



JAK2-Mutation Promoted Thrombosis: From Basic Science to Treatment Approaches

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Agenda Overview



- Introduction to JAK2 mutation
- Pathophysiology of JAK2-mutation promoted thrombosis
- Current treatment approaches



Mechanisms of JAK2 Mutation

JAK2 Pathway Activation

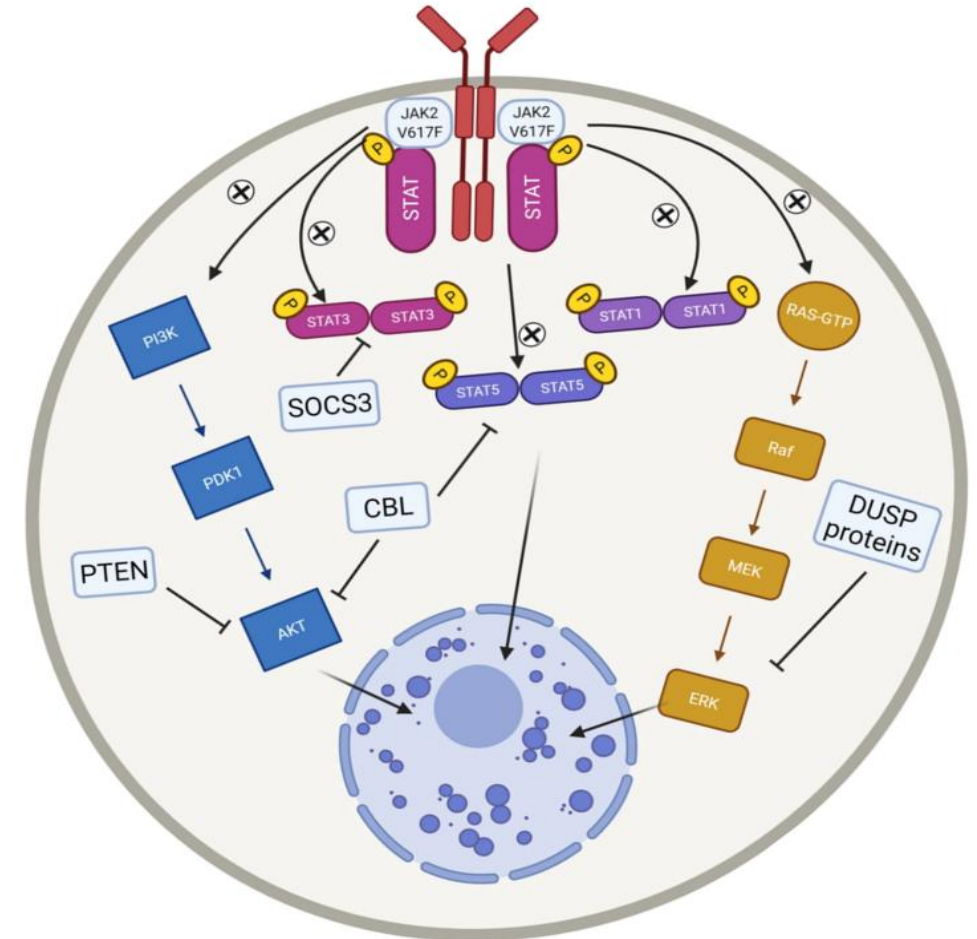
The V617F mutation causes constant activation of the JAK2 signaling pathway, disrupting normal cell signaling processes.

Excessive Blood Cell Proliferation

Constitutive activation of JAK2 leads to increased proliferation of blood cells, contributing to blood disorders.

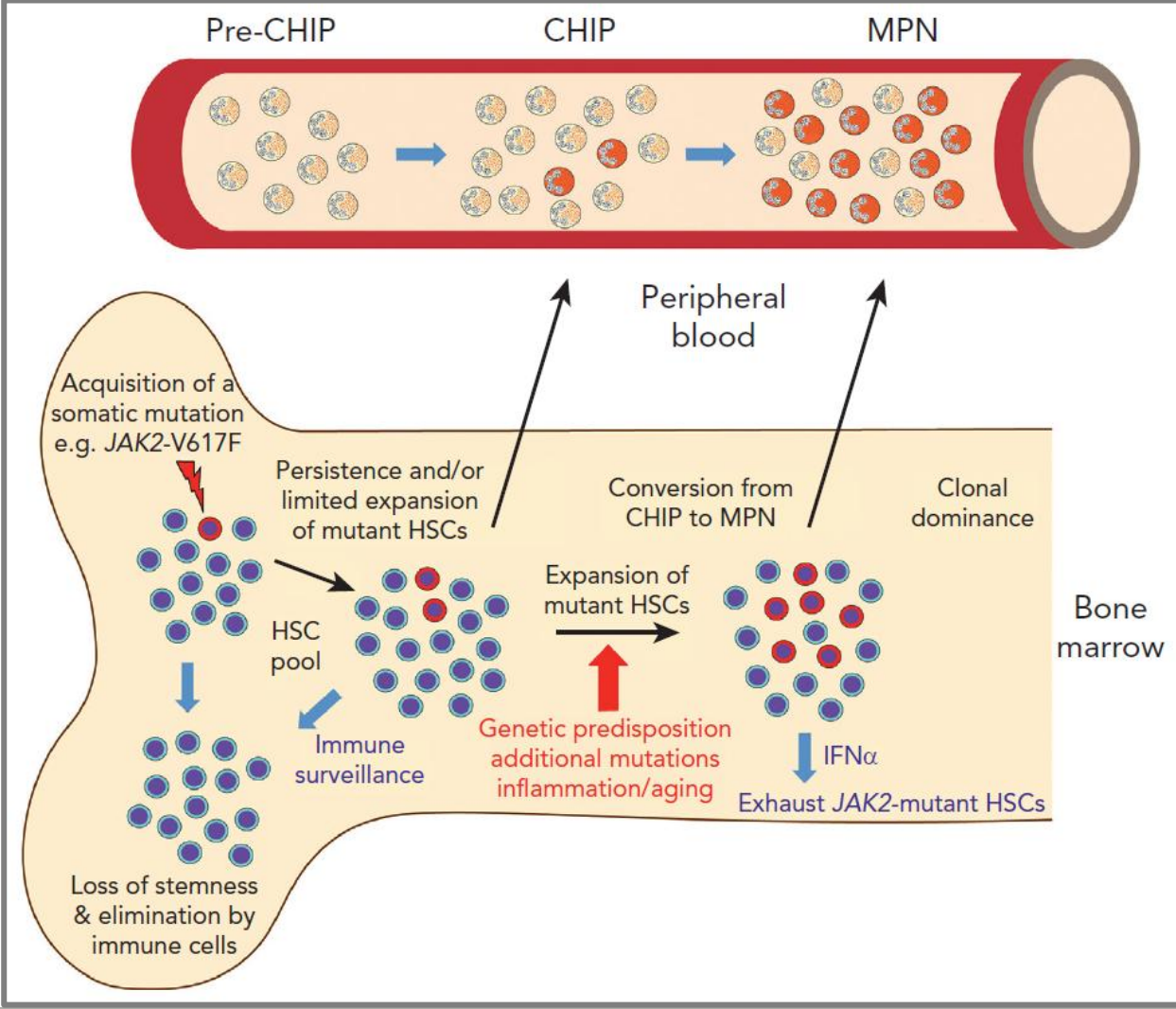
Risk of Thrombosis

Increased blood cell production raises the risk of thrombosis, a serious health condition characterized by blood clots.





Evolution of MPNs originating from a single HSC that acquired a disease driver mutation





Pathophysiology of JAK2-Mutation Promoted Thrombosis

Thrombosis risk varies significantly across different types of MPNs

Polycythemia Vera (PV):

- Annual incidence of about 2.6% to 5.5% per patient-year.
- Both arterial and venous thromboses are common, with arterial events like stroke and myocardial infarction being more frequent.

Essential Thrombocythemia (ET):

- The risk of thrombosis from 2-4% per patient-year.
- Arterial thromboses, such as transient ischemic attacks and myocardial infarctions, are more prevalent than venous events.

Primary Myelofibrosis (PMF):

- Thrombosis risk in PMF is approximately 2.2% per patient-year.

Prefibrotic Myelofibrosis:

- This subtype has a thrombosis risk similar to PMF, around 2.1% per patient-year.

The presence of the JAK2 V617F mutation further amplifies the risk of thrombosis across all MPN types. Additionally, factors like age, history of thrombosis, and elevated blood cell counts can influence individual risk levels.



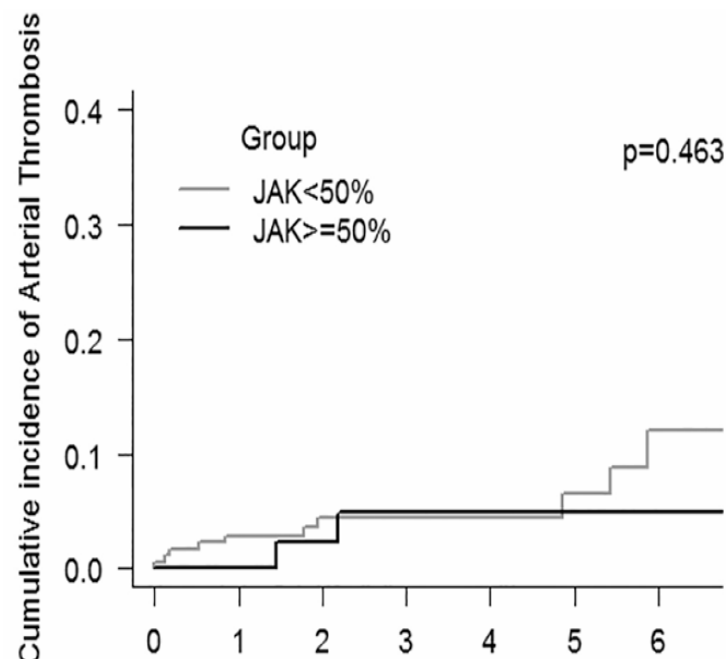
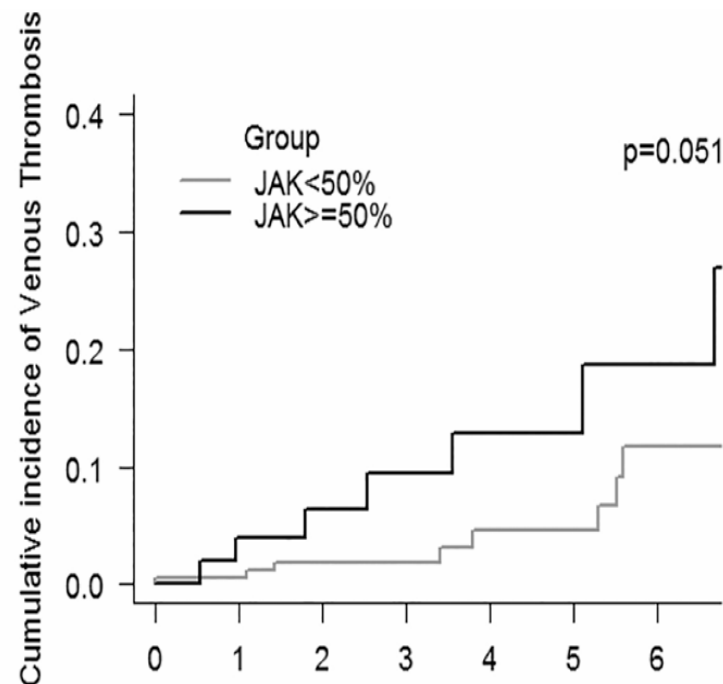
How JAK2 Mutation Leads to Thrombosis

- Individuals with JAK2V617F-mutant CHIP have 12 times the risk of coronary heart disease and ischemic stroke compared to individuals without any CHIP-associated mutation.
- ECs carrying the JAK2V617F mutation can be detected in isolated liver or spleen ECs in many patients with MPNs^{1,2}.
 - JAK2V617F-mutant spleen ECs co-exist in the same patients and mutant ECs comprise 40% to 100% of all splenic ECs.

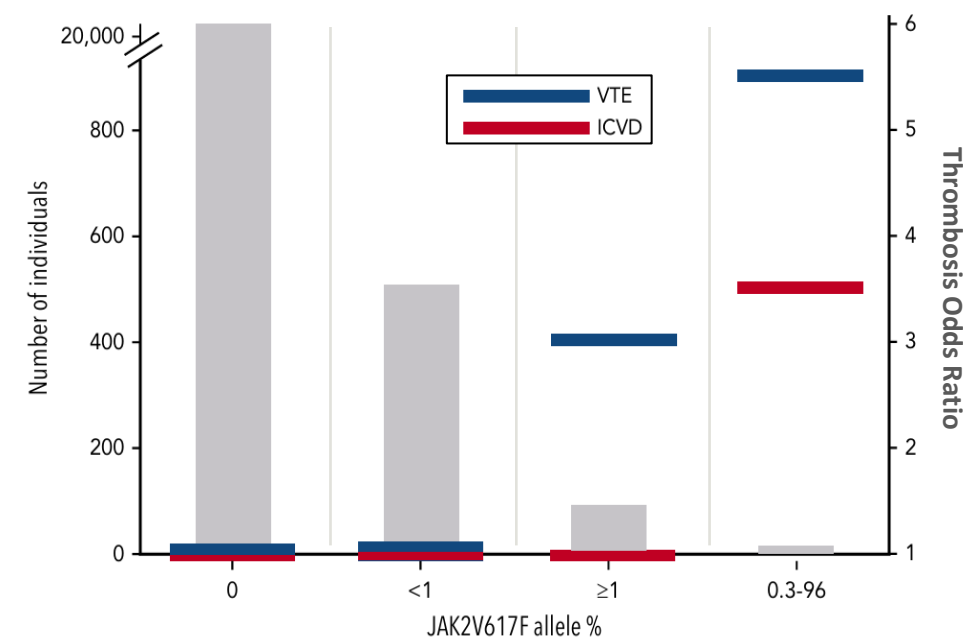
JAK2V617F variant allele frequency and thrombosis risk



Cumulative incidence of venous and arterial thrombosis



	Non Mutated	JAK2V617F <1%	JAK2V617F ≥1%	JAK2V617F MPN
WBC (10 ⁹ /L)	7.3	7.5*	8.7*#	11.0*
Hg (gm/dL)	14.0	14.2	14.3*	13.5*
Platelet (10 ⁹ /L)	250	264*	361*#	370*



Prevalence, relative thrombosis risk, and average blood counts stratified by JAK2^{V617F} VAFs from the Danish Suburban Population Study.

	Years	184	153	105	81	54	39	22
JAK < 50%								
JAK ≥ 50%								

	Years	184	150	105	80	54	37	21
JAK < 50%								
JAK ≥ 50%								

JAK2 Mutation Leads to Thrombosis

Constitutive Activation: continuous signaling through pathways that stimulate blood cell production, particularly red blood cells, platelets, and white blood cells.

Increased Blood Cell Production

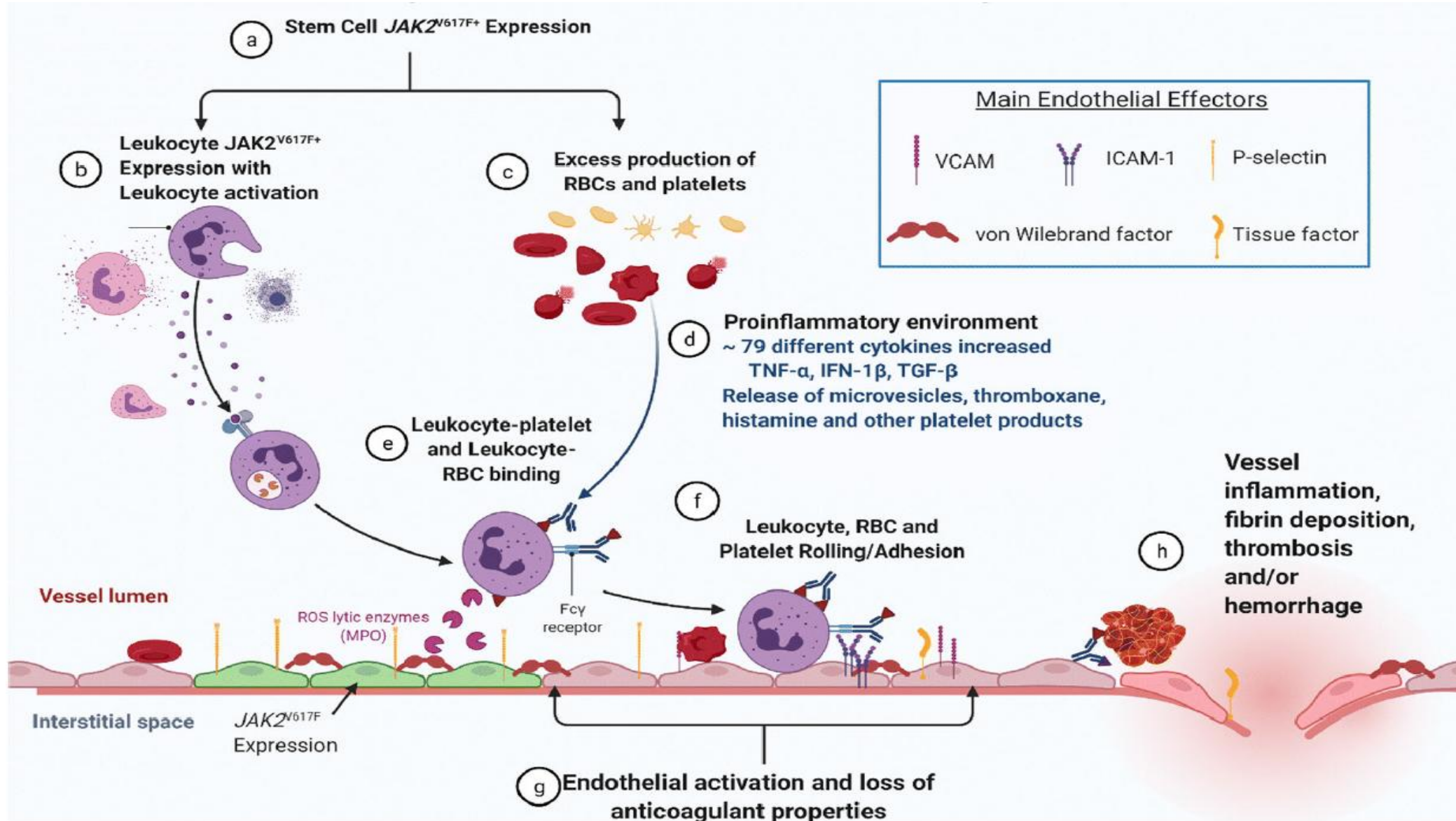
Platelet Activation: The activated JAK2 signaling pathway in platelets leads to increased sensitivity and responsiveness to various agonists, such as thrombin and collagen. This hyperreactivity can lead to enhanced platelet aggregation.

Altered Hemodynamics: An increase in red blood cell mass thickens the blood, leading to hyperviscosity.

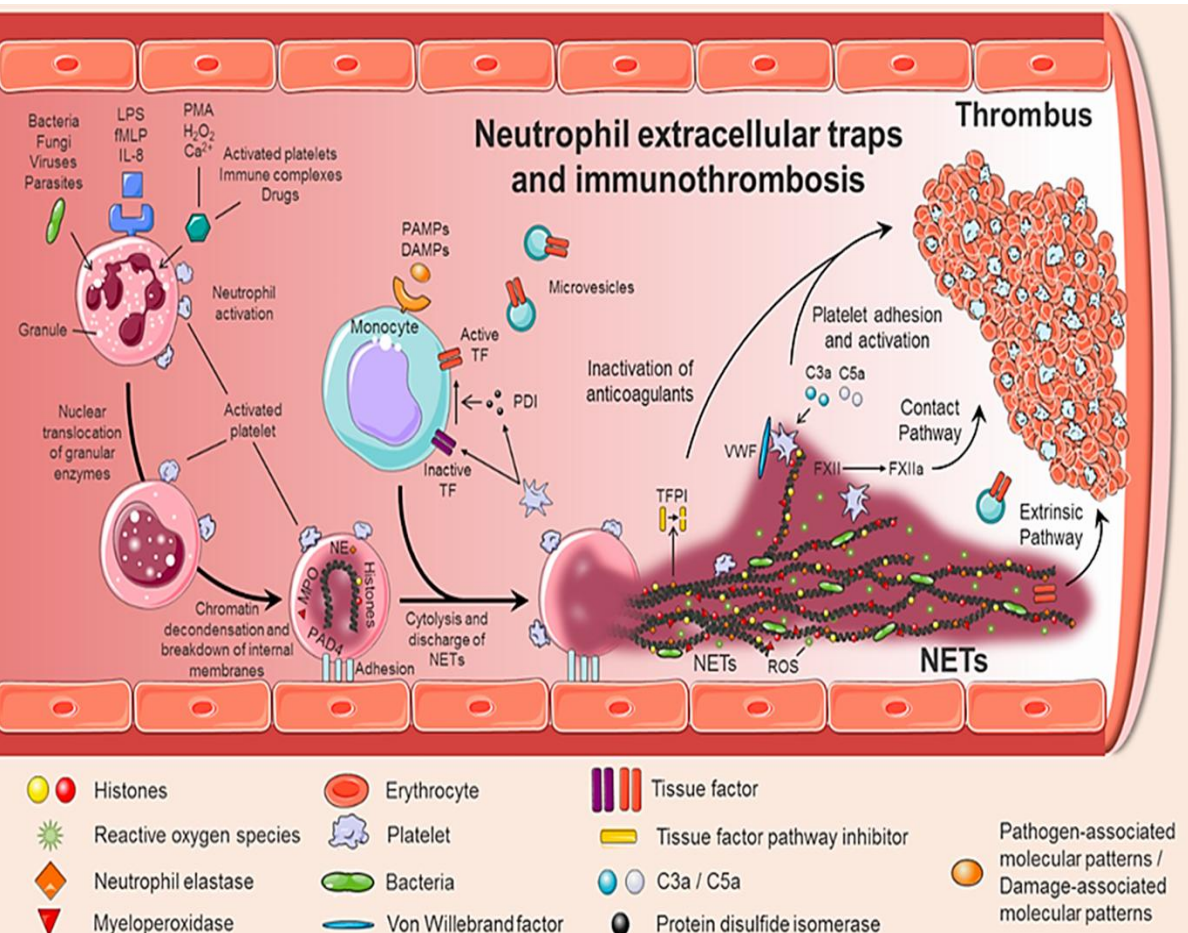
Inflammatory Mediators: The presence of the JAK2 mutation can also be associated with an inflammatory state, characterized by increased levels of cytokines and other mediators that can promote vascular inflammation and endothelial dysfunction, further increasing the risk of thrombosis.

Endothelial Dysfunction

Proposed mechanism for MPN-related coagulation activation



Generation of neutrophil extracellular traps (NETs)



1. Scaffold for Platelet Adhesion

- NETs, provide a structural framework for platelets to adhere to, initiating clot formation.
- Interaction facilitates the formation of platelet aggregates at sites of vascular injury or inflammation.

2. Activation of the Coagulation Cascade

- Directly activate clotting factors, promoting thrombin generation and fibrin deposition, enhances the coagulation cascade, driving the formation of stable clots.

3. Platelet-NET Interaction

- Platelets bind to NETs through receptors like TLR4, amplifying platelet activation and aggregation. This creates a feedback loop that promotes thrombus growth.

4. Role of ROS (Reactive Oxygen Species)

- NETosis is often driven by increased ROS production, which promotes endothelial injury and enhances pro-thrombotic pathways.



Role of NETs in Thrombotic Pathogenesis

1.DNA in NETosis and Coagulation: DNA released during NETosis activates Factor XII (FXII), triggering the intrinsic coagulation pathway. This leads to fibrin formation and thrombus development.

2.Histones and Platelets:

- ❑ Histones H3 and H4 in NETs activate platelets via TLR2/TLR4 signaling, promoting aggregation and thrombin generation.
- ❑ They also reduce thrombomodulin-dependent protein C activation, further enhancing coagulation.

3.Granule Proteases (Elastase and Cathepsin G):

- ❑ These enzymes degrade natural anticoagulants like TFPI (tissue factor pathway inhibitor) and antithrombin (AT), fostering thrombogenesis.
- ❑ Elastase promotes platelet adhesion by exposing von Willebrand factor (vWF), while Cathepsin G activates platelets via PAR4.

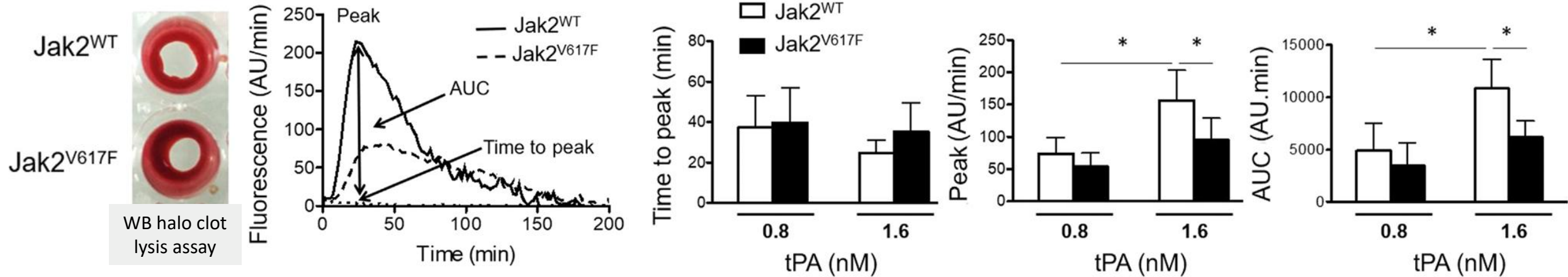
4.Tissue Factor (TF) and NETs:

- ❑ TF present in NETs, derived from neutrophils and monocytes, activates the extrinsic coagulation pathway, enhancing thrombus formation.
- ❑ Autophagy assists neutrophils in capturing and releasing TF during NETosis, further driving thrombin generation and platelet activation.

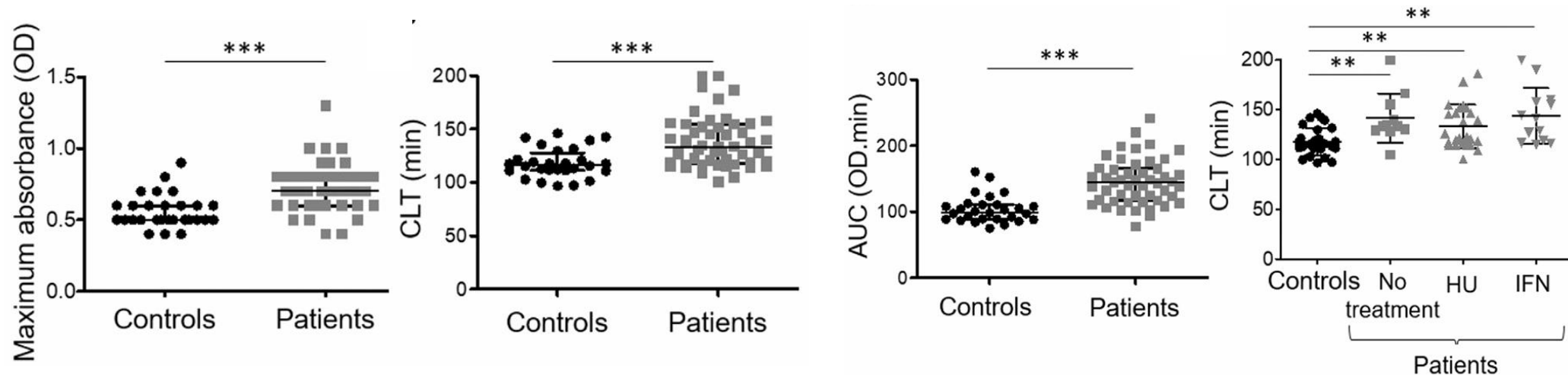


Impaired fibrinolysis in JAK2^{V617F}-related myeloproliferative neoplasms

Impaired tissue-type plasminogen activator (tPA)–induced fibrinolysis of whole blood (WB) clots from Jak2V617



Clot lysis assay in Jak2V617F-positive patients compared with controls





Arterial thrombosis

The incidence of arterial thrombosis in individuals with the JAK2 mutation

- Polycythemia Vera (PV): Arterial thrombosis, including stroke and myocardial infarction, is a major complication. The annual incidence of arterial events in PV is approximately 2.5 to 3.5% per patient-year.
- Essential Thrombocythemia (ET): Arterial thrombosis is also common in ET, with an incidence of 2 to 3% per patient-year, which is higher than venous thrombosis rates.
- Primary Myelofibrosis (PMF): While less common than in PV or ET, arterial thrombosis still occurs, particularly in high-risk patients.

The JAK2 V617F mutation significantly increases the risk of arterial thrombosis compared to JAK2-negative cases, due to its effects on platelet activation, endothelial dysfunction, and inflammation



Effect of JAK2^{V617F} CHIP on CAD

Cohort	No JAK2 ^{V617F} CHIP		JAK2 ^{V617F} CHIP		Adjusted HR (95% CI)	P-value	Adjusted HR (95% CI)
	Participants (n)	Incidence rate per 1,000 person-years (95% CI)	Participants (n)	Incidence rate per 1,000 person-years (95% CI)			
UK Biobank	417,108	5.42 (5.35 to 5.49)	113	17.26 (9.50 to 25.02)	2.32 (1.48 to 3.63)	< .001	
All of Us	83,744	7.73 (7.35 to 8.11)	55	29.15 (0.58 to 57.71)	2.26 (0.85 to 6.04)	.10	
MGB Biobank	41,143	4.07 (3.73 to 4.40)	52	25.38 (0.51 to 50.26)	2.88 (1.07 to 7.75)	.04	
Meta-analysis					2.38 (1.63 to 3.48)	< .001	

Cohort	Participants (n)	Incidence rate per 1,000 person-years (95% CI)	Adjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	
No CHIP						
UK Biobank	417,108	5.42 (5.35 to 5.49)	Ref.	Ref.		
All of Us	83,744	7.73 (7.35 to 8.11)	Ref.	Ref.		
MGB Biobank	41,143	4.07 (3.73 to 4.40)	Ref.	Ref.		
Meta-analysis			Ref.	Ref.		
Non-JAK2 ^{V617F} CHIP at VAF >10%						
UK Biobank	9,357	8.28 (7.70 to 8.85)	1.10 (1.30 to 1.18)	.008		
All of Us	2,554	15.43 (12.35 to 18.52)	1.08 (0.88 to 1.33)	.48		
MGB Biobank	1,982	6.45 (4.50 to 8.40)	0.92 (0.67 to 1.27)	.61		
Meta-analysis			1.09 (1.02 to 1.16)	.01		
JAK2 ^{V617F} CHIP with no other driver genes at VAF >10%						
UK Biobank	97	16.58 (8.46 to 24.70)	2.26 (1.38 to 3.69)	.001		
All of Us	47	26.25 (-3.45 to 55.95)	1.99 (0.64 to 6.20)	.23		
MGB Biobank	34	31.71 (-4.17 to 67.60)	4.41 (1.41 to 13.78)	.01		
Meta-analysis			2.43 (1.60 to 3.69)	< .001		
JAK2 ^{V617F} CHIP with ≥1 other driver gene at VAF >10%						
UK Biobank	16	22.08 (-2.91 to 47.06)	2.70 (0.87 to 8.38)	.09		
All of Us	8	43.60 (-41.85 to 129.04)	3.80 (0.53 to 27.07)	.18		
MGB Biobank	18	15.88 (-15.24 to 47.00)	1.28 (0.18 to 9.18)	.81		
Meta-analysis			2.49 (1.04 to 6.00)	.04		

- 2.4-fold increase in incident CAD events in association with JAK2^{VF} CHIP ($P < 0.001$)
- 2.5-fold increase in incident CAD events in association with JAK2^{VF} CHIP combined with additional CHIP mutations.
- Non JAK2^{VF} CHIP was associated with a 1.09-fold increase in CAD events

Multivariable-adjusted logistic regression model including age, square of age, sex, self-reported race, principal components of ancestry 1 to 10, smoking status, body mass index (BMI) systolic blood pressure, antihypertensive medication-use, hyperlipidemia, cholesterol-lowering medication use, and prevalent diabetes mellitus as covariates.



Venous thromboembolism

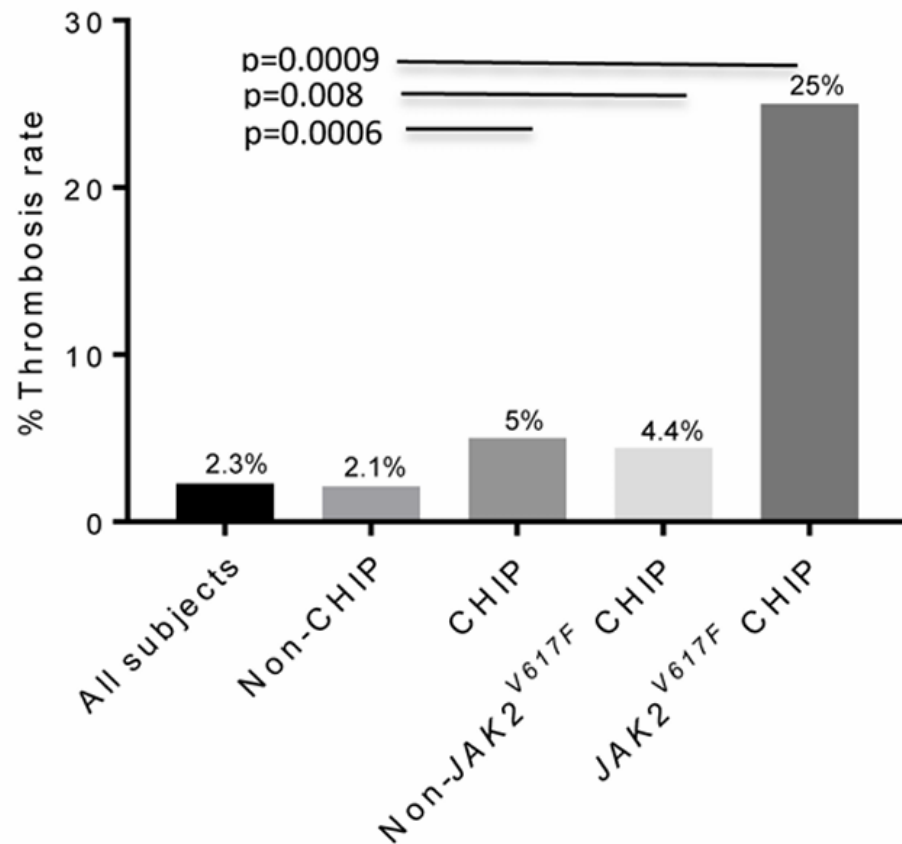
The incidence of venous thrombosis in individuals with the JAK2 mutation, particularly in the context of myeloproliferative neoplasms (MPNs), is significant:

- Polycythemia Vera (PV):
 - VTE occurs at an annual rate of approximately 1.5% to 2.5% per patient-year, with splanchnic vein thrombosis (e.g., portal vein thrombosis) being a notable complication.
- Essential Thrombocythemia (ET):
 - VTE incidence is slightly lower, around 1% to 2% per patient-year, but still higher than in the general population.
- Primary Myelofibrosis (PMF):
 - VTE is less common compared to PV and ET, but splanchnic vein thrombosis remains a serious concern.

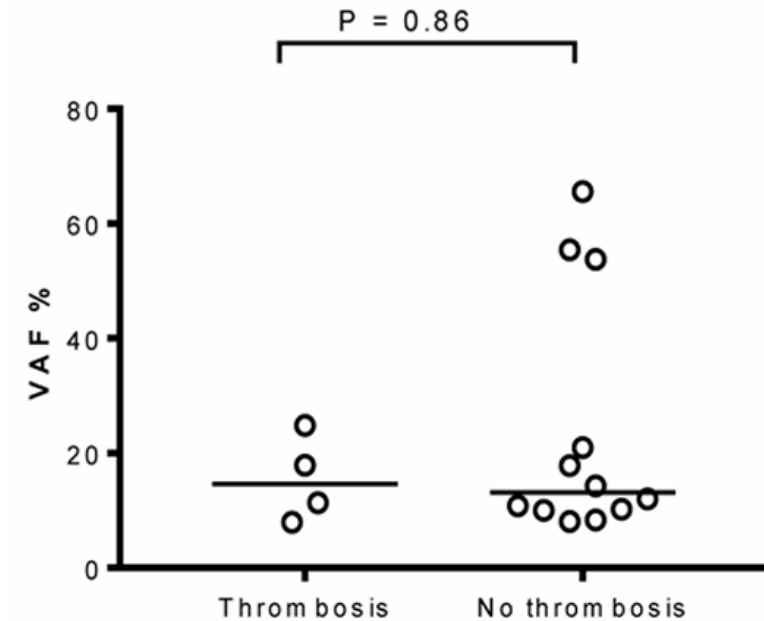
The JAK2 V617F mutation is a strong independent risk factor for venous thromboembolism (VTE), with studies showing a 4- to 6-fold increased risk compared to individuals without the mutation



Rates of venous thrombosis in patients with or without CHIP and/or JAK2^{V617F} mutation.



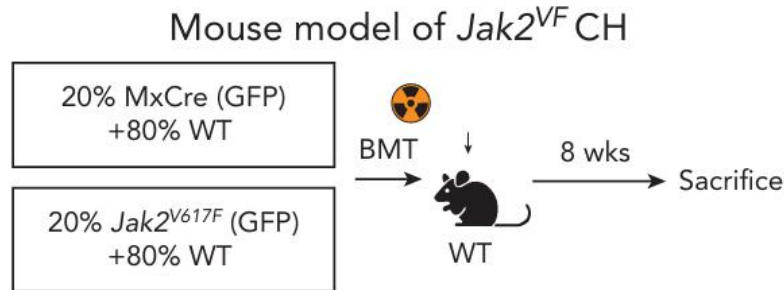
Variant allele frequency (VAF) of individuals with JAK2^{V617F} CHIP separately according to the incidence of VTE



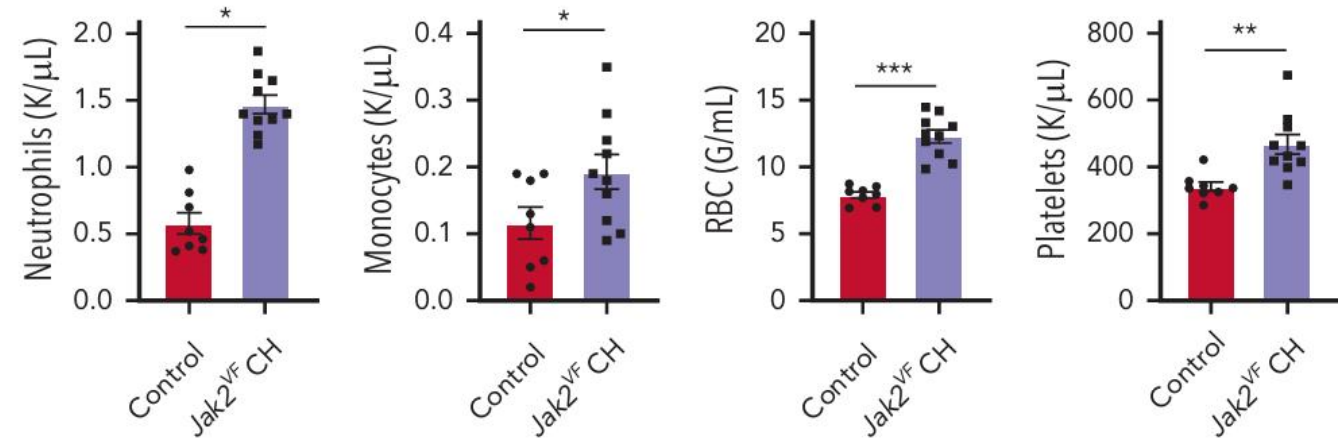
Accelerated arterial thrombosis and increased platelet activity in $Jak2^{VF}$



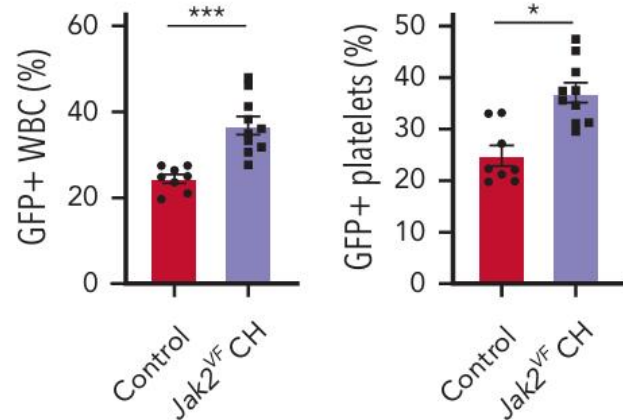
Experimental design of $Jak2^{VF}$ CH mouse model



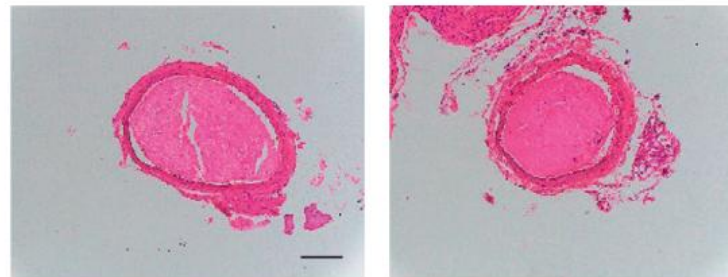
Peripheral blood cell counts



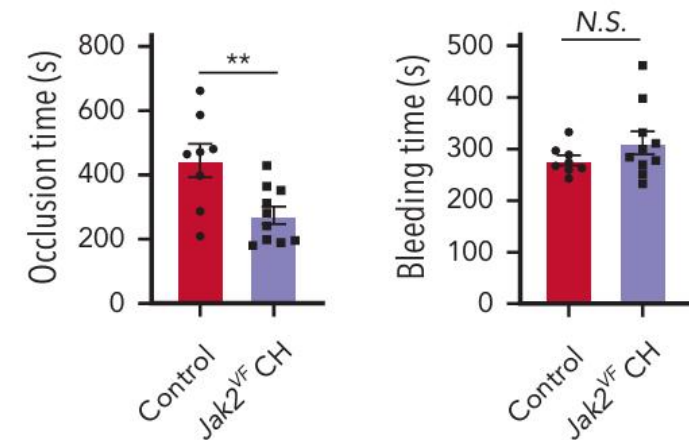
$Jak2^{VF}$ chimerism in peripheral blood as indicated by fraction of GFP+ WBCs and platelets.



FeCl₃-induced carotid artery thrombotic occlusion time (sec)



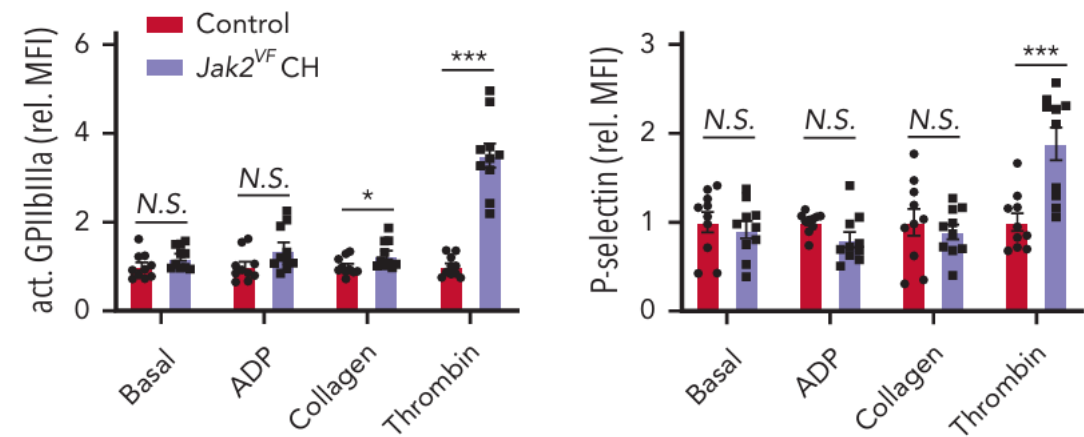
Tail bleeding time (seconds)



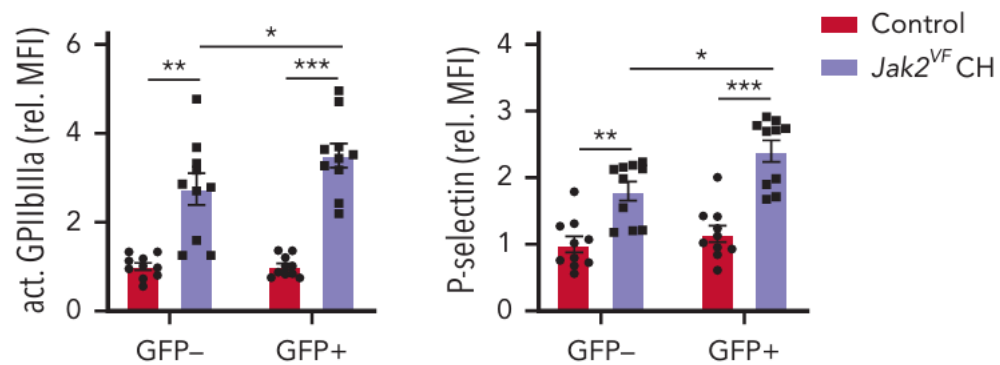
Accelerated arterial thrombosis and increased platelet activity in Jak2^{VF} CH



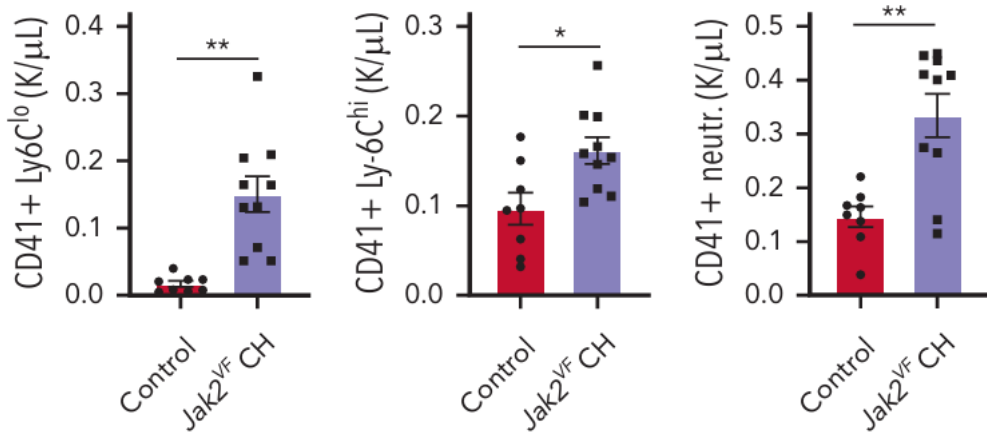
Platelet activation as measured by levels of active GPIIb/IIIa conformation and P-selectin surface expression



Platelet activation in GFP+ (mutated in Jak2^{VF} CH) and GFP- thrombin-stimulated platelets (GPIIb/IIIa activation and P-selectin)

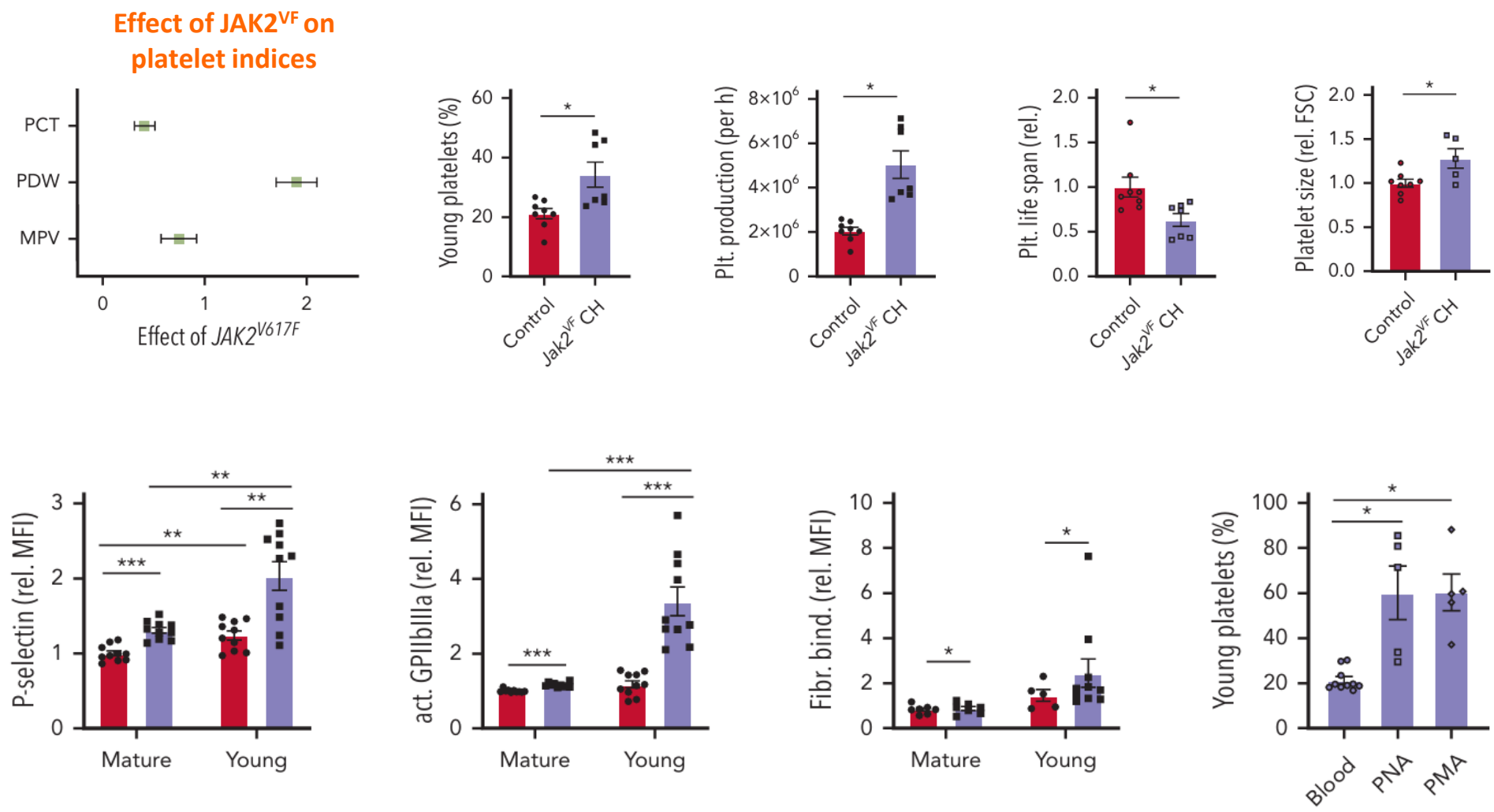


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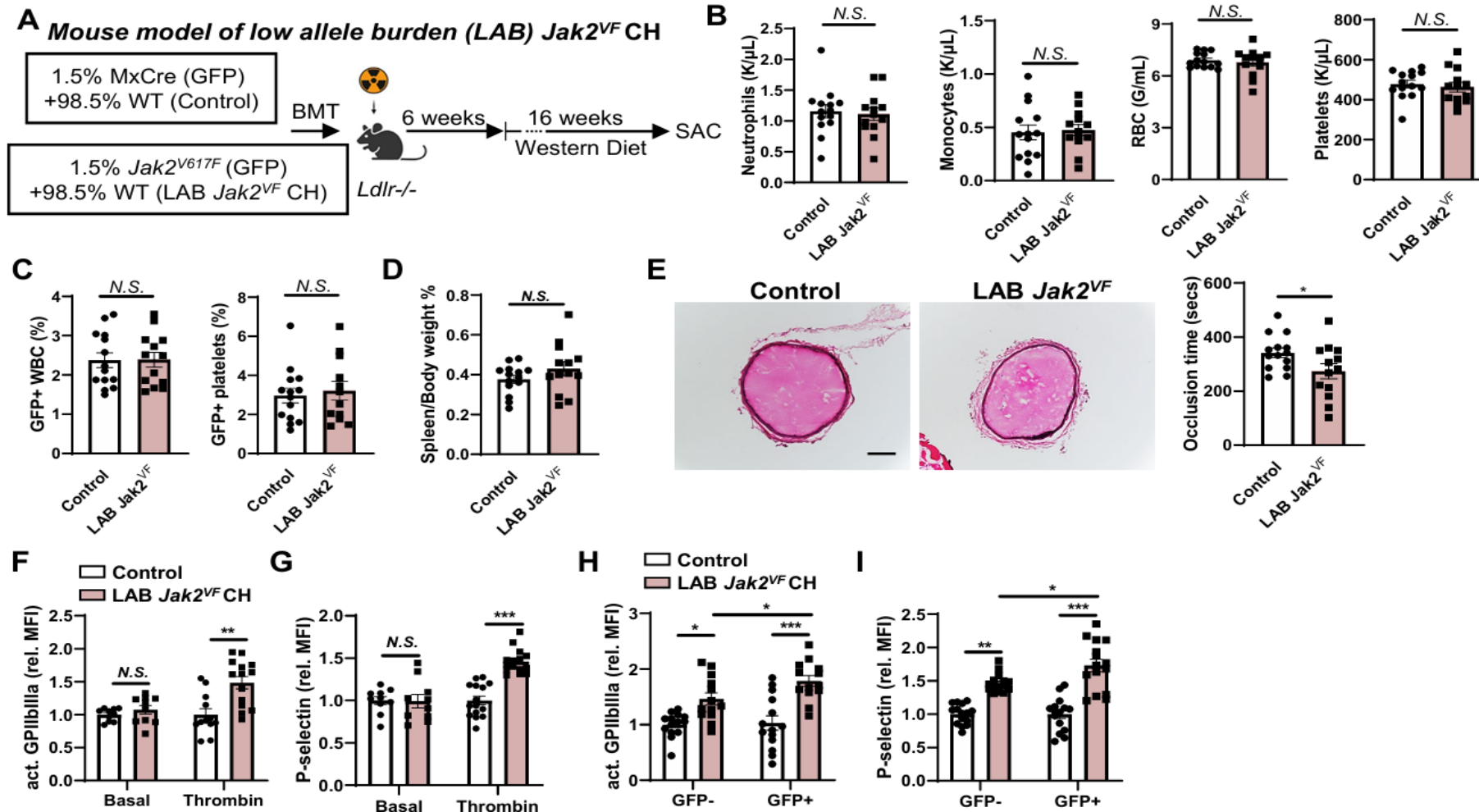


Platelet-leukocyte heteroaggregates in the peripheral blood, indicated by number of CD41+ Ly6C-low and Ly6C-high monocytes, and neutrophils

Accelerated production of hyperreactive young platelets in Jak2^{VF} CH



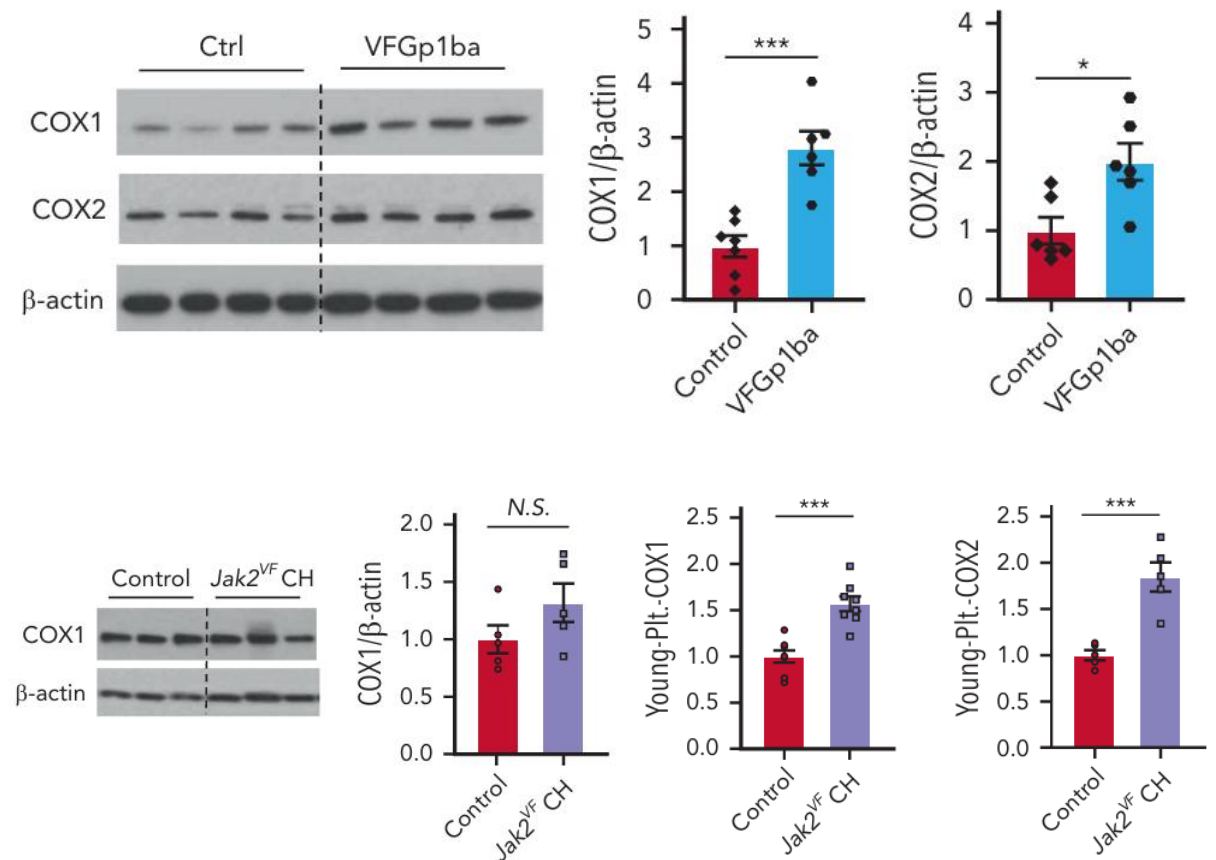
Accelerated arterial thrombosis and increased platelet activity in low allele burden (LAB) $Jak2^{VF}$ clonal hematopoiesis



A. Experimental design of LAB $Jak2^{VF}$ CH mouse model. **B.** Peripheral blood cell counts **C.** $Jak2^{VF}$ chimerism in peripheral blood as indicated by fraction of GFP-positive white blood cells (WBC) and platelets. **D.** Spleen/Body weight ratio **E.** FeCl₃-induced carotid artery thrombotic occlusion time (seconds) **F, G.** Platelet activation as measured by levels of active GPIIb/IIIa conformation (JON/A, F) and P selectin surface expression **H, I.** Differential analysis of activation in GFP-positive (mutated in $Jak2^{VF}$ CH) and GFP-negative thrombin stimulated platelets (GPIIb/IIIa activation/JON/A, H; P-selectin, I).

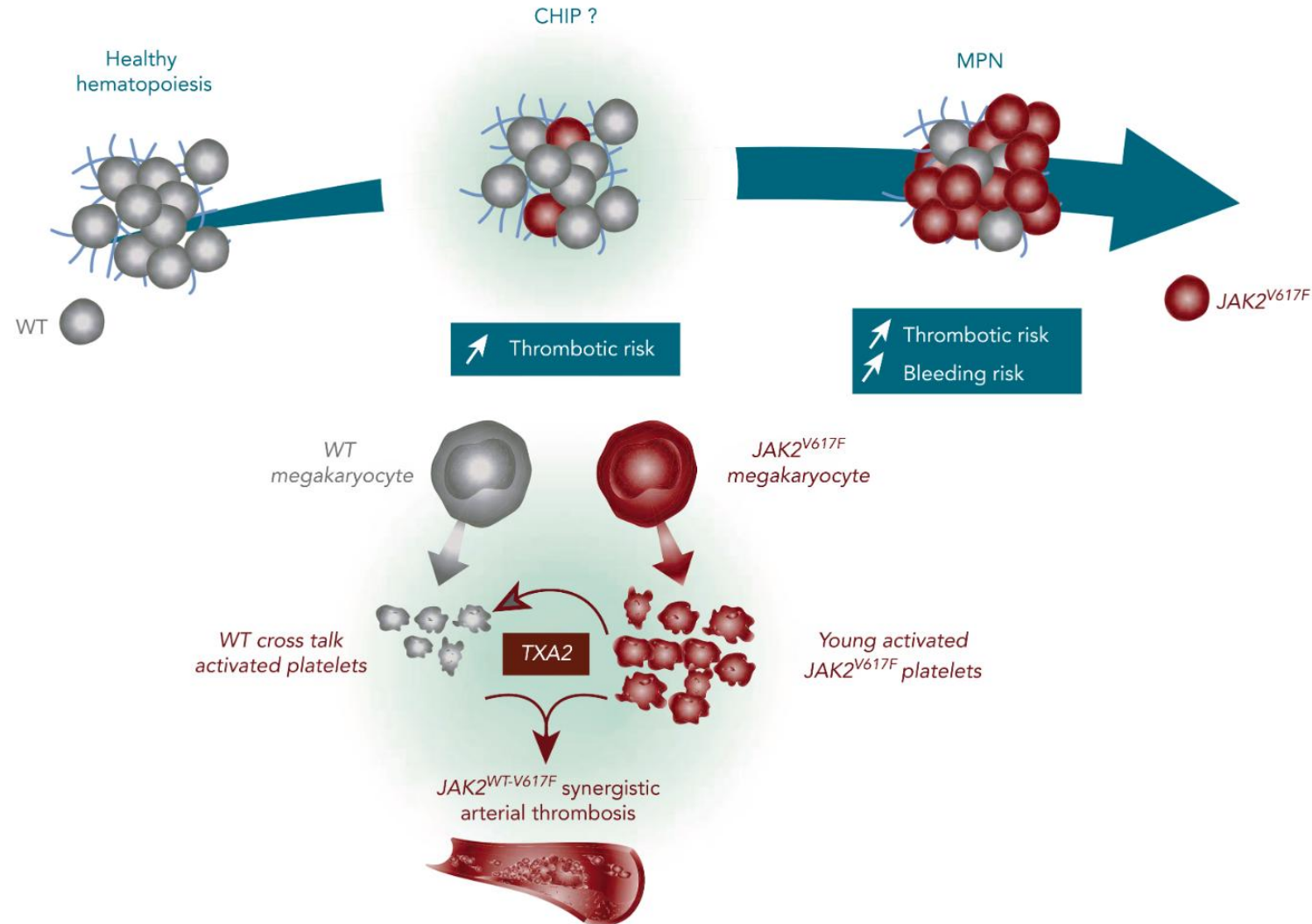


Increased COX-1 and COX-2 expression and cPLA2 activation in Jak2VF platelets

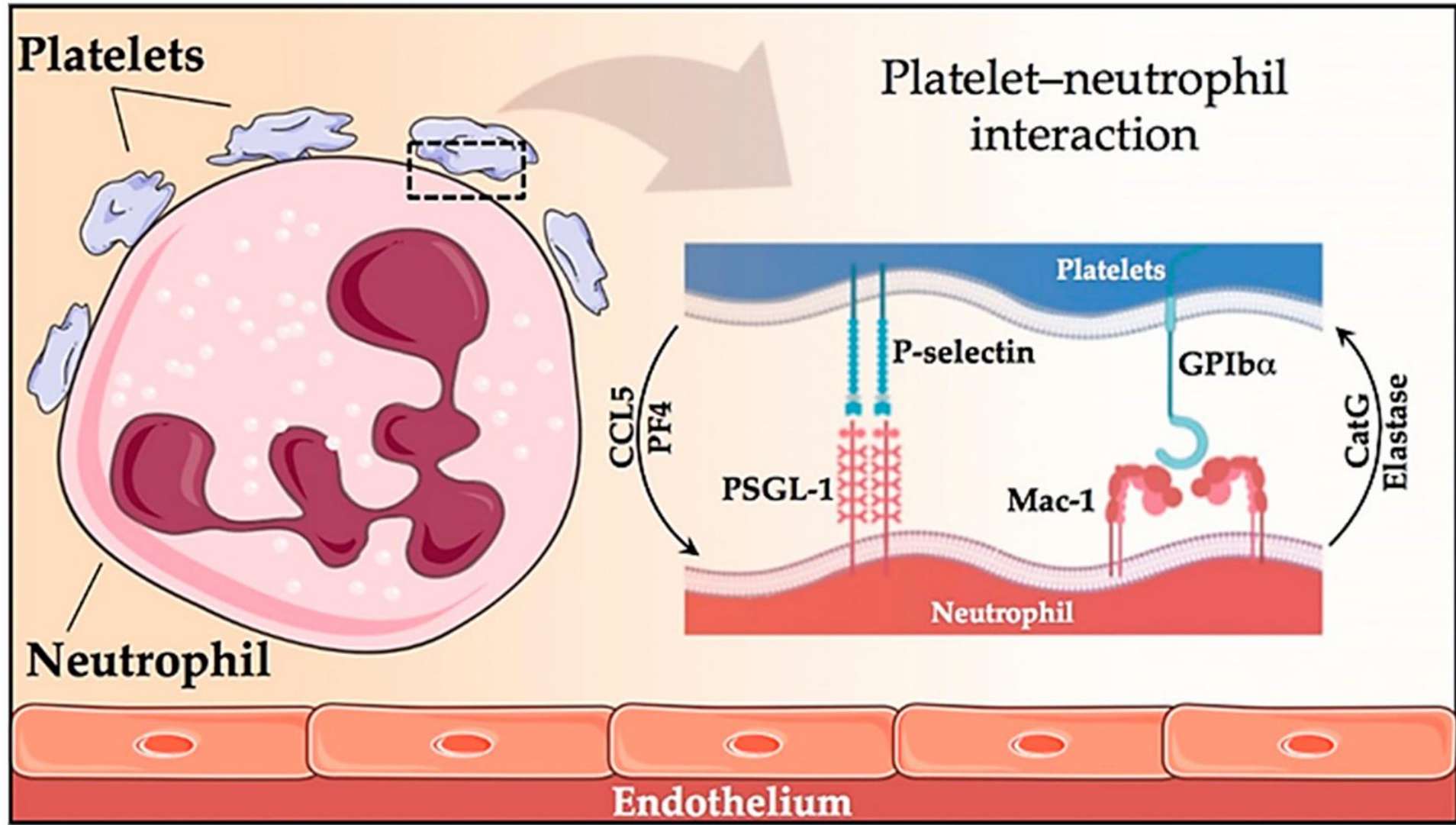


- Role of COX-1 and COX-2:** Enhanced expression of both COX-1 (2.8-fold) and COX-2 (2-fold) in Jak2^{VF} platelets and young platelets of Jak2^{VF} CH mice suggests their involvement in aspirin resistance, commonly observed in MPNs.
- Arachidonic Acid & TXA2 Pathway:** Elevated phosphorylation of cPLA2, a critical enzyme for liberating arachidonic acid, drives thromboxane A2 synthesis and platelet activation.
- Upregulated Signaling Pathways:** Increased phosphorylation of PLC γ 2, Syk, and ROS production (via NOX2) contribute to amplified platelet activation in Jak2VF platelets, highlighting a cascade that potentiates platelet reactivity.

JAK2^{V617F} CHIP is associated with an increased arterial thrombosis risk, resulting from a thromboxane A2-mediated JAK2^{V617F} to JAK2^{WT} platelets activating cross talk.

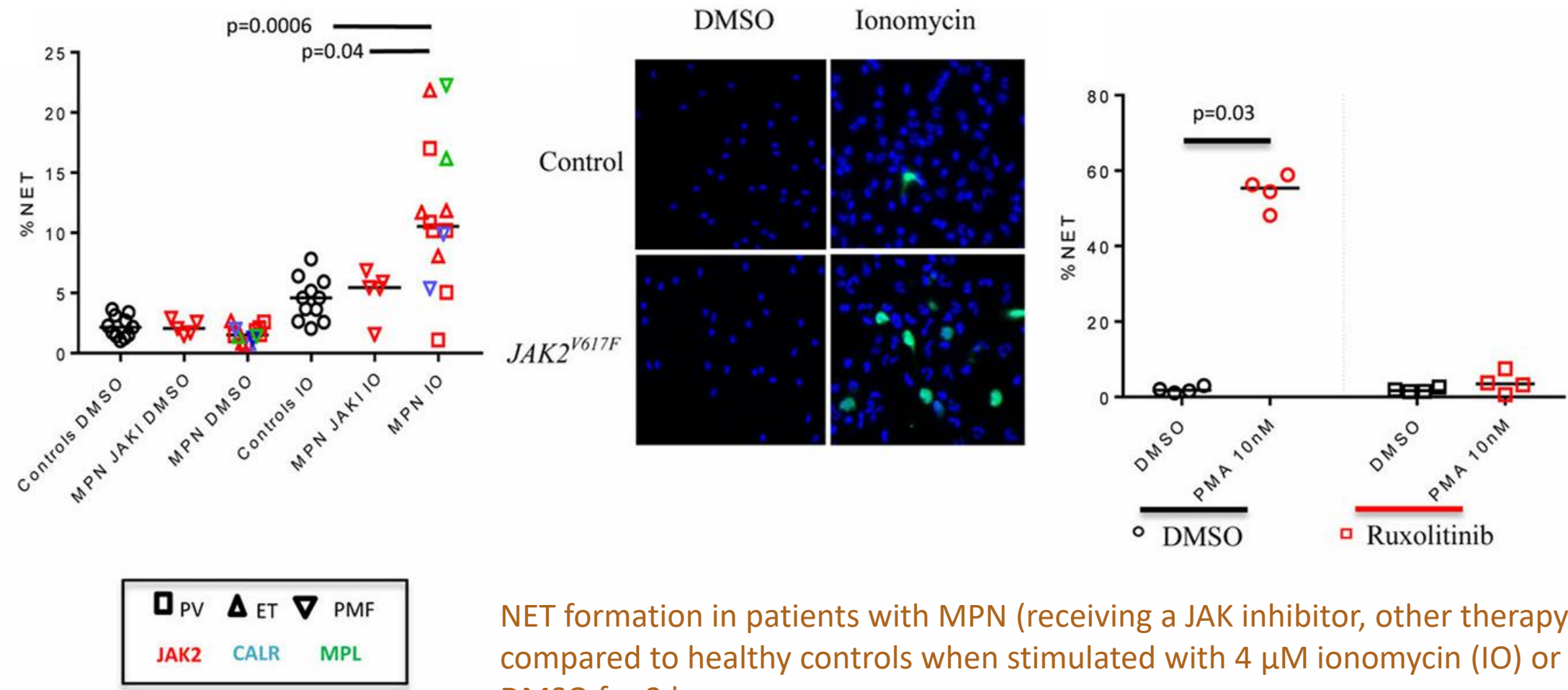


Platelet-neutrophil interaction





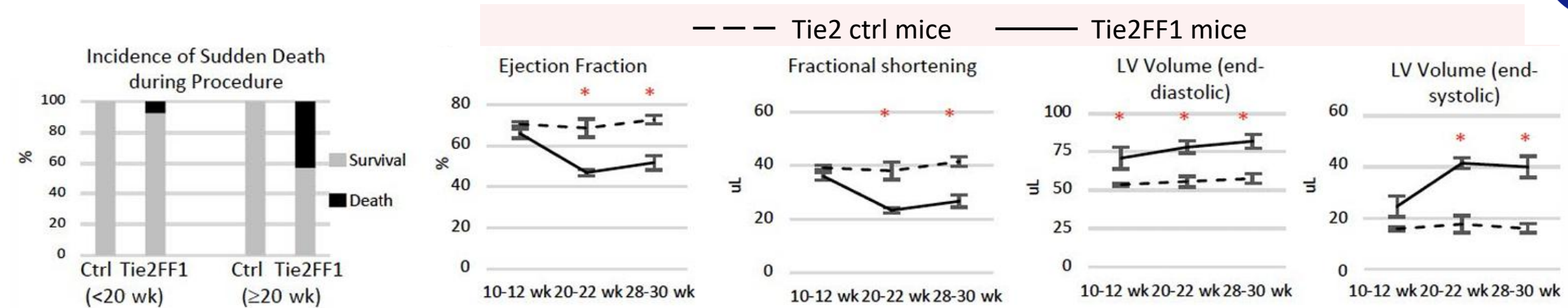
Neutrophils derived from patients with MPNs are associated with an increase in NET formation and a prothrombotic, NET-rich phenotype



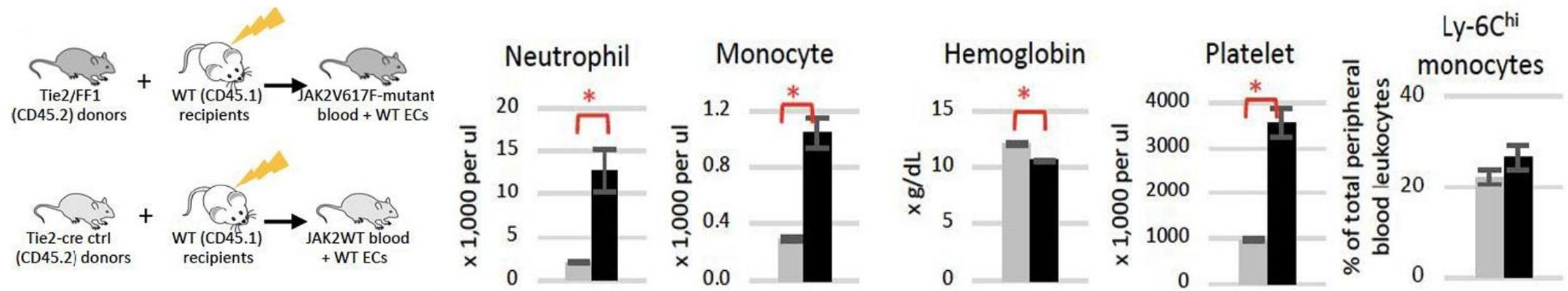
NET formation in patients with MPN (receiving a JAK inhibitor, other therapy) compared to healthy controls when stimulated with 4 μ M ionomycin (IO) or DMSO for 2 hours.



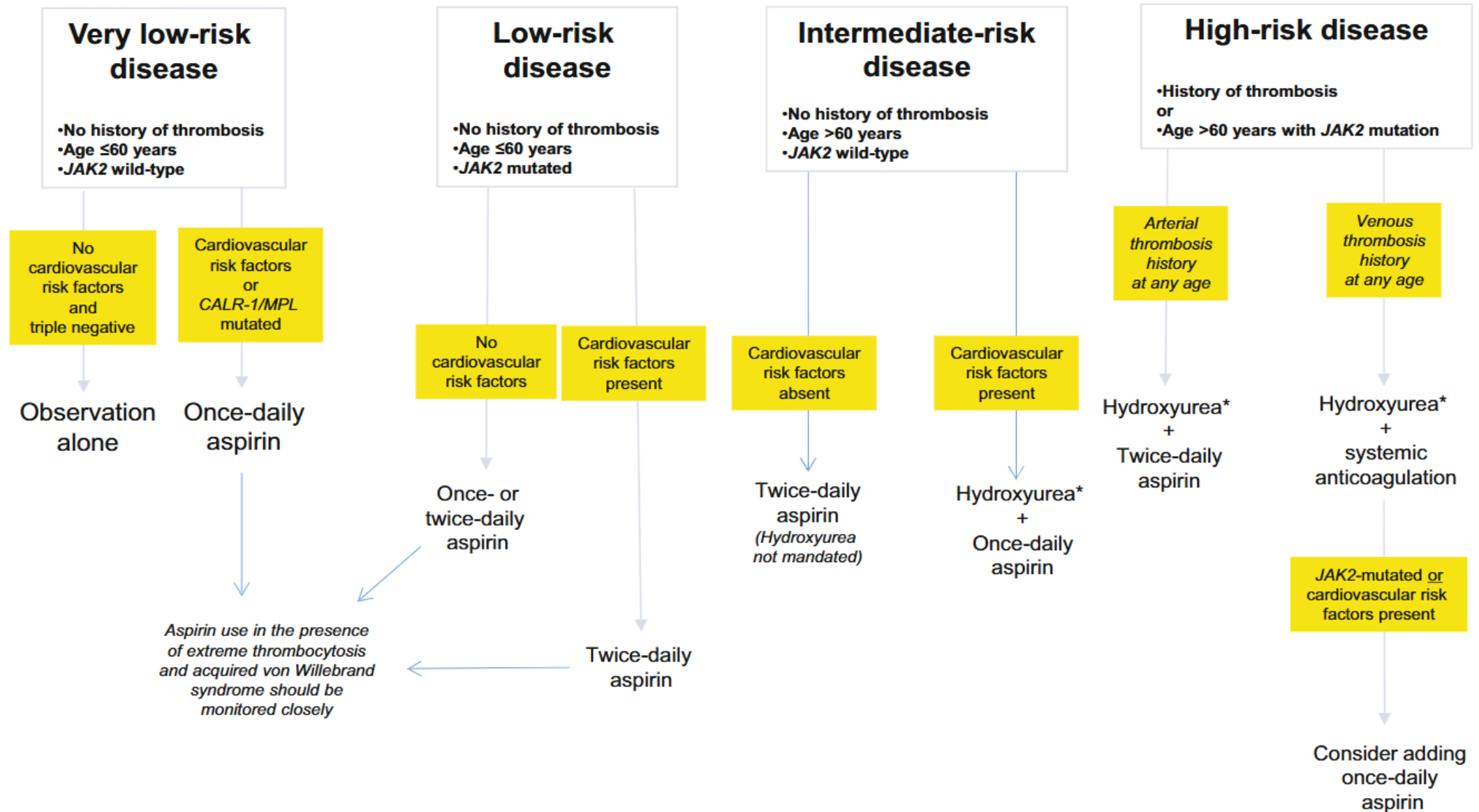
Endothelial JAK2^{V617F} mutation leads to thrombosis, vasculopathy, and cardiomyopathy



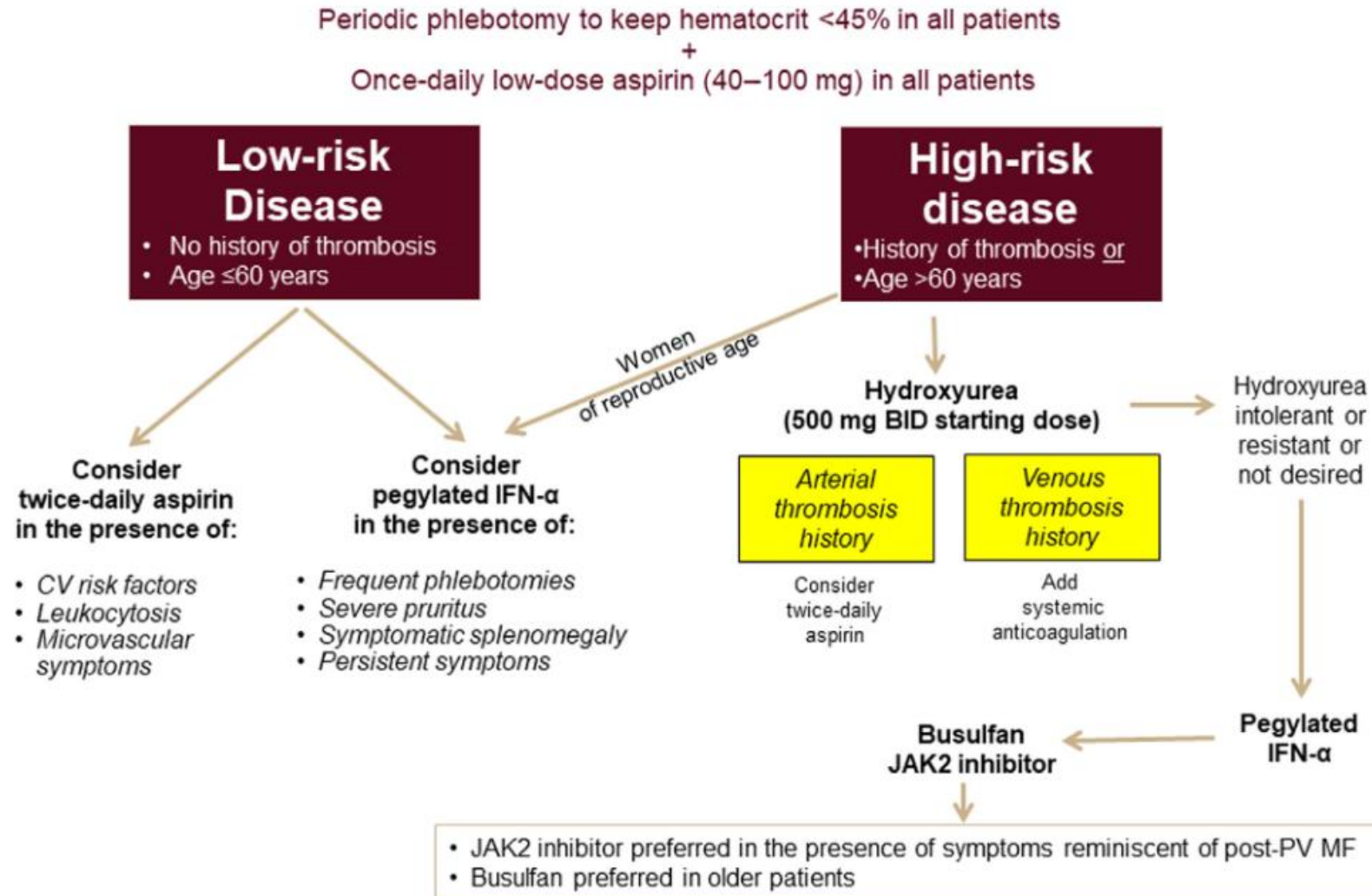
JAK2V617F-mutant ECs are required to develop the cardiovascular complications in Tie2FF1 mice



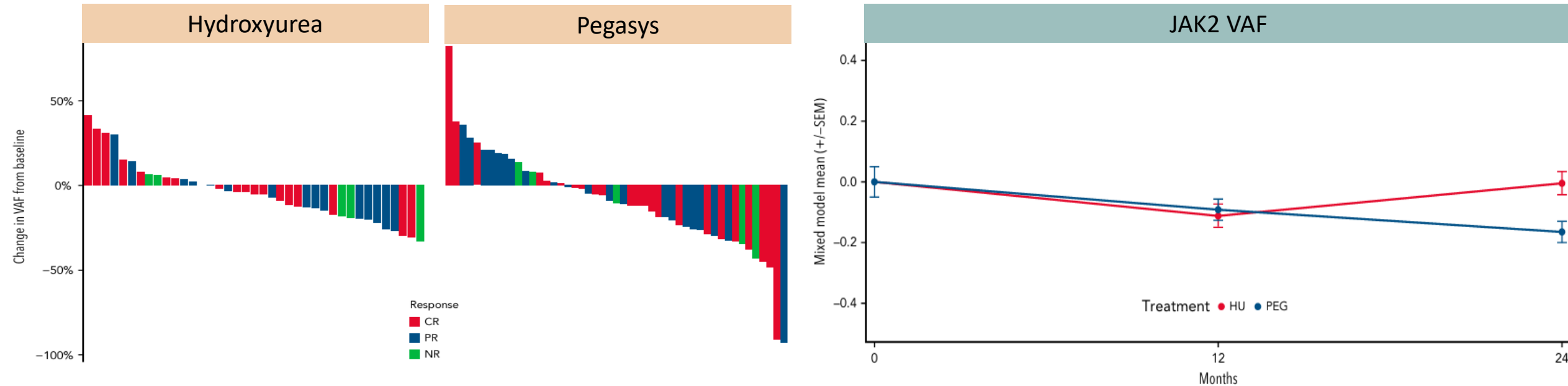
Thrombosis risk stratification in essential thrombocythemia (ET)



Treatment approach in polycythemia vera



Kinetics of JAK2V617F allele burden of patients treated in the MPD-RC 112 trial.



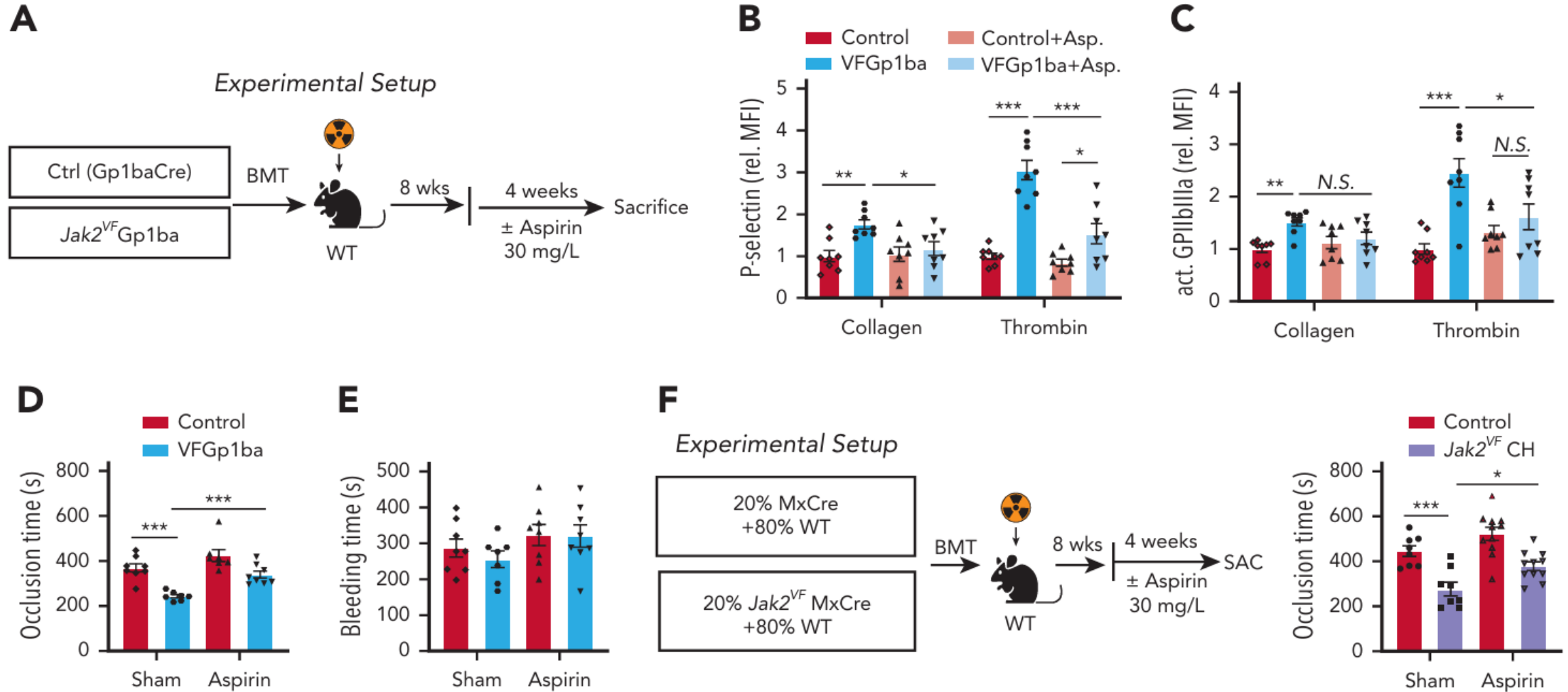
Complication-free survival: 5 events (HU: 3; PEG: 2) 5 events (HU: 3; PEG: 2) occurred during the trial with a hazard ratio 0.60 (95% CI, 0.10-3.62).

PEG - