Ruolo del Profilo Molecolare nella Stratificazione Prognostica e Target Therapy: nella Policitemia Vera

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DISCLOSURE

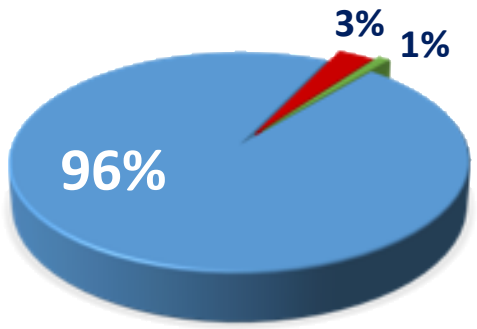
In qualità di RELATORE, ai sensi dell'art.76 sul Conflitto di Interessi dell'Accordo Stato-Regioni del 2 febbraio 2017, dichiaro che negli ultimi due anni ho avuto i seguenti rapporti di finanziamento con soggetti portatori di interessi commerciali in campo sanitario:

Advisory Board e Lectures: NOVARTIS, ABBVIE, BMS, GSK, INCYTE

Dichiaro, inoltre, che i contenuti formativi esposti sono indipendenti da interessi commerciali.

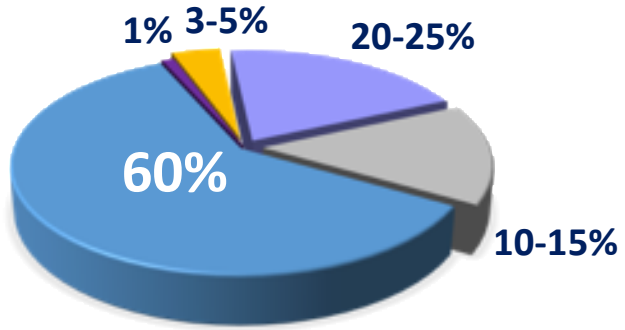
Spectrum of Driver Mutations in MPN

PV



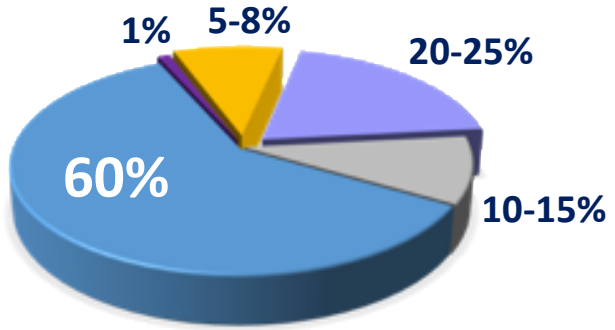
- *JAK2* V617F
- *JAK2* Exon12
- Others (*SH2B3*)

ET



- *MPL* (W515X)
- Non-canonical *MPL* and *JAK2* Mutations
- Unknown (Triple Negative»)

MF

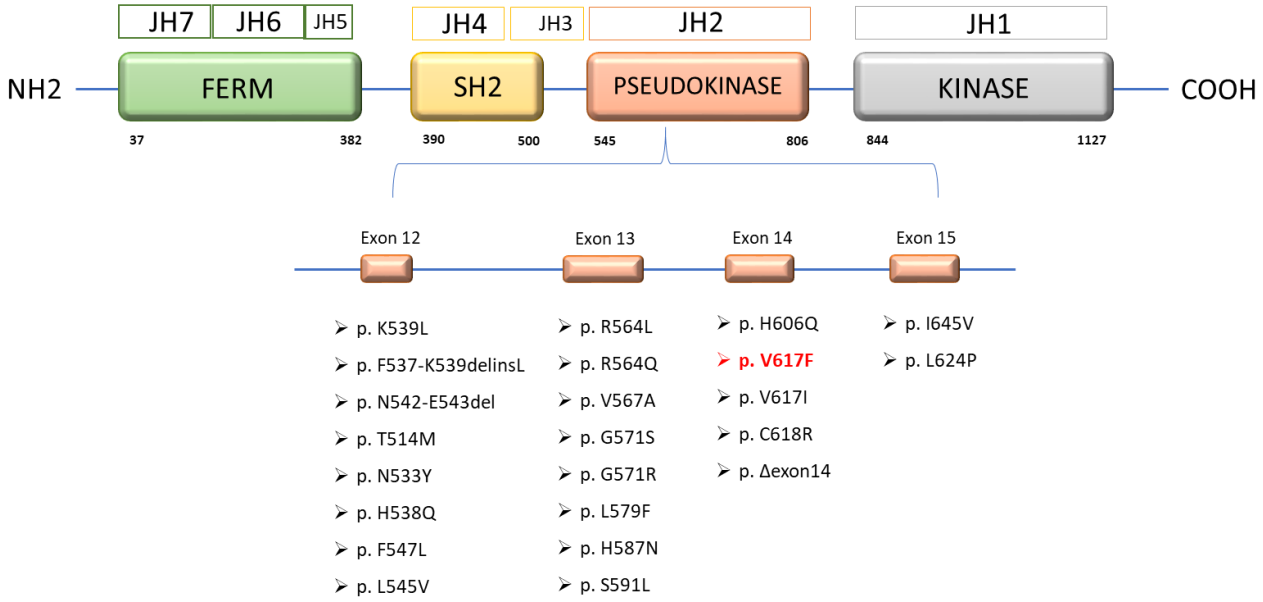


- *CALR* mut
  - Type1/Type1-like
  - Type2/Type2-like

James et al. Nature 2005; 434: 1144-8 ; Baxter et al. Lancet 2005; 365: 1054-61; Levine et al. Cancer Cell 2005; 7:387-97; Kralovics et al. NEJM. 2005: 352:1779-90; Klampfl T, et al. *NEJM* 2013;369(25):2379-90; Nangalia J, et al. *NEJM* 2013;369(25):2391-405; Milosevic JD et al. Blood 2016;127:325-332; Cabagnols J et al. Blood 333-342

JAK2 Canonical and Non-Canonical Mutations in MPNs

- Mutations in *JAK2* result in constitutive activation of the JAK2-associated receptors.



Higher *JAK2*V617F allele burden is usually associated with:

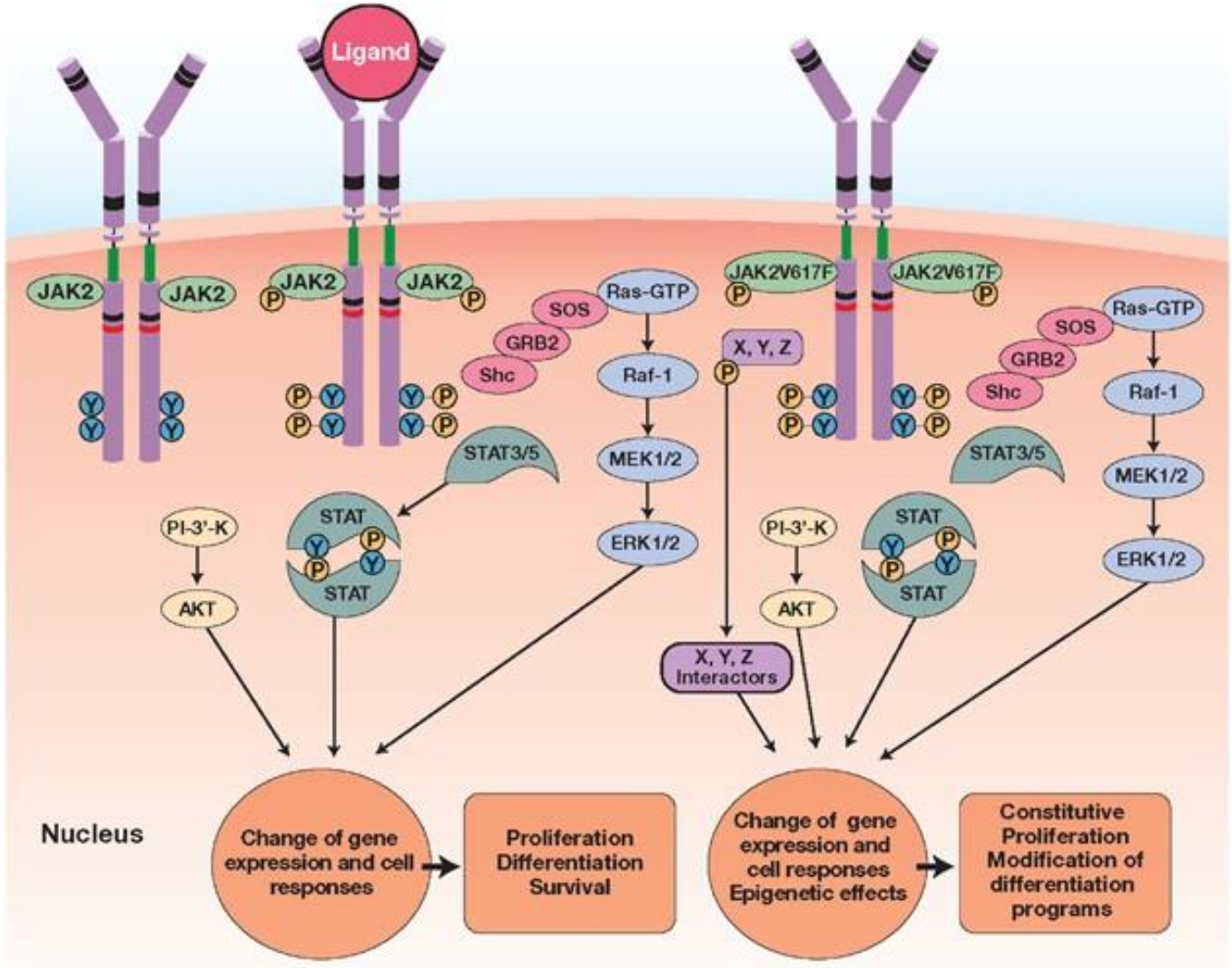
- Higher hemoglobin
- Higher leukocytes
- Lower platelets
- Larger spleen
- Pruritus
- Older age
- In ET/PV, a VAF >50% is a risk factor for for venous thrombosis, large splenomegaly and evolution to sMF

JAK2 Exon 12 mutations:

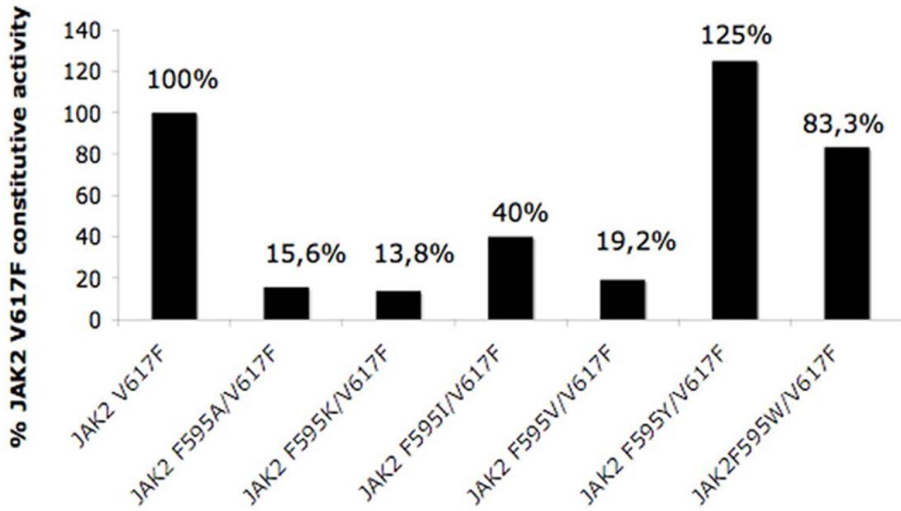
- Typically isolated erythrocytosis
- Younger age
- Higher hemoglobin than *JAK2V617F*
- Homozygosity is relatively rare
- Usually normal WBC and PLT counts
- Similar rate of MF, AML, thrombosis and hemorrhage as *JAK2V617F*

James C et al. Nature, 434 (2005), pp. 1144-1148; Baxyer EJ et al. Lancet, 365 (2005), pp. 1054-1061; Kralovics R et al. N Engl J Med, 352 (2005), pp. 1779-1790; Vannucchi AM et al, Leukemia 2007; 21:1952; Scott LM, Am J Hematol. 2011;86(8):668-76;Tefferi A et al, Leukemia 2013;27(9):1874-81; ; Rotunno G, et al. Blood. 2014; 123:1552-5; Rumi E, et al. Blood. 2014; 123:1544-51; Tefferi A, Leukemia. 2014; 28:1472-7; Guglielmelli P Et al. Blood Cancer J. 2021 Dec 11;11(12):199

*JAK2*V617F Constitutively Activates the JAK2/STAT3-5 Pathway



Residue F595, located in the middle of the αC helix of JH2, is indispensable for the constitutive activity of JAK2 V617F



Kralovics R et al. N Engl J Med. 2005;352:1779–1790; Vainchenker WW et al. Semin Cell Dev Biol, 2008, vol. 19 4(pg. 385-393); Dusa A et al. PLoS One. 2010 Jun 16;5(6):e11157

Non-Driver Mutations in Chronic Phase

Gene (%)	PV	ET	Pre-PMF	Overt MF
N=	*133*	*183*	*278*	*383*
ASXL1	12%	11%	18%	34%
EZH2	0	3%	4%	12%
SRSF2	3%	2%	9%	11%
IDH1/2	2%	1%	3%	3%
TET2	22%	16%	24%	17%
LNK/SH3B3	2%	0	5%	3%
ZRSR2	5%	3%	0	4%
SF3B1	3%	5%	5%	8%
SETBP1	2%	2%	0	0
DNMT3A	2%	6%	4%	4%
CSF3R	3%	3%	9%	4%
NRAS	0	1%	4%	9%
CBL	1%	1%	7%	4%
U2AF1	0	1%	3%	6%
RUNX1	2%	2%	3%	1%
TP53	1%	2%	4%	3%

Vannucchi AM et al, Leukemia 2013; 27:1861-9. Tefferi A et al, Bood Adv 2016; 1:21-30; Tefferi A et al, Blood Adv 2016; 1:105-111; Guglielmelli P et al, Blood; 2017:129:3227-3236

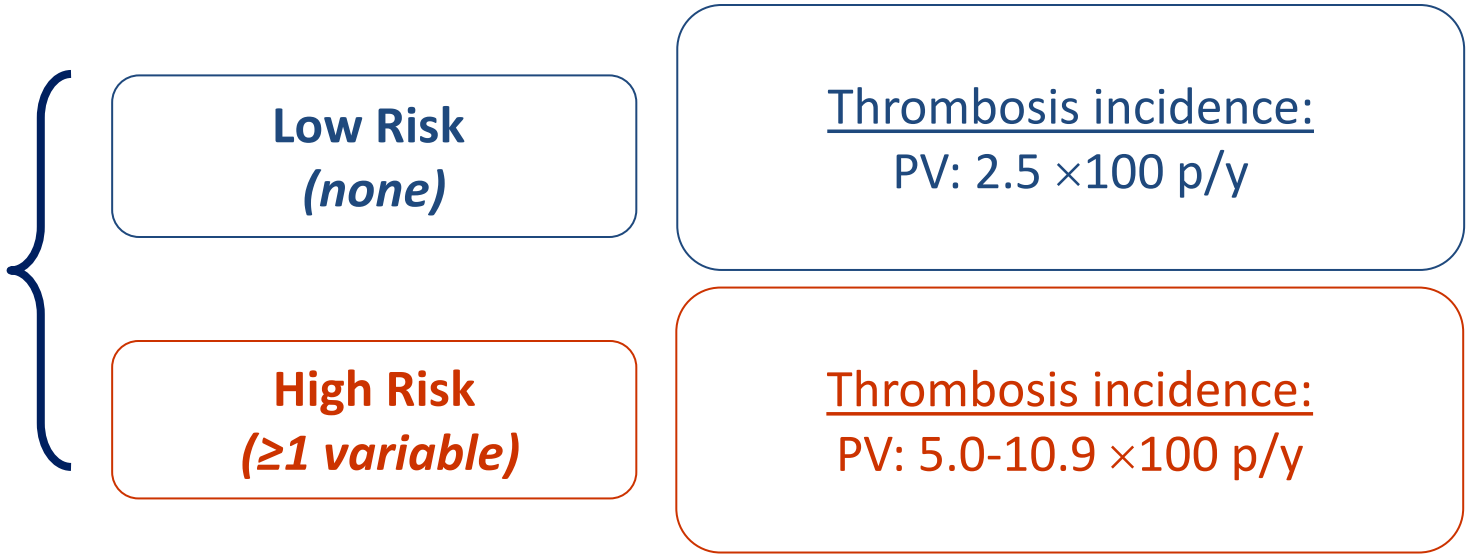
Endpoints of Prognostication

Polycythemia Vera
• To predict first occurrence and/or recurrence of thrombotic events and bleeding complications
• To predict the risk of evolution to myelofibrosis
• To predict the risk of acute leukemia
• Duration of survival

Prediction of Thrombosis in PV : Conventional Risk Model

Prognostic Variables

1. **Age over 60 y**
2. **Previous thrombosis**



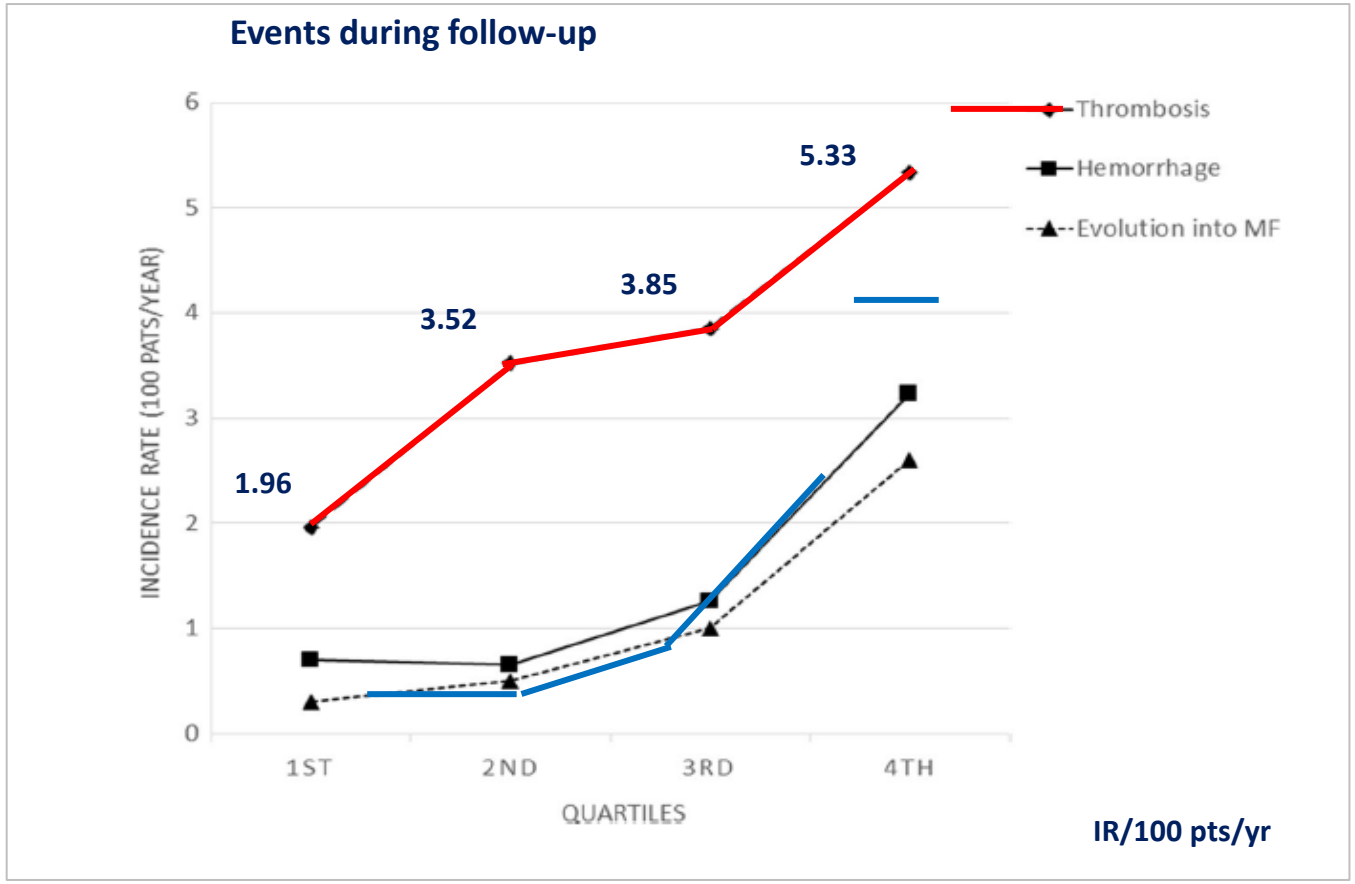
- Thrombocytosis is NOT associated with thrombosis risk, and conversely extreme thrombocytosis (Plt count >1,500 $\times 10^9$/L) may predict for bleeding.

Barbui T et al, J Clin Oncol 2011;29(6):761-70; Marchioli R et al, J Clin Oncol 2005;23(10):2224-32;Carobbio A et al, Blood 2008;112(8):3135-7

Thrombosis and Hemorrhage are More Common in MPN Patients with High *JAK2*V617F Allele Burden

JAK2V617F VAF (%) Quartiles:

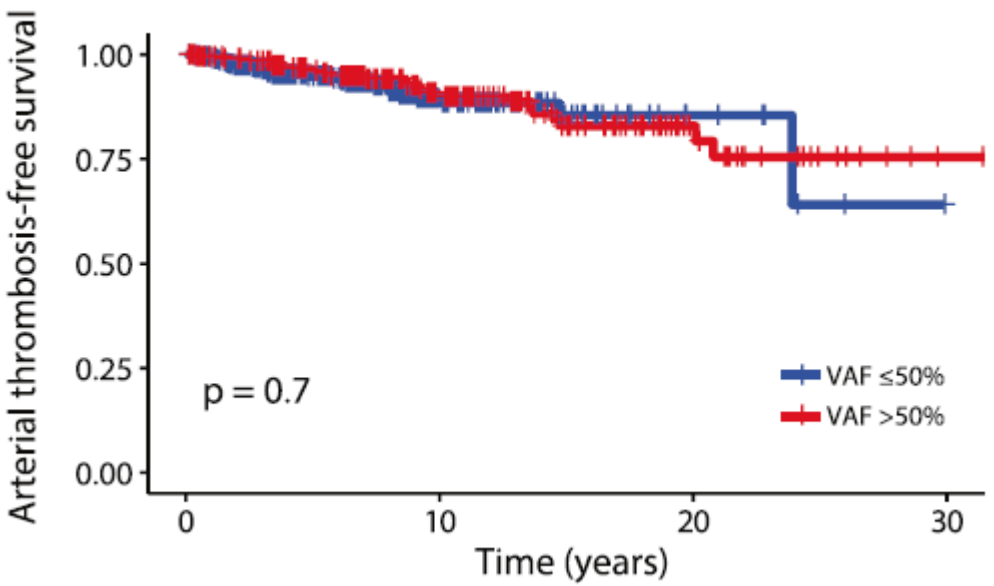
- 1st <25%
- 2nd 26-50%
- 3rd 51-75%
- 4th >75%

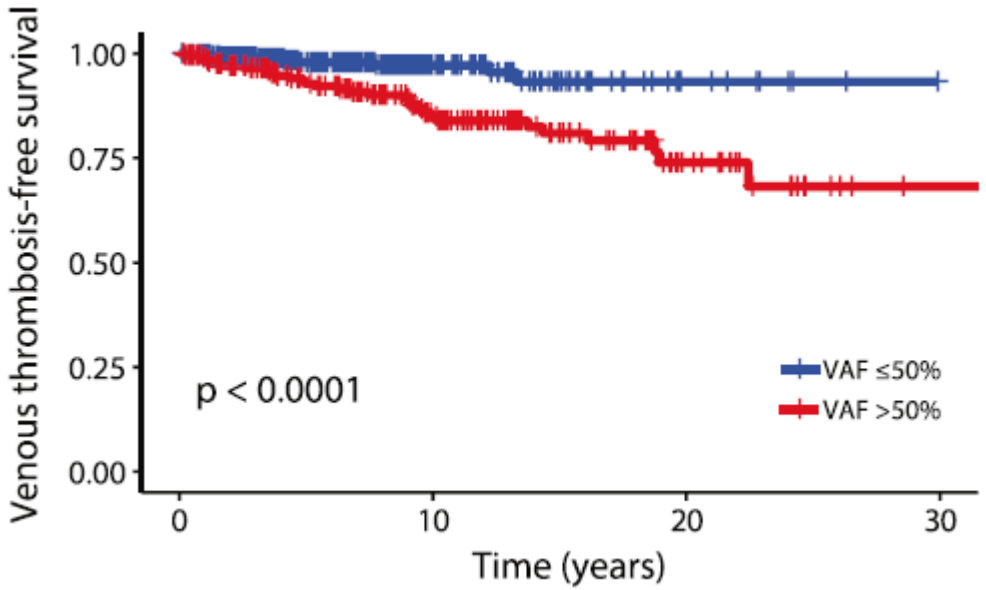


N=245, [ET=121, PV=124]

Bertozzi I et al, Ann Hematol 2017;42:E639; Vannucchi AM et al, Blood 2007; 110:840; Vannucchi AM et al, Leukemia 2007; 21: 1952

*JAK2*V617F VAF >50% Identifies PV Patients with High Risk for Venous Thrombosis



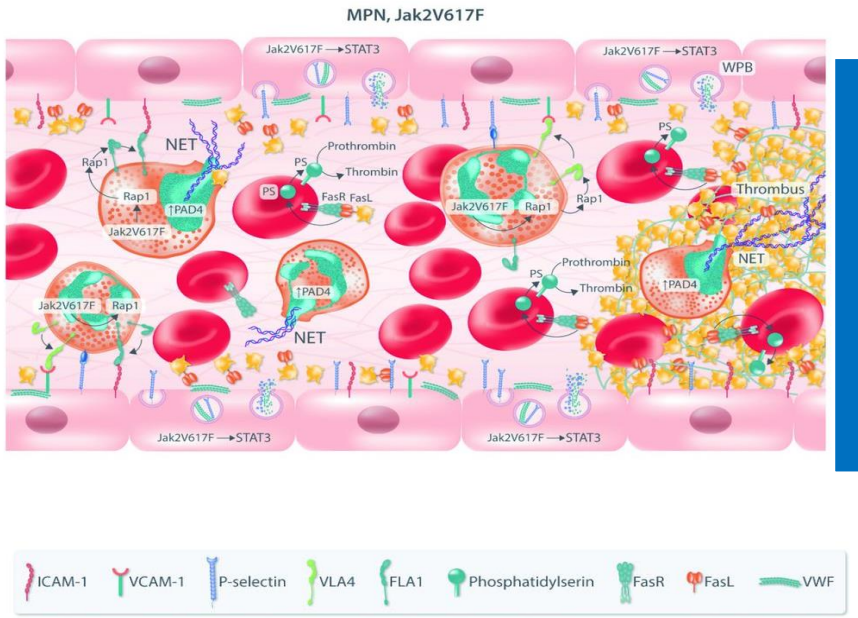


Multivariable analysis:

- *JAK2*V617F VAF > 50% (HR 3.8, p = 0.001) and previous VT (HR 2.2; p = 0.04) as independent risk factors for future VT
- Diabetes (HR 2.4; p = 0.02), hyperlipidemia (HR 2.3; p = 0.01) and previous AT (HR 2; p = 0.04) were independent risk factors for future AT

Guglielmelli P, Loscocco GG et al. Blood Cancer J. 2021 Dec 11;11(12):199.

Is the Guilty JAK2V617F + VAF *per se*, or the Resulting Downstream Changes?



JAK2V617F

Cell Proliferation

PLT

Increase in endothelial cell Weibel-Palade body degranulation of P-selectin , von Willebrand factor and thromboxane

WBC

Activation of the integrins LFA1 and VLA4; and increased neutrophil extracellular trap (NET) formation.

RBC

A red blood cell-platelet interaction through FasL/FasR> externalization of phosphatidylserine

Cytokines and other pro-inflammatory products

Endothelial Dysfunction

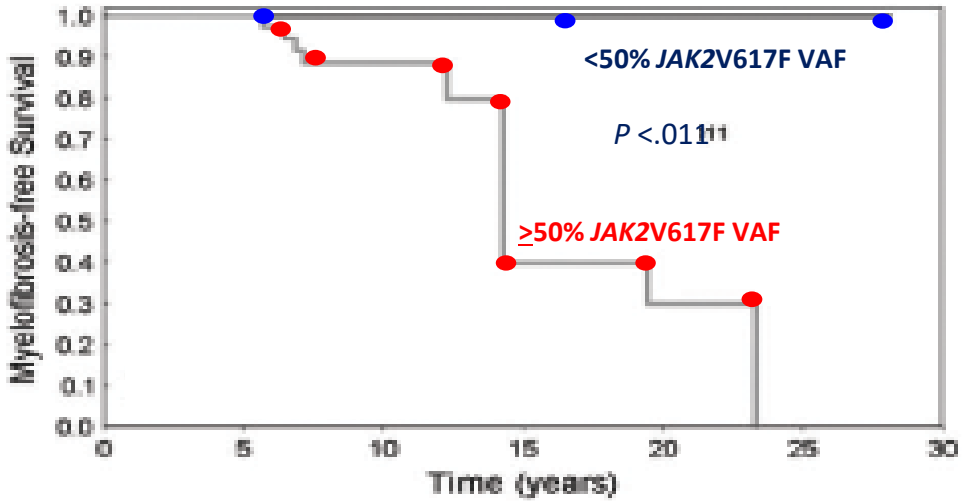
- Leukocyte-platelets interation
- RBCs-platelets interation
- Coagulative activation

Bar-Natan M, Hoffman R. Haematologica 2019;104:3-6; Vannucchi AM et al, Leukemia 2007; Spivak J, NEJM 2004; Barbui et al, Haematologica 2011; Landolfi et al Haematologica 2011; Carobbio et al. JCO 2008;26:2732-2736; Carobbio et al. Blood Adv 2019;3:1729-1737

Accumulation of *JAK2* V617F Mutated Alleles is Associated with Evolution to Myelofibrosis

- Rate of transformation to myelofibrosis depending on the *JAK2*V617F VAF

	WT	Hetero	Homo	*P*
PV	0	2%	12%	<0.01
ET	2%	5%	14%	<0.01



- In 338 PV patients prospectively followed, a 10%, difference in allele burden between two samples corresponded to a 40% increase in risk of post-PV MF

Vannucchi AM, Blood 2007; 110:840-6; Passamonti T, Leukemia 2010; 24:1574-79; Tefferi A, Cancer 2006; 106:331; Silver RT, Leuk Res 2010;

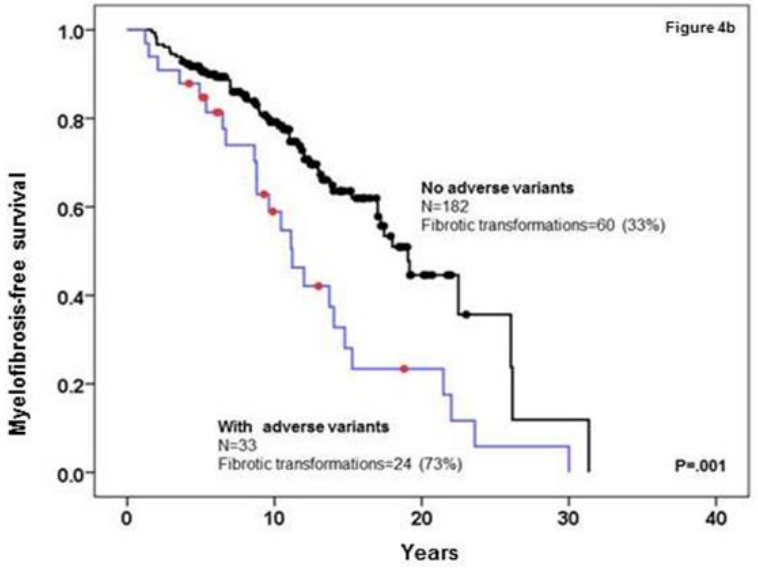
The Acquisition of Additional Mutations in Myeloid Genes is a Common Feature During Progression to sMF

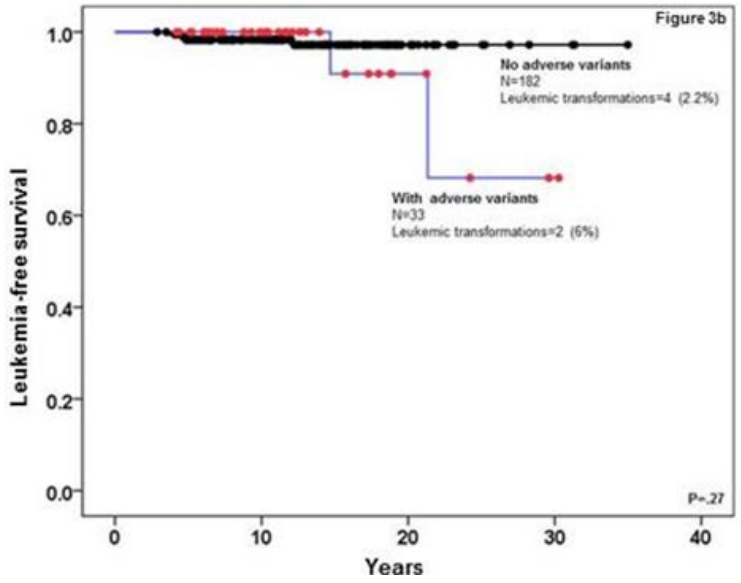
Gene (%)	PV -----→	PPV-MF	ET -----→	PET-MF
N=	*133*	*158*	*183*	*134*
ASXL1	12%	17%	11%	29%
EZH2	0	4%	3%	10%
SRSF2	3%	1%	2%	4%
IDH1/2	2%	6%	1%	1%
TET2	22%	23%	16%	17%
LNK/SH3B3	2%	3%	0	0
ZRSR2	5%	8%	3%	0
SF3B1	3%	16%	5%	5%
SETBP1	2%	6%	2%	6%
DNMT3A	2%	0	6%	5%
CSF3R	3%	0	3%	8%
NRAS	0	0	1%	2%
CBL	1%	0	1%	16%
U2AF1	0	16%	1%	7%
RUNX1	2%	3%	2%	0
TP53	1%	0	2%	9%

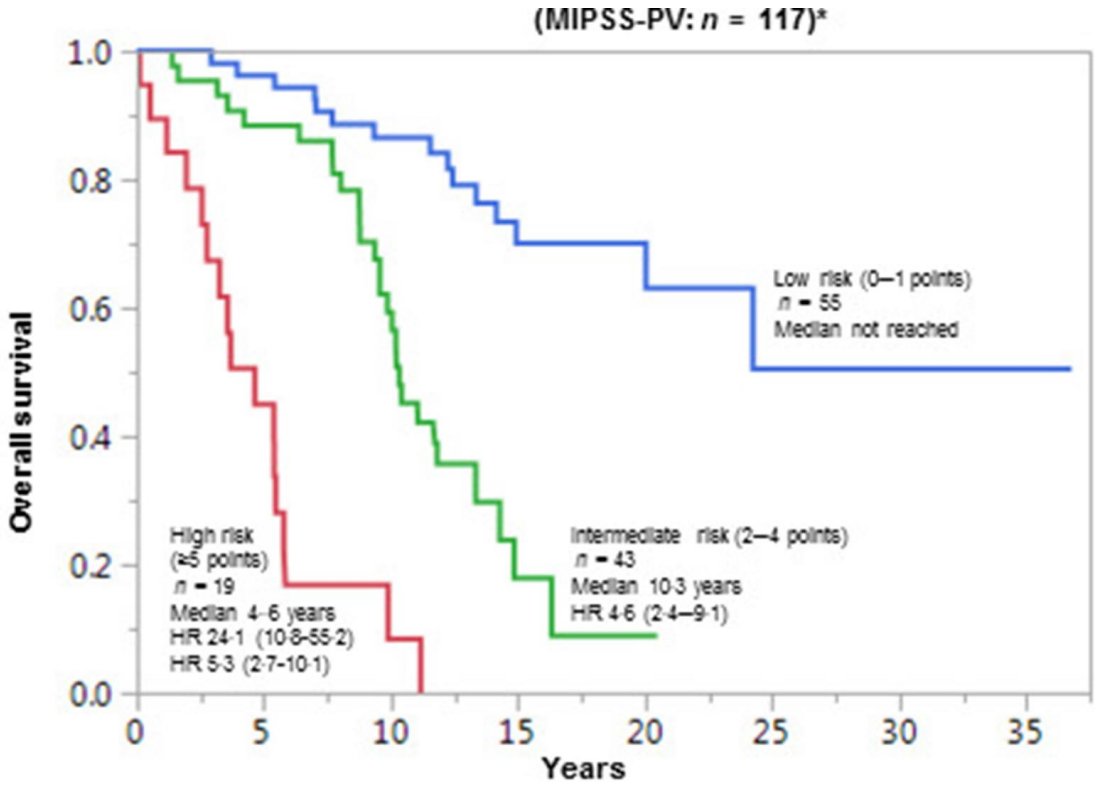
Vannucchi AM et al, Leukemia 2013; 27:1861-9. Tefferi A et al, Bood Adv 2016; 1:21-30; Tefferi A et al, Blood Adv 2016; 1:105-111; Guglielmelli P et al, Blood; 2017:129:3227-3236

Impact of Myeloid-genes Mutations on Survival in PV

PV: *ASXL1,* ***SRSF2,*** *IDH2*



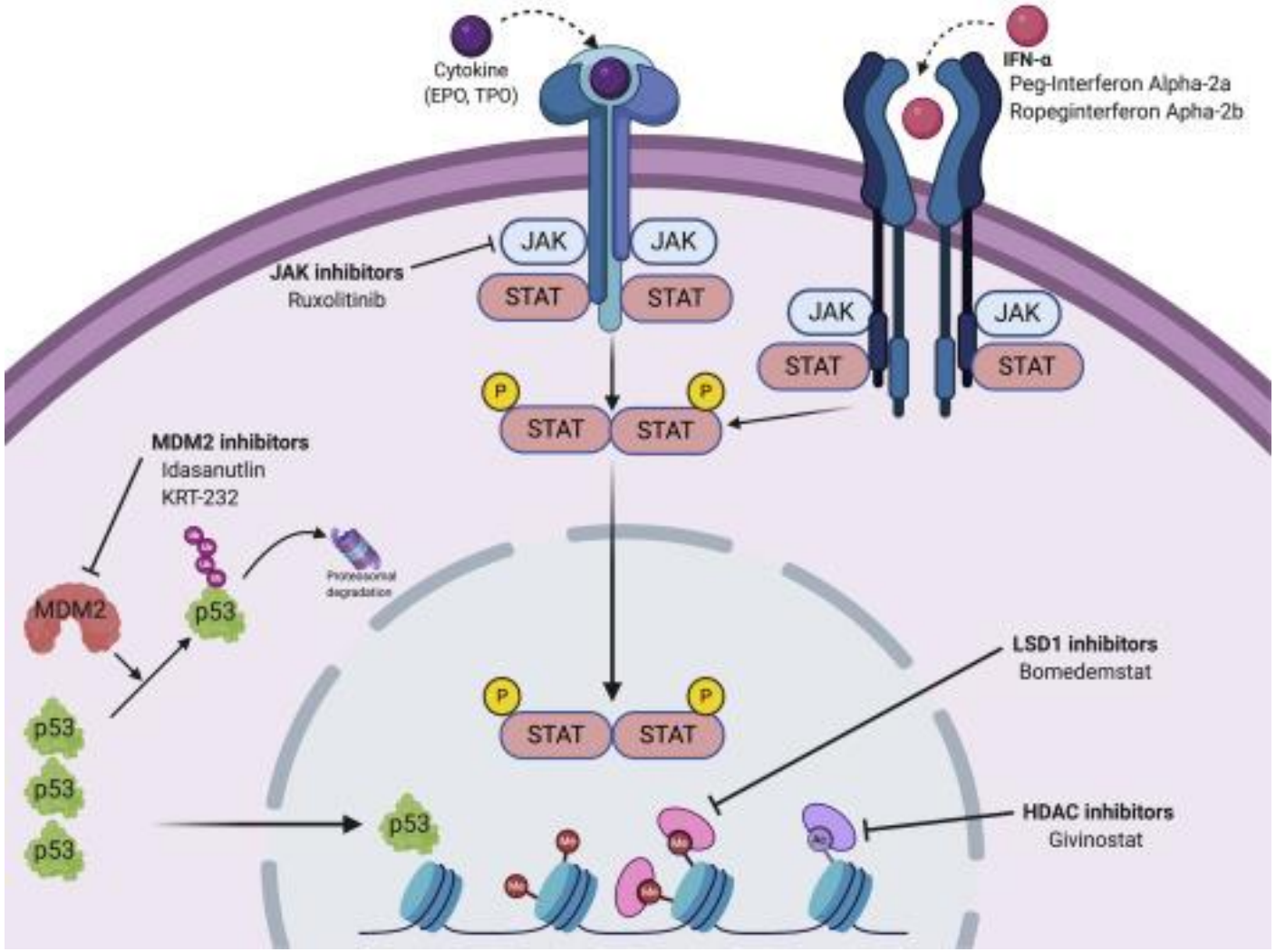




MIPSS-PV was based on four risk factors: presence of adverse mutations (*SRSF2*) (three points); age >67 years (two points); leukocyte count ≥15 × 10^9/l (one point) and thrombosis history (one point).

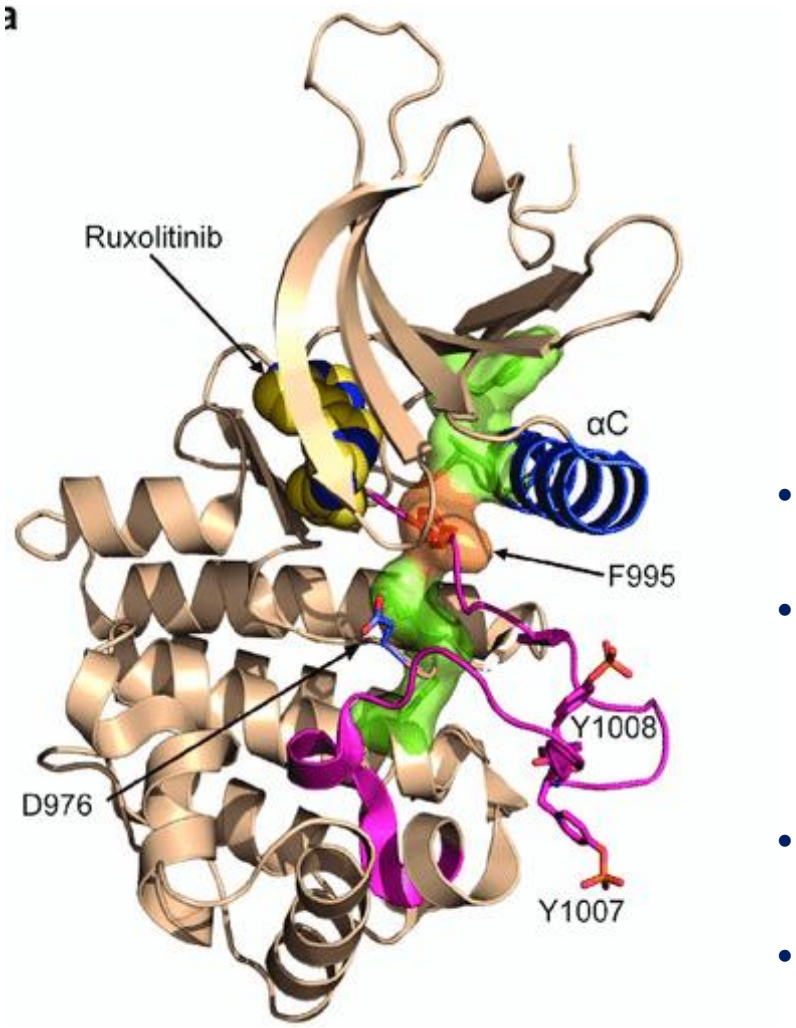
Ayalew Tefferi et al. Blood Adv 2016;1:21-30; Tefferi A et al. Br J Haematol. 2020

Agents that May Lead to Disease Modification in Polycythemia Vera



Kishtagari A et al. Hematol Oncol Clin N Am 35 (2021) 295–303

Strategies Targeting the JAK2 Pathway in PV



Ruxolitinib is a “type I” ATP-competitive inhibitor

The mutated JH2 pseudokinase domain does not bind ruxolitinib directly

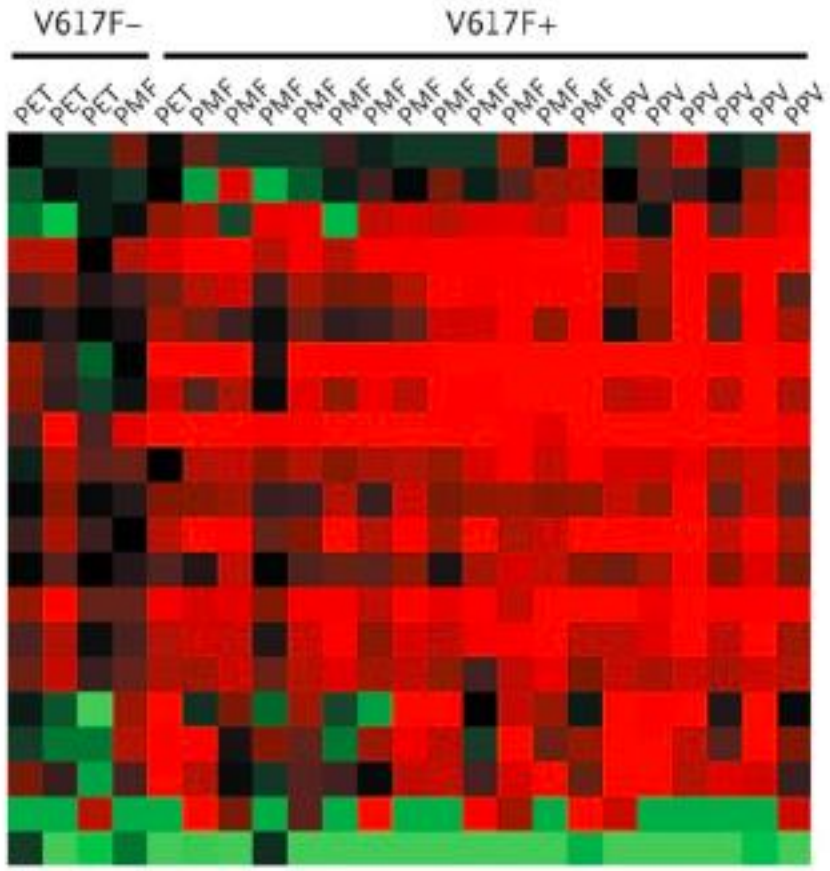
Bind and stabilize the kinase-active conformation of JAK2 and JAK1

- Selective growth impairment of PV erythroid progenitor colonies through a dose-dependent apoptosis
- Reduced cell proliferation. > downstream hypophosphorylation of STAT pathway.

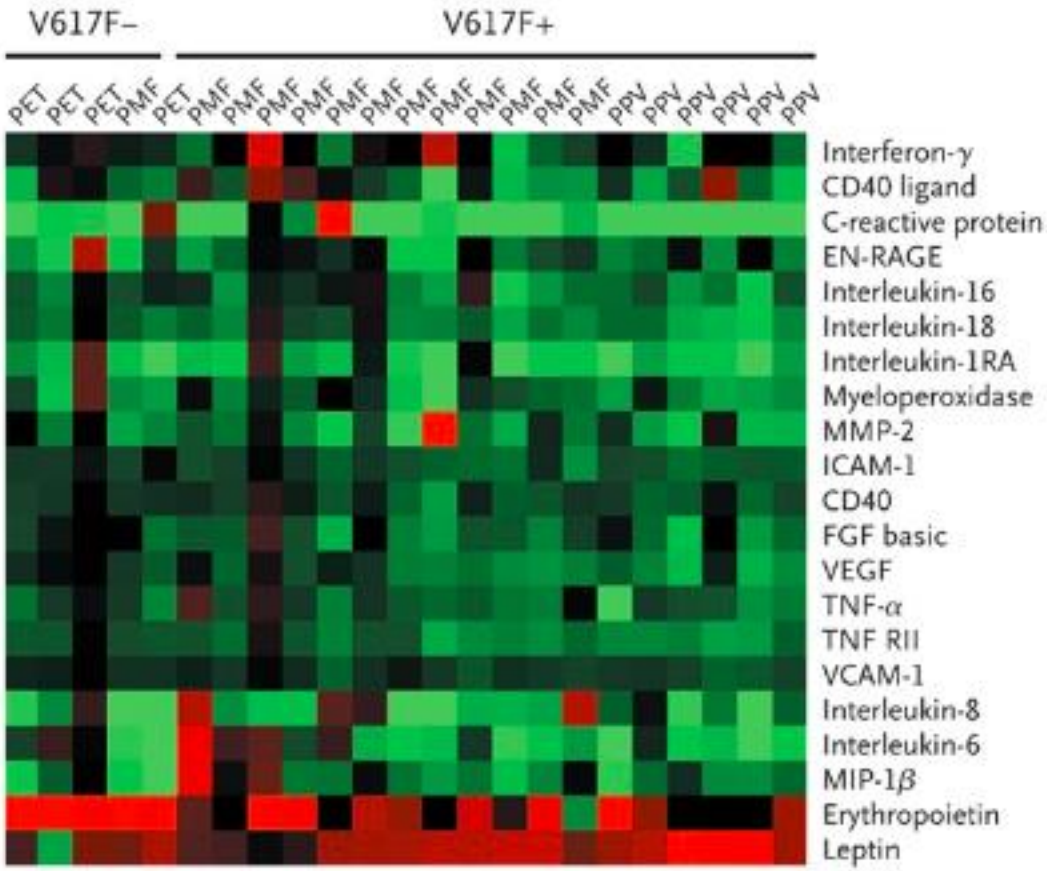
- Due to its anti-JAK activity it has been reported to improve splenomegaly and constitutional symptoms
- The ubiquitary inhibition explains the hematological side effects (anemia and thrombocytopenia) and the immunosuppressive effects

Plasma levels of several proinflammatory cytokines are reduced in Ruxolitinib Treated Patients

Plasma Levels
At baseline vs Healthy Controls



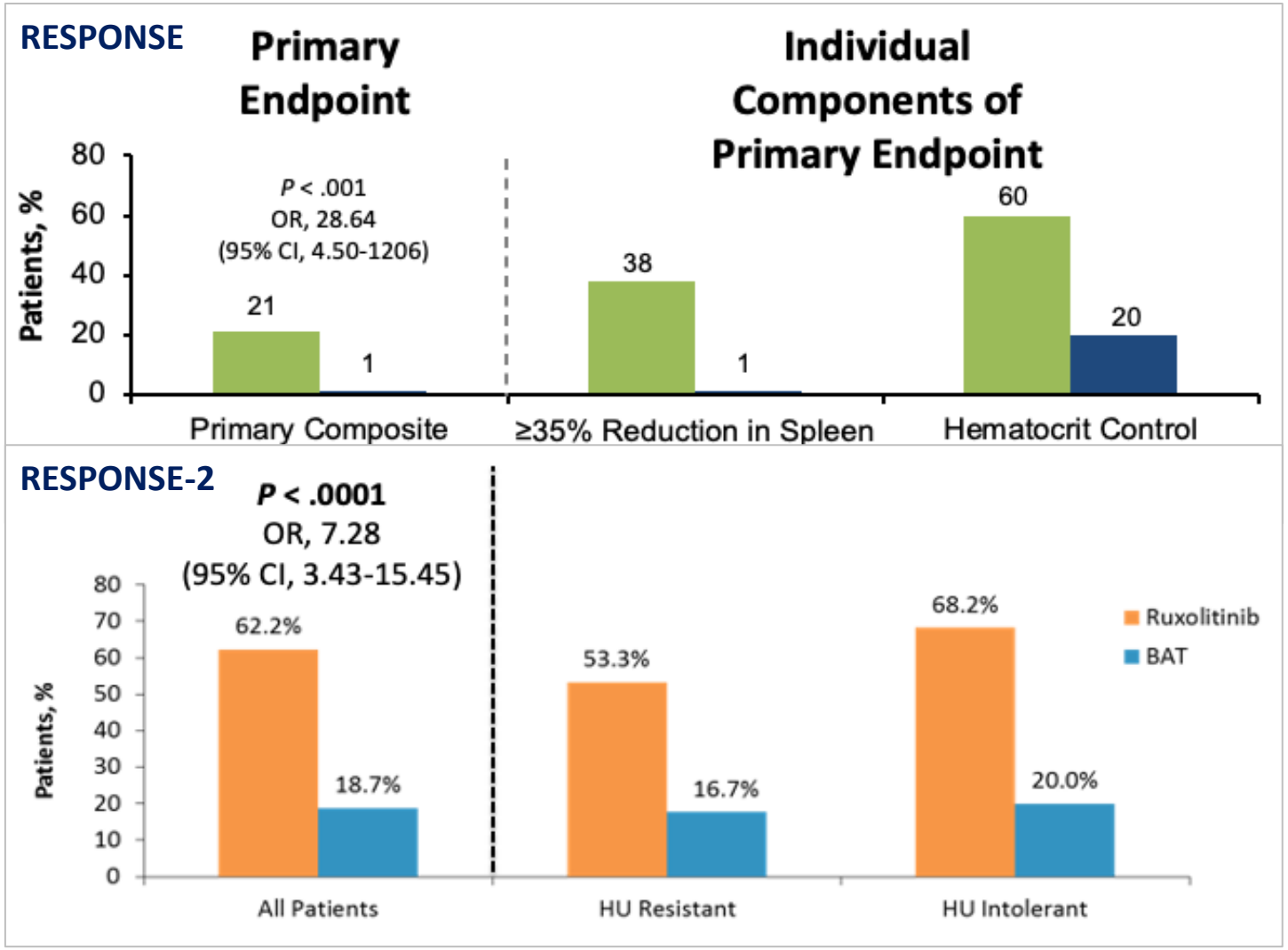
Plasma Levels
after 28 days of Ruxo treatment vs baseline



Mesa R et al, Cancer 2007; 109:68- 71. Scherber R et al, Blood 2011; 118:401-8. Geyer H et al, Blood 2014 123:3803-3810. Verstovsek S, NEJM 2010; 363:1117-27

Phase III Trial RESPONSE and RESPONSE-2 in PV

Hematocrit control ± Spleen Volume Reduction



- Led to the approval of **ruxolitinib** for PV patients R/R to HU

Vannucchi AM et al, NEJM 2015: 372:426-35 Passamonti F et al, Lancet Oncol 2017; 18:88-99

Ruxo in Real-Word Treatment of PV Patients R/R to HU

- N= 377 patients with R/R to HU, 105 treated with ruxolitinib and 272 BAT (60%HU, no active treatment 8%, IFN 4%)
- Median duration of Ruxo treatment: 2 y (0.1-8 y)
- Permanent discontinuation: 16 % (17 patients)

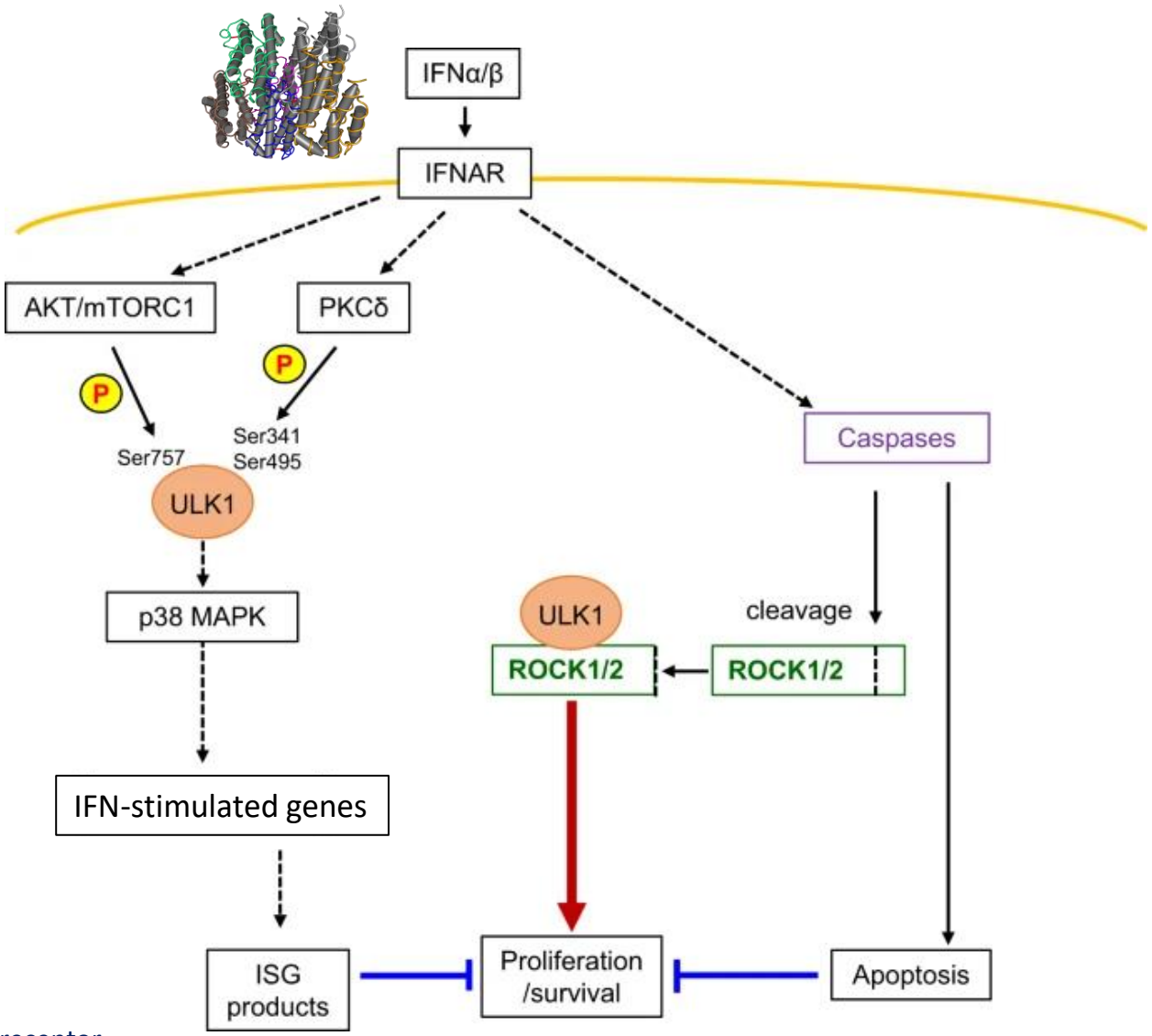
TABLE 3. Incidence of Thrombosis and Major Bleeding in 377 Patients With Polycythemia Vera Who Were Treated With Ruxolitinib or BAT After Developing Resistance/Intolerance to Hydroxyurea

	Ruxolitinib (251 Person-y)		BAT (1272 Person-y)		
	No. of Events	Incidence Rate[a]	No. of Events	Incidence Rate[a]	*P*
Arterial thrombosis[b]	1	0.4	29	2.3	.03
Venous thrombosis[c]	2	0.8	14	1.1	.7
Major bleeding[d]	2	0.8	11	0.9	.9

Abbreviations: BAT, best available therapy; CI, confidence interval; IRR, incidence rate ratio.
[a]Events per 100 person-years.
[b]IRR, 0.18; 95% CI, 0.02-1.3; $P = .09$ (adjusted by propensity score).
[c]IRR, 1.1; 95% CI, 0.3-3.9; $P = .9$ (adjusted by propensity score).
[d]IRR, 0.9; 95% CI, 0.2-4.9; $P = .9$ (adjusted by propensity score).

Alvarez-Larran A et al, Cancer 2022;

Signaling Feedback Circuit Regulation of IFN-mediated Anti-neoplastic Responses



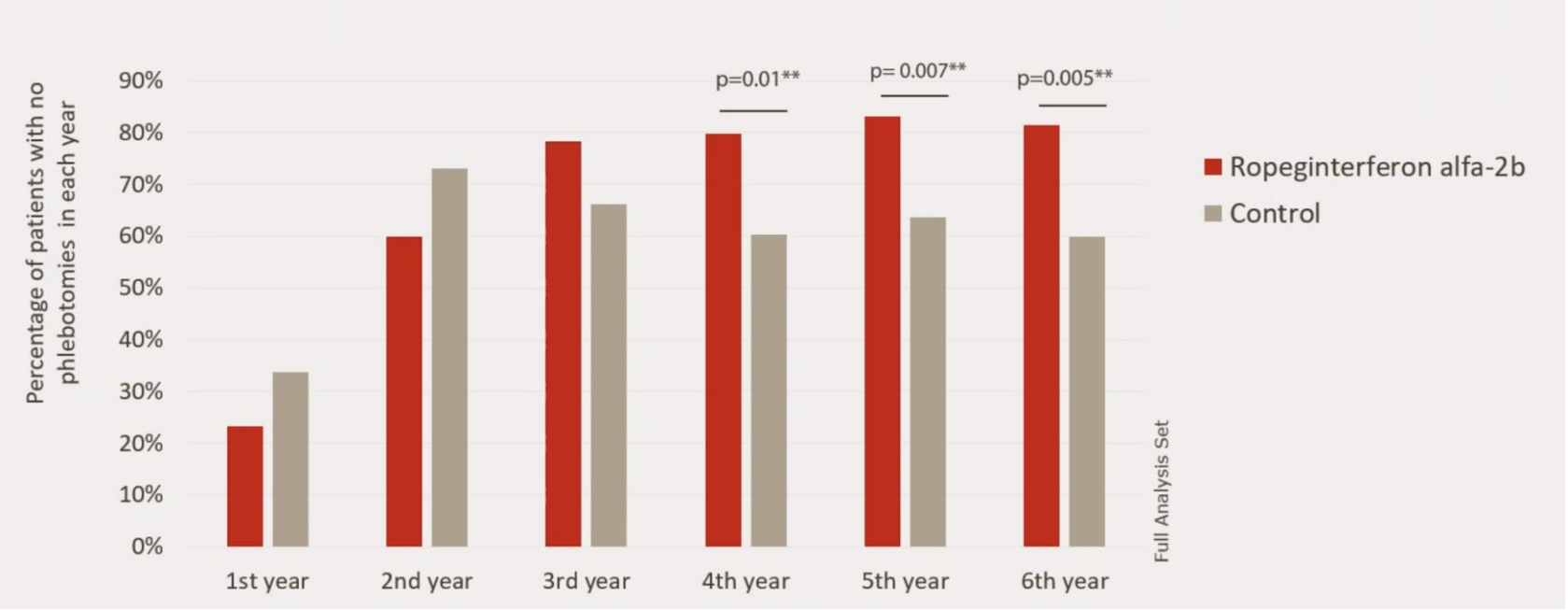
ULK1: Unc-51-like kinase 1
IFNAR: Type I IFNs bind their transmembrane receptor
ROCK : Rho-associated coiled-coil protein kinase 1

Healy FM, et al. Front Oncol. 2021. ;Saleiro D et al. Nature Communications 2022; 13: 1750

Higher response rates for Ropeginterferon alfa-2b versus control treatment at 6 years

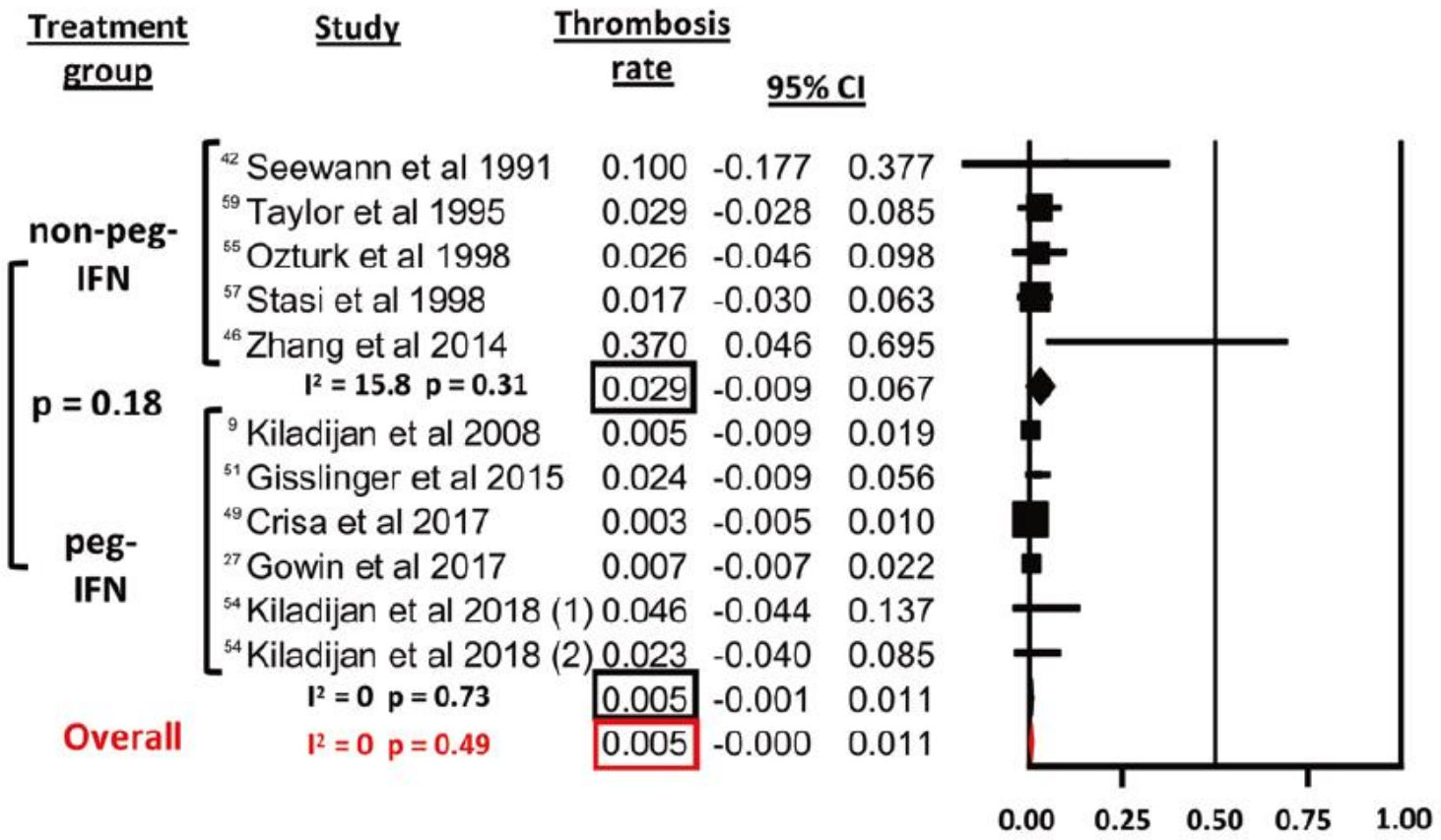
In the 6th year of treatment, no phlebotomies were required to maintain hematocrit <45% in 81.4% of patients receiving ropeginterferon alfa-2b compared with 60.0% of patients in the control arm (p=0.005).

	Ropeginterferon alfa-2b N=95		Control N=74		RR (95% CI)	P-value
CHR*	48/88	54.6%	22/63	34.9%	1.55 (1.07 to 2.26)	**p=0.02**



Gisslinger et al, Lancet Haematol . 2020 Mar;7(3):e196-e208Kiladjian JJ et al. Leukemia 2022, 36, pages1408–1411 (2022)

Interferon Reduces Thrombotic Risk in Patients with PV



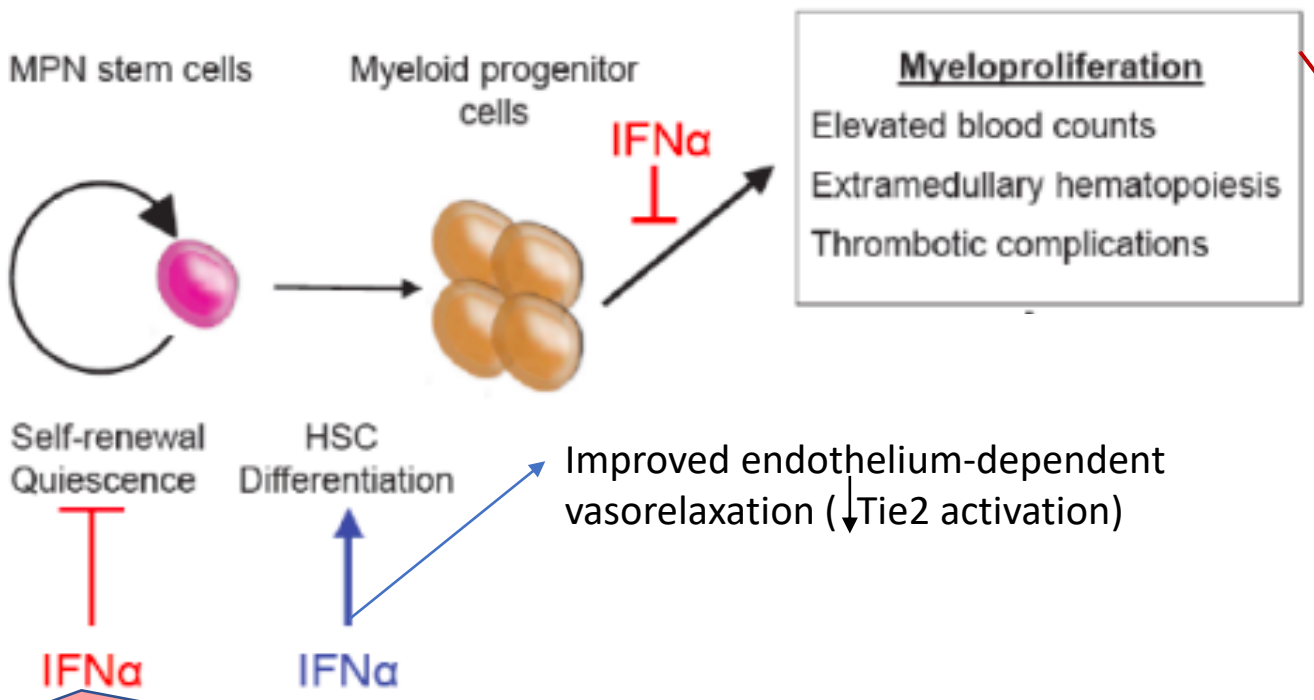
The rate of thromboembolic

- complications was uniformly low at 0.5% per patient year (95% CI 0.0–1.1%;
- The rate was not statistically significantly different (p=0.18) between peg- and non PEG-IFN

(n= 629)
Systematic review and metanalysis

Jan Philipp Bewersdorf et al, Leukemia 2020

Inhibition of Several Pathways Reduce Prothrombotic Activation in MPNs



Ruxolitinib

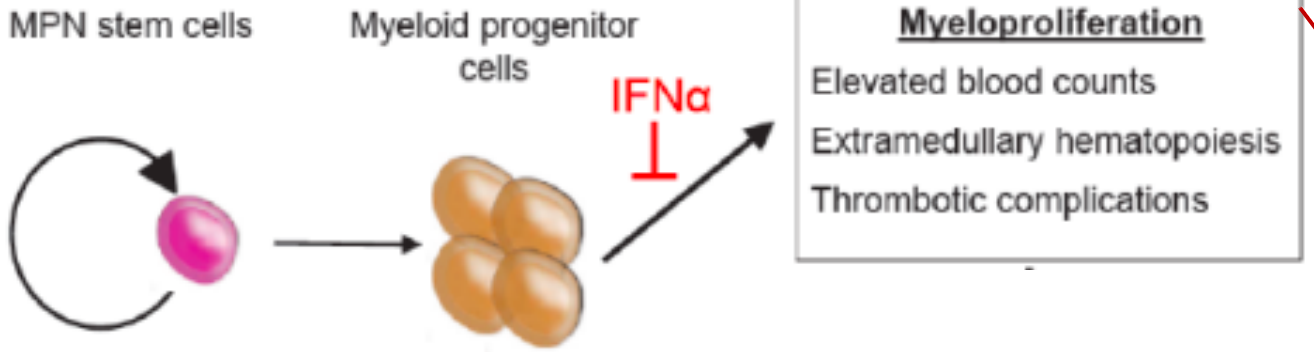
- Reduction of Neutrophil extracellular traps (NET)
- reduces endothelial prothrombotic activation and leukocyte–endothelial proadhesive interactions
- reduced expression of VWF, VCAM-1 and P-selectin (↓ Endothelial Pro-Adhesive Interactions)
- Reduce several proinflammatory cytokines

↑ STAT1 phosphorylation:

- increased induction of ROS
- DNA damage
- reduction in quiescence

Wolach O et al. SCIENCE TRANSLATIONAL MEDICINE 2018 Vol 10, Issue 436; Beckman JD et al. J Thromb Haemost. 2023;21:1366–1380

Inhibition of Several Pathways Reduce Prothrombotic Activation in MPNs



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- Reduction of Neutrophil extracellular traps (NET)
- reduces endothelial prothrombotic activation and leukocyte–endothelial proadhesive interactions
- reduced expression of VWF, VCAM-1 and P-selectin (↓ Endothelial Pro-Adhesive Interactions)
- Reduce several proinflammatory cytokines

Improved endothelium-dependent vasorelaxation (↓Tie2 activation)

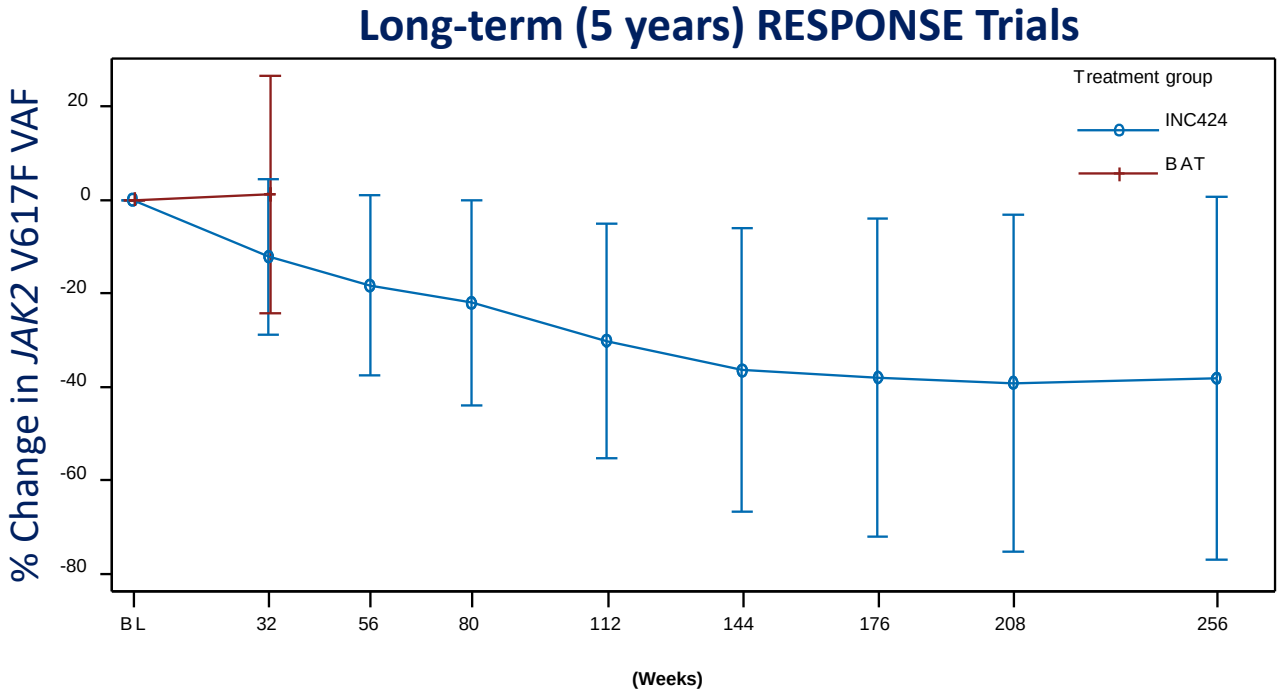
↑ STAT1 phosphorylation:

- increased induction of ROS
- DNA damage
- reduction in quiescence

IFNa + Ruxolitinib → **Reduction of *JAK2* Mutant Allele**

Wolach O et al. SCIENCE TRANSLATIONAL MEDICINE 2018 Vol 10, Issue 436; Beckman JD et al. J Thromb Haemost. 2023;21:1366–1380

*JAK2*V617F VAF Changes in PV Patients Treated with Ruxolitinib

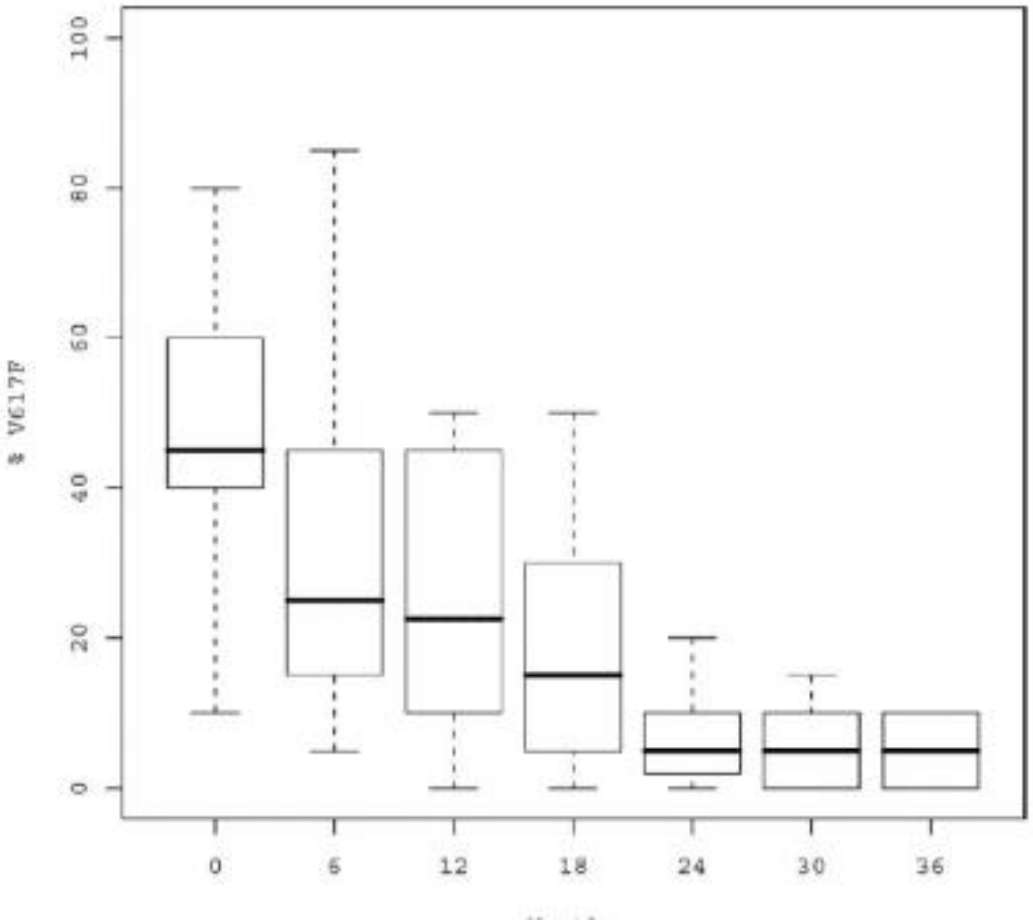


In the ruxolitinib arm, the mean percent change from baseline in VAF was **–38.12%** (SD: 38.64, n = 66) at week 256. It was **–22.88%** (SD: 40.5, n = 64) at week 224 in the crossover population.

In the **MPN-SVT** Mynerva trial at a median of 5.5 yr of treatment reduction of *JAK2*V617F VAF>50% was documented in 40% of the pts, although it was not correlated with clinical parameters

Vannucchi AM et al, AOHE, 2017; 96:1113-20; Kiladjian JJ et al. Lancet Haematol . 2020 Mar;7(3):e226-e237; Paoli C et al. Abstrcat 1662P, Orlando ASH2019.

IFN-alpha induces high rates of CHR and *JAK2*V617F VAF reduction

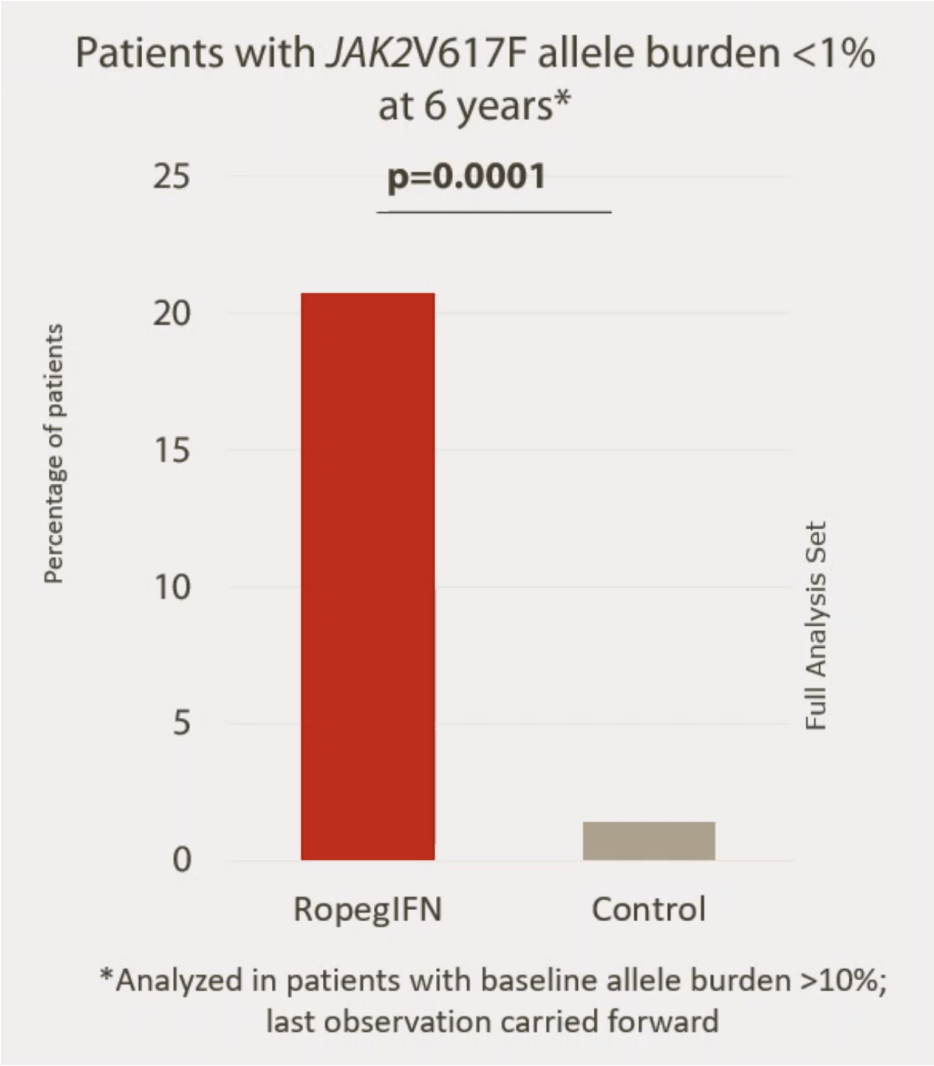


- A molecular response does not always accompany a hematologic response
- the presence of somatic mutations affected outcomes, with a higher frequency of mutations in genes outside of JAK2, most commonly, TET2, DNMT3A, and ASXL1, in patients failing to achieve a CMR (56%) versus those achieving CMR (30%);
- responses to IFN have also been reported in patients with CALR-mutated ET

Kiladjian JJ et al. Blood 2008;112:3065; Kuriakose E et al. Haematologica 2012;97:538-42; Verger E et al Blood 2015;126:1762

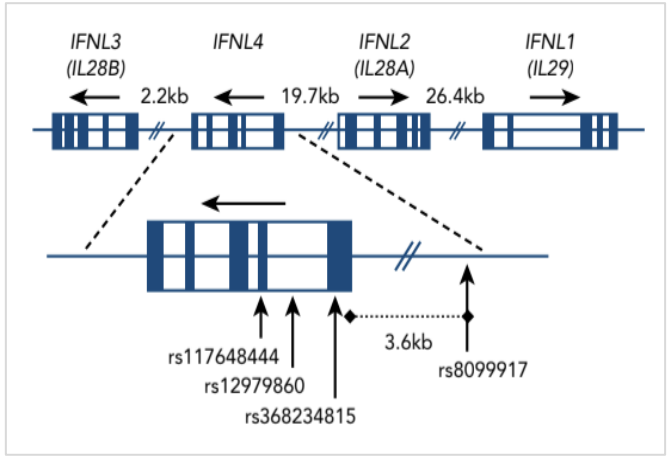
Final PROUD/CONTI data
Effect of Ropeginterferon alfa-2b on *JAK2* Mutant Allele

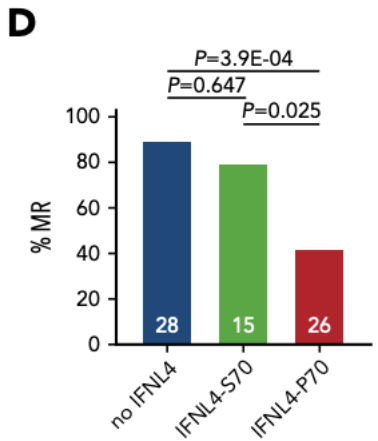
- After 6 years of treatment, the JAK2V617F allele burden decreased to <1% in 20.7% of patients in the ropeginterferon alfa-2b arm.
- In contrast, only 1.4% of patients in the control arm achieved an allele burden <1% at 6 years of treatment (p=0.0001).
- The molecular response rate was higher in low-risk versus high-risk patients (84.4% vs 49.0%;p=0.0009)
- Molecular response was achieved more rapidly in low-risk patients (12 months vs 18 months ; p=0.03)

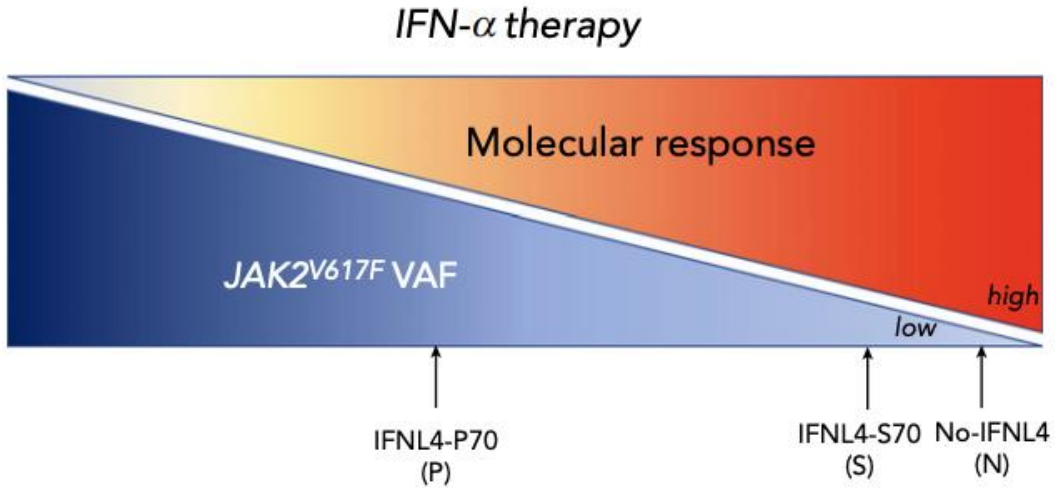


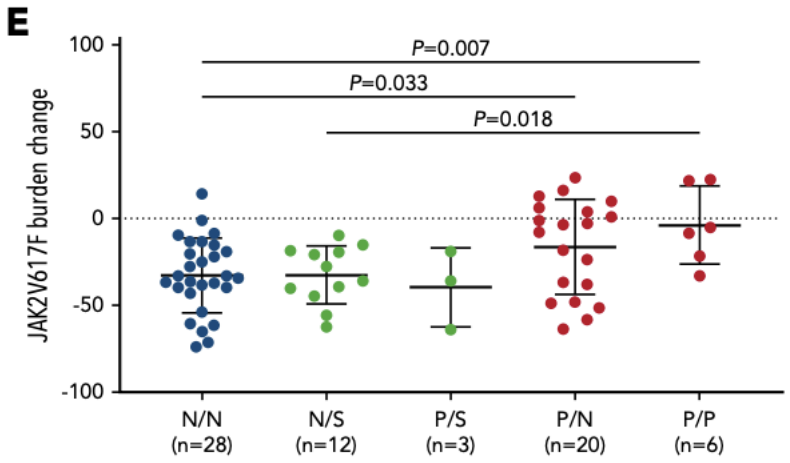
Gisslinger et al, Lancet Haematol . 2020 Mar;7(3):e196-e208Kiladjian JJ et al. Leukemia 2022, 36, pages1408–1411 (2022)

Germline Genetic Factors Influence the Outcome of Interferon-a Therapy in Polycythemia Vera









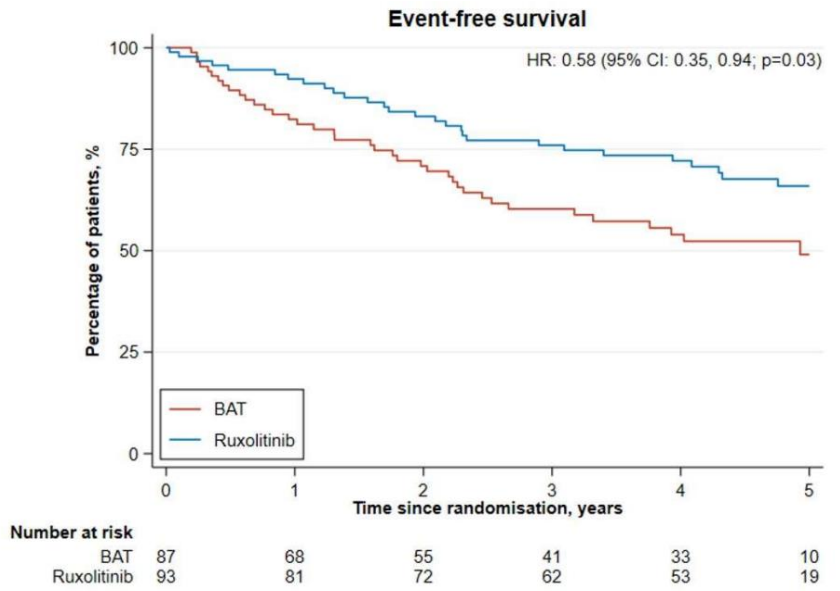
- Harboring no-IFNL4 (or the S variant, with impaired activity) had a positive impact on the rate of Molecular Response (MR) at 36-mo in the PROUD-PV and CONTINUATION-PV trial.
- No correlation with obtainement of Hemato Response.
- *IFNL4* encodes for type III IFN-lambda4.

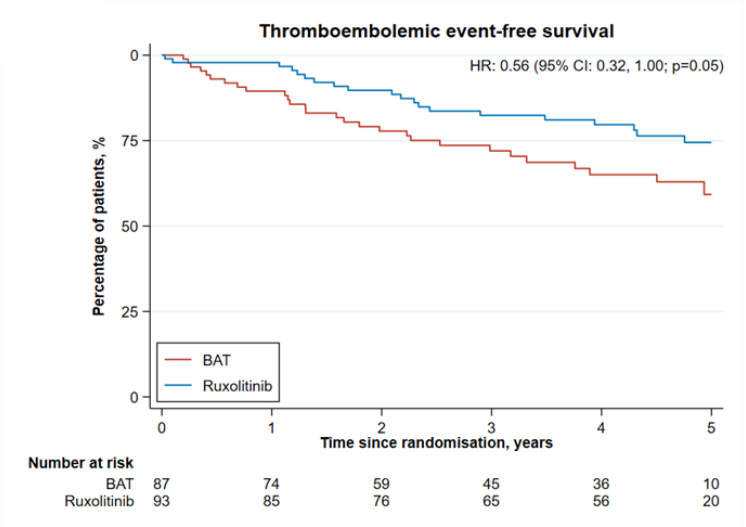
N, no IFNL4; **S**, IFNL4-Serine70; **P**, IFNL4-Proline70.

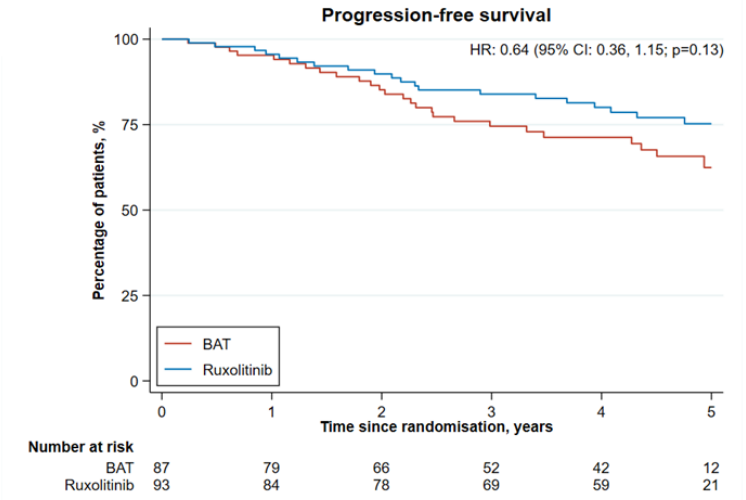
Jager R et al, Blood 2021; 137:387-91; Plo I, W Vainchenker, Blood 2021; 137:291-3.

Ruxolitinib versus Best Available Therapy for PV Intolerant or resistant to HU: Final Results of Majic Randomized Phase II Trial

180 evaluable high-risk PV patients resistant/intolerant to HU: 93 Ruxo vs 87 BAT





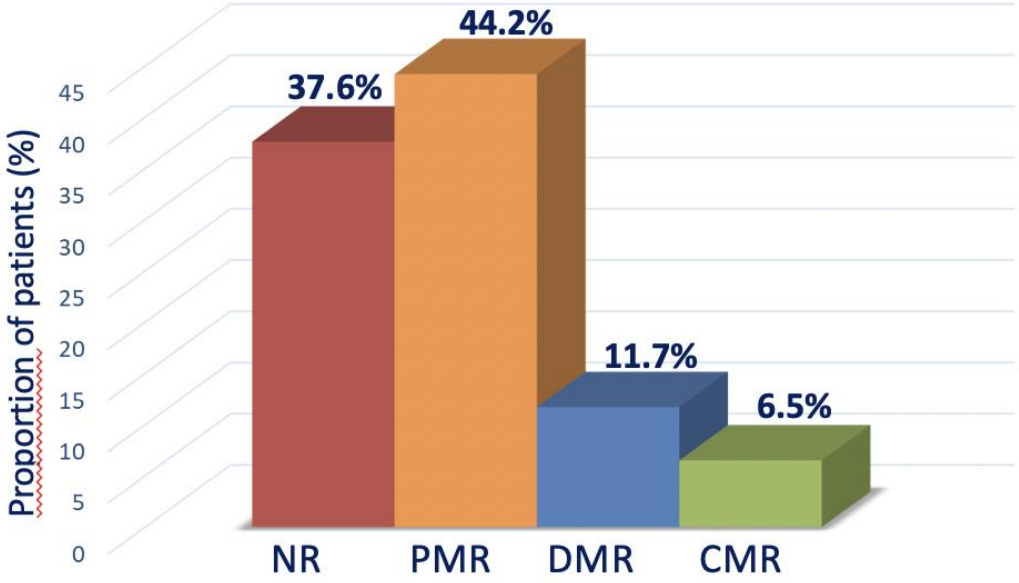


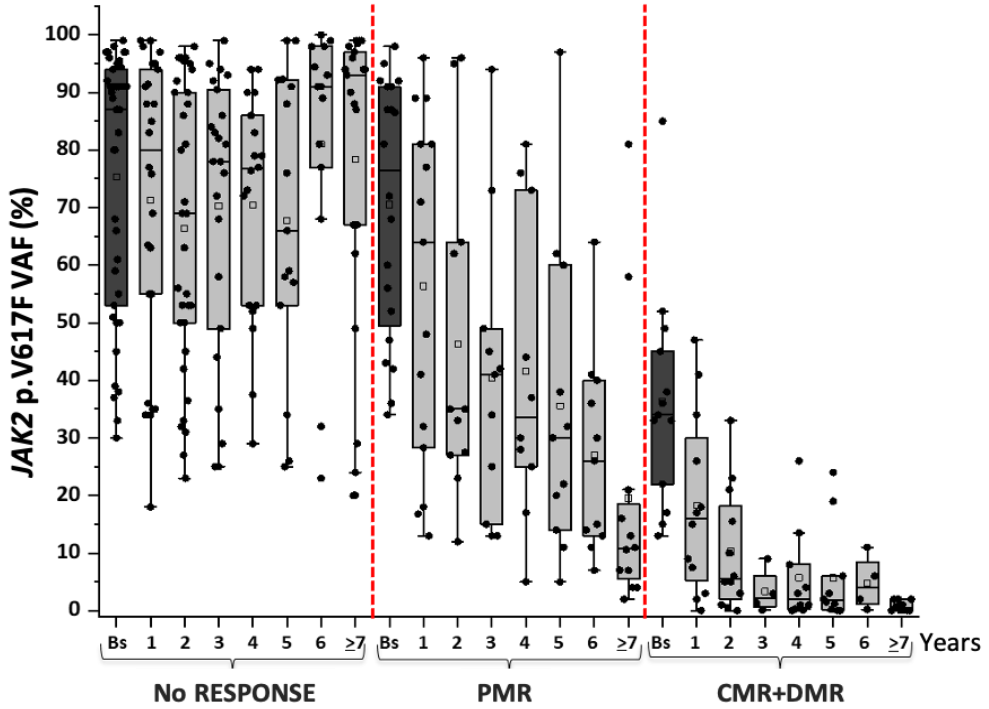
- No significant difference was observed between Ruxo and BAT in regards to hemorrhages or OS
- Event free survival (major hemorrhage/ thrombosis, transformation or death) was superior for both ruxolitinib & for attaining a CR within 1 yr (HR 0.41; p=0.01)
- Molecular response at 1 year correlated with superior EFS (all except thrombosis)
- Additional mutations (independent of age) were associated with less likelihood of molecular response and worse EFS (es. ASXL1).

Harrison CN, et al. J Clin Oncol. 2023

*JAK2*V617F Molecular Response in Ruxolitinib Long-term Treated Patients with PV

77 patients : 64 PV, 13 ET long-term treated (median, 8.8 years) with ruxolitinib.



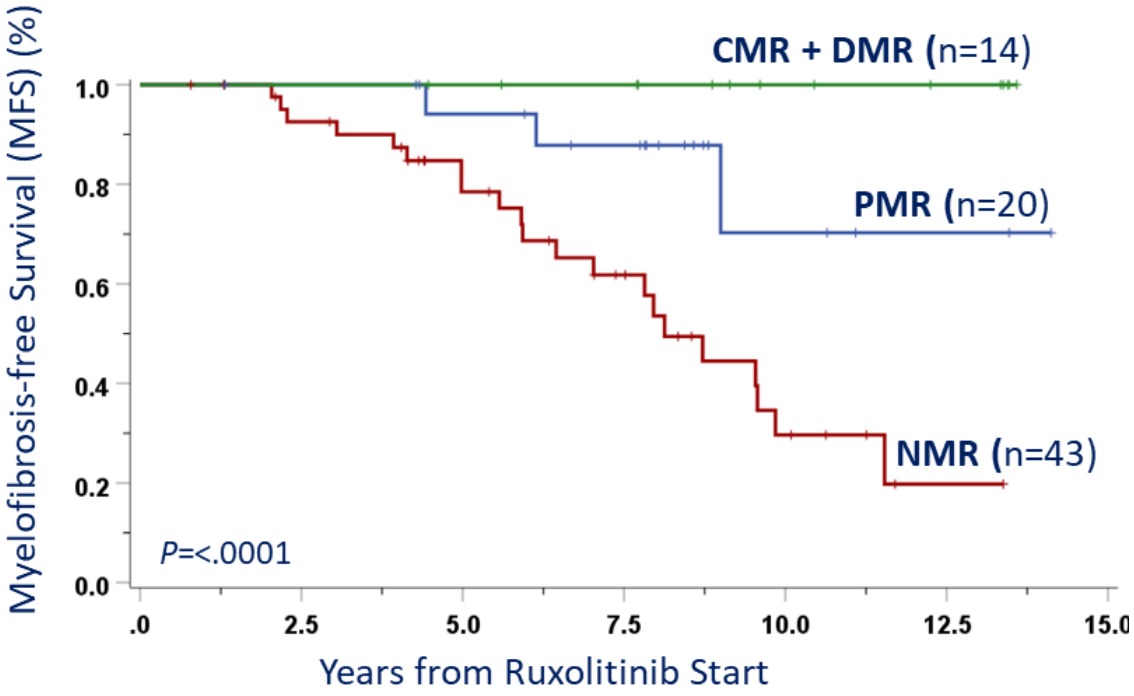


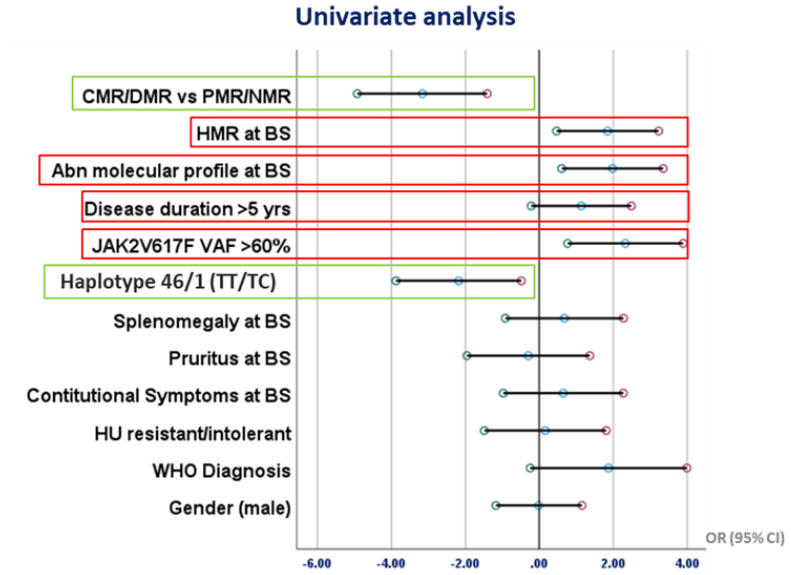
- There was no correlation between molecular response and response of Hct, platelets and spleen length reduction.
- A baseline JAK2V617F VAF level of <60% was associated with a significantly greater likelihood to obtain CMR+DMR (37.1% vs 2.4%; P<0.0001) as well as PMR (60% vs 38.2%, P=0.01).

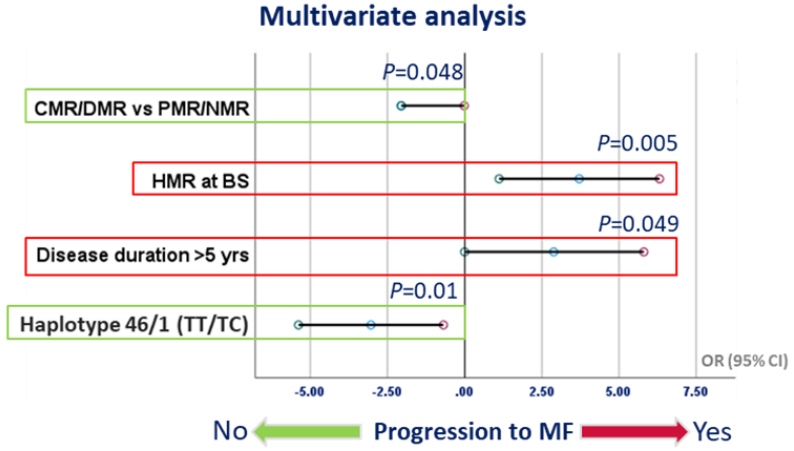
- Median time to CMR and DMR was 4.6y (1.1-7.6y) and 5.0y (2.1-12.1y), respectively.
- Median duration of CMR + DMR was 8y (7-12y).
- All CMR + DMR pts have ongoing molecular responses at data cutoff.

Guglielmelli P. et al, ASH 2022 Abstract n. 741

JAK2V617F Molecular Response to Is Associated with Lower Risk of Progression to Secondary Myelofibrosis

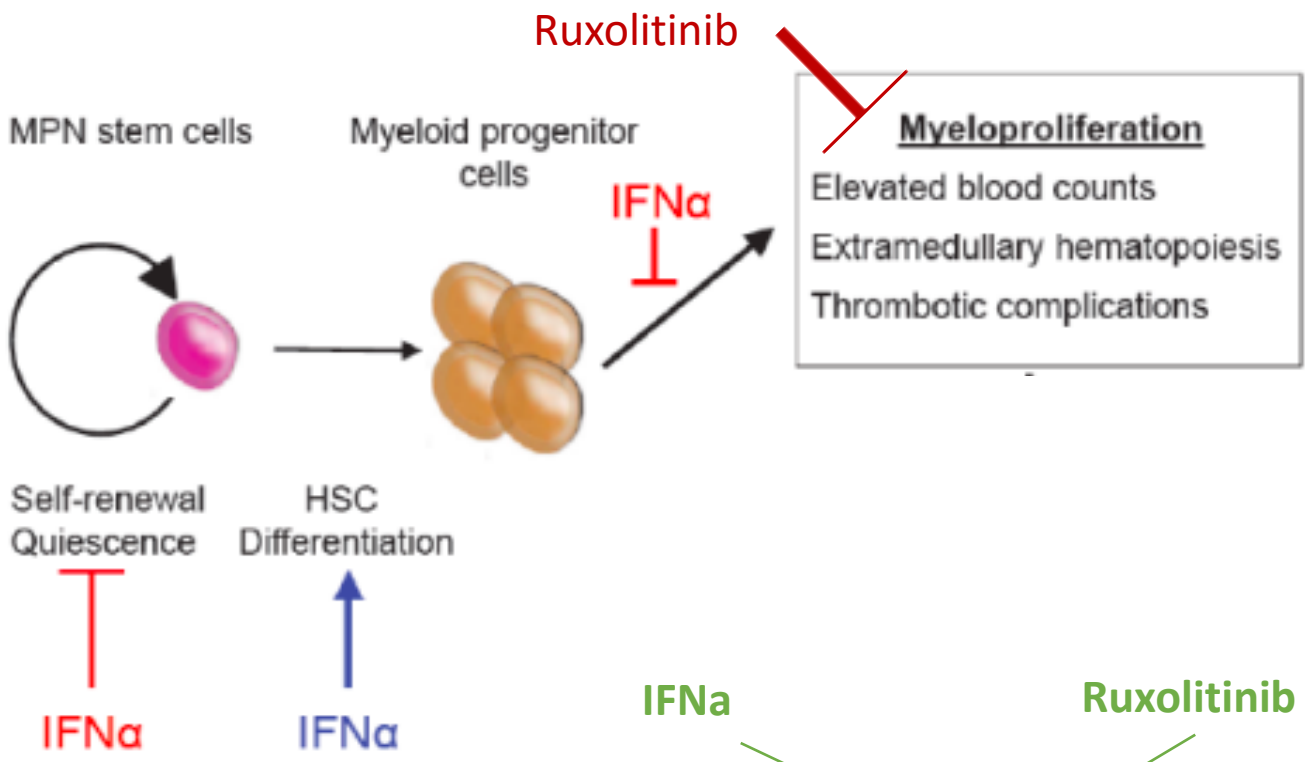






Guglielmelli P. et al, ASH 2022 Abstract n. 741

Attainment of Molecular Response May be a Surrogate of Disease Modifications in PV



- Reduce Blood cells
- Reduce Thrombosis

- Reduce BM fibrosis
- Reduce Risk of Progression in sMF

- ??? Reduce AML progression ???

IFNa + Ruxolitinib → **Reduction of *JAK2* Mutant Allele** → **JAK/STAT Inhibition**

Acknowledgments

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