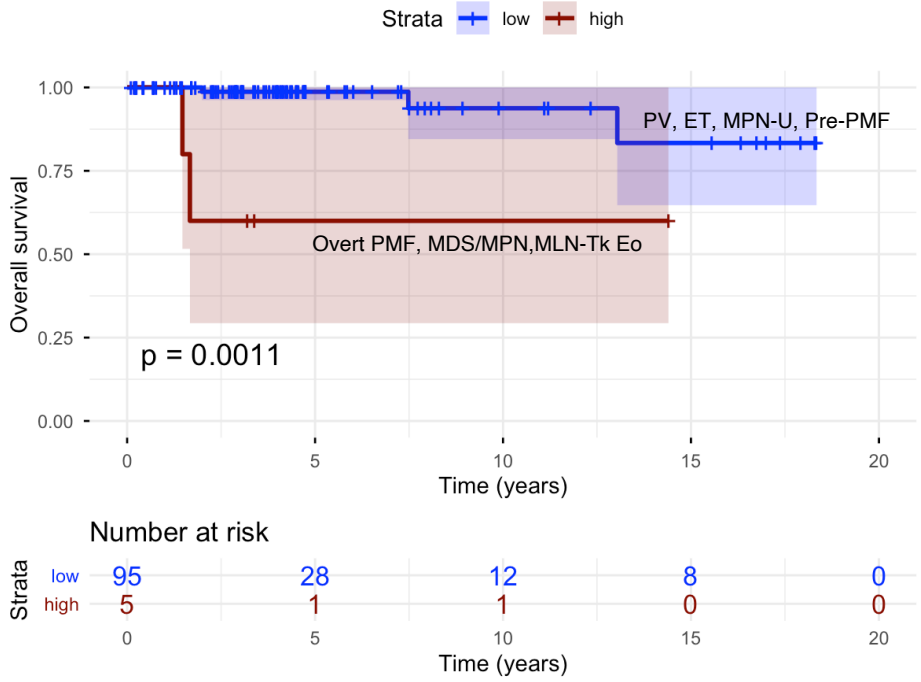


Miscellaneous group:

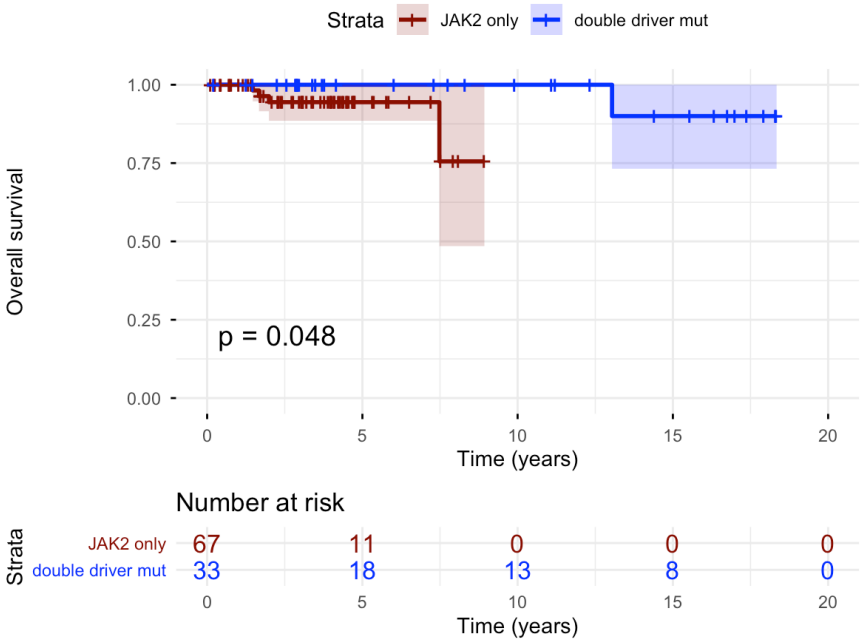
- Splenomegaly (n=4)
- Atypical thrombosis (n=7)
- Leukocytosis (n=6)
- Erythrocytosis with suspected systemic mastocytosis (n=1)

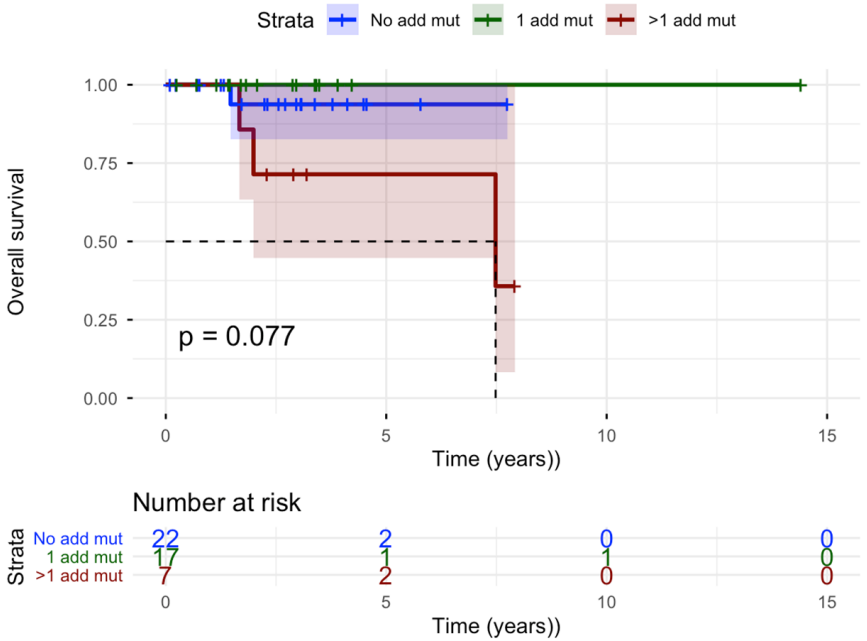
Survival according to diagnosis



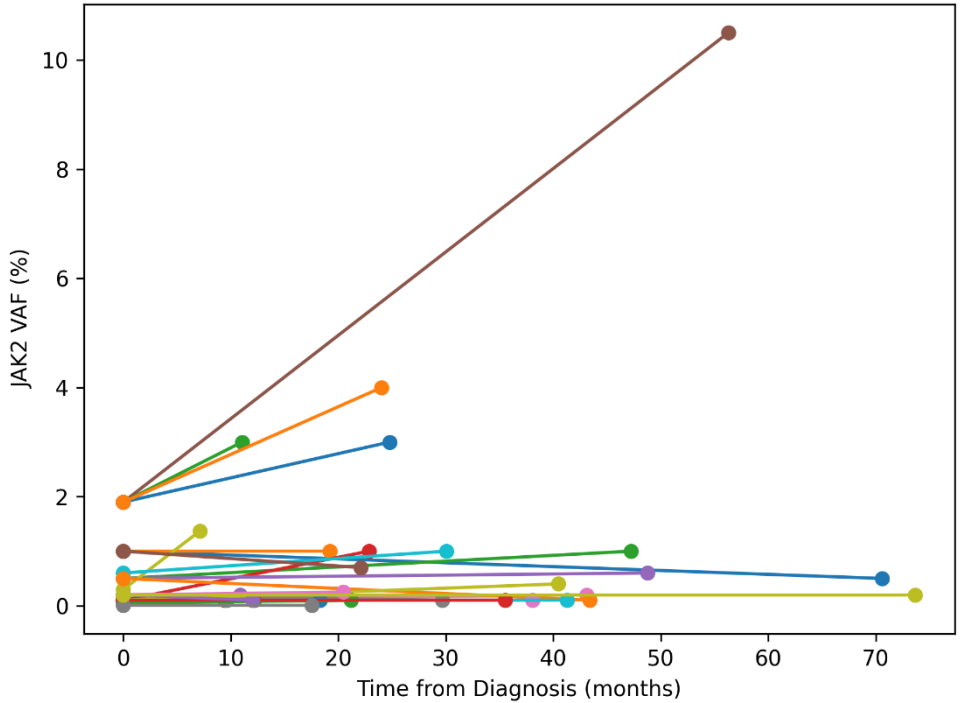


Survival according to molecular features



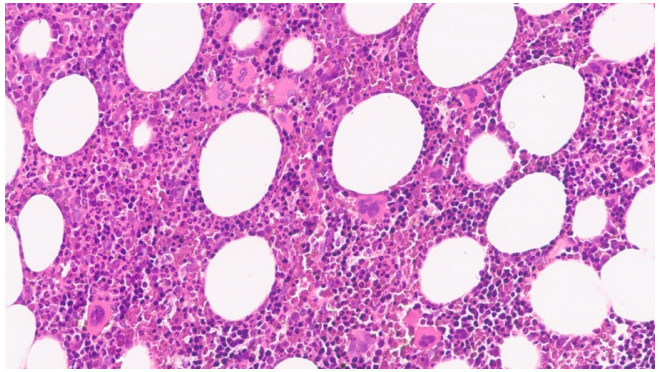


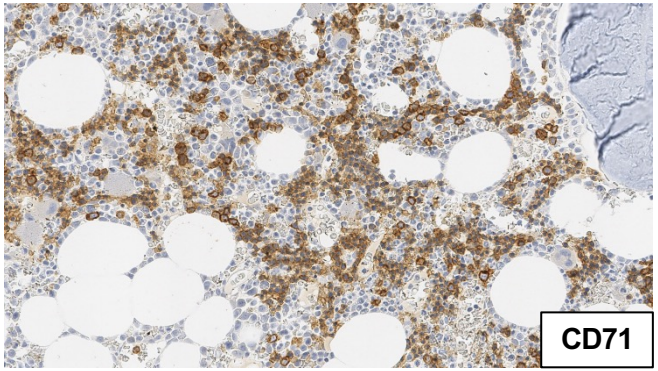
Longitudinal analysis of *JAK2* V617F VAF in 30 cases

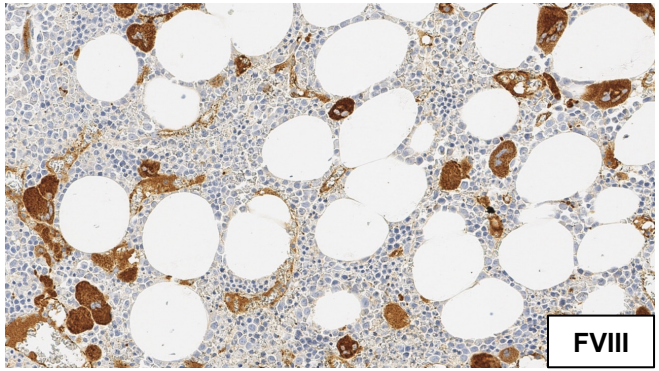


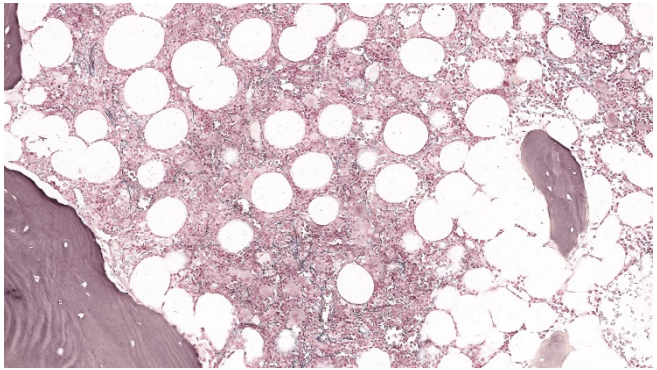
- **Median follow-up: 24.0 months**
- **Range: 7.1–73.7 months**

M, 79aa; Erythrocytosis; WBC: 6.77, Hb: 18.5, Htc: 53.6, PTL: 454; LDH: 202; normal EPO; *JAK2*^{V617F} [VAF: 1%], PV

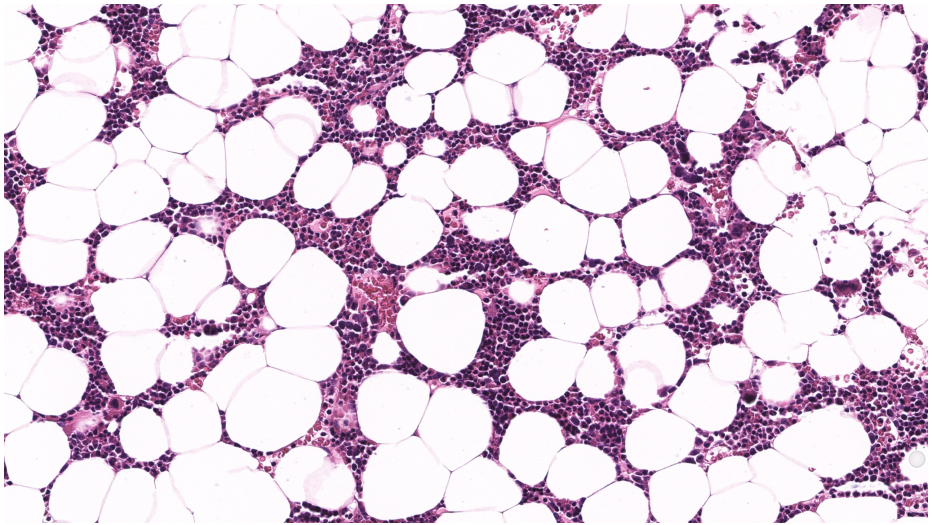


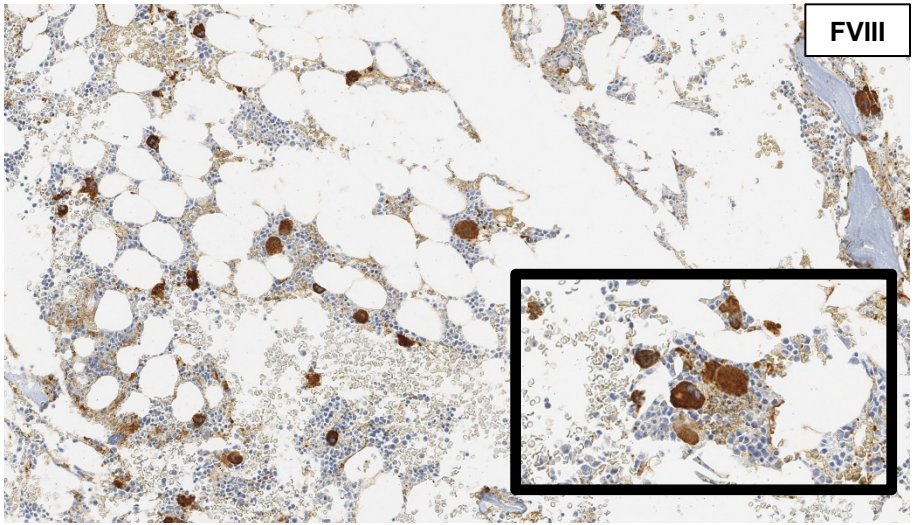




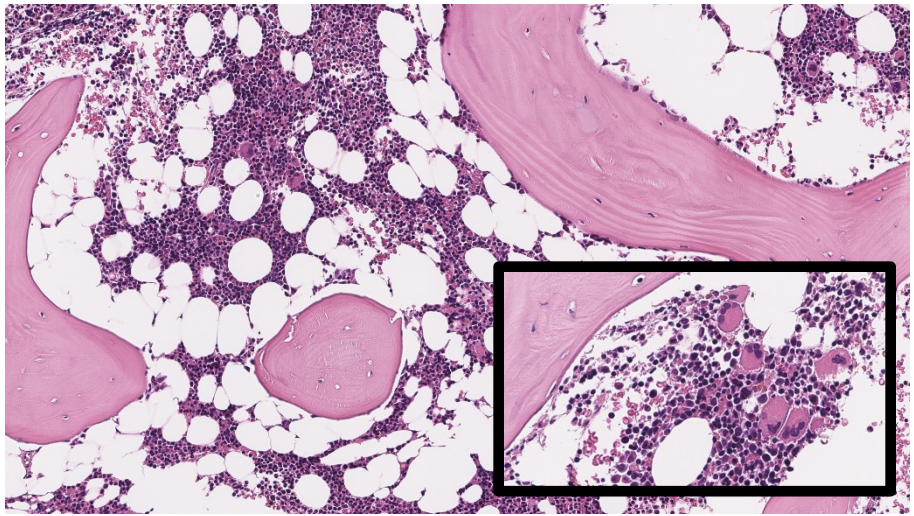


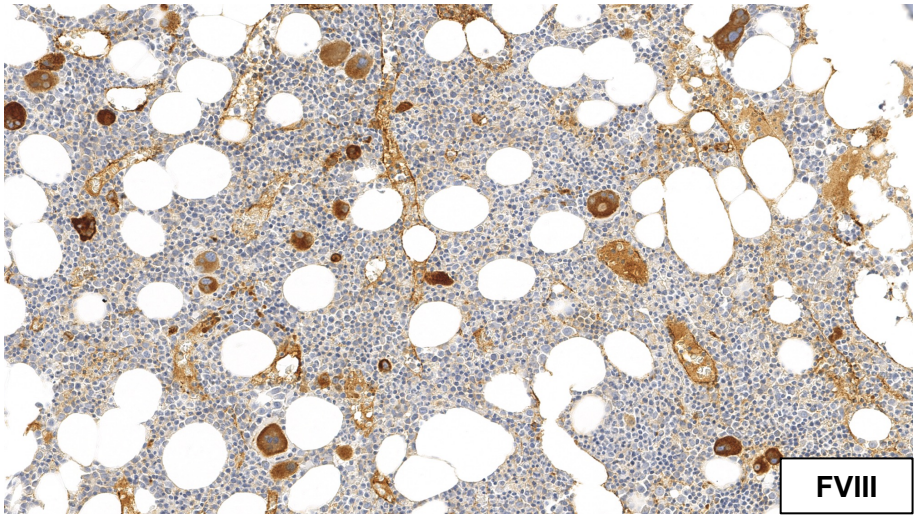
M, 62aa; Atypical thrombosis; WBC: 5.73, Hb: 12.8, Htc: 39.4, PTL: 223; LDH: 296; *JAK2*^{V617F} [VAF: 0.1%], MPN-U





F, 49aa; Thrombocytosis; WBC: 9.42, Hb: 13.1, Htc: 39.8, PTL: 507; LDH: 146; *JAK2*^{V617F} [VAF: 0.1%], ET





Background (4)

HiJAKing the Hematopoietic System: A Low-Frequency JAK2V617F Clone Drives Myeloproliferative Neoplasm (MPN) Pathology

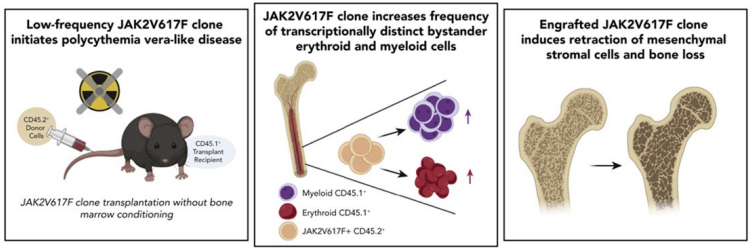
Context of Research

Given the wide interindividual variability in JAK2V617F clonal MPN there is a critical need for models that replicate sustained low variant allele frequency (VAF) to study early MPN progression.

Aim of This Study

To determine the impact of a low-frequency JAK2V617F clone on both the naïve hematopoietic system and the bone marrow stroma, we developed a traceable murine MPN model.

Findings



Visual Abstract figure was created in BioRender. Bonal, D. (2025) https://BioRender.com/hft4a95

Conclusions: A low-frequency MPN-driving clone in unconditioned recipients not only impacts hematopoiesis-supporting stroma, but profoundly and uniquely alters the transcriptomic and phenotypic profiles of unmutated bystander cells.

blood Visual Abstract

*Bonal et al. DOI: 10.1182/**blood**.2024027125*

Bonal DM, *Blood*. 2025;146(20):2428-2442

Conclusions

- This study highlights the importance of using **highly sensitive assays** to detect *JAK2* V617F (VAF limit of at least 0.1%).
- In pts presenting with erythrocytosis, histopathological diagnostic clues to MPN may be subtle as in the early phase of the disease with a final diagnosis **of MPN-U, with PV-like morphology**, in 3/4 cases (mostly those with a VAF <1%).
- **68%** of pts with thrombocytosis **have another driver mutation** (*CALR* and *MPL*); accordingly, it is imperative searching for all driver mutations in such cases.
- These data support that LOW VAF *JAK2*-mutated clonal hematopoiesis may represent an early phase of MPN rather than a CHIP.

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Metastatic disease: the key unmet need in oncology

Mynerva

AIRC

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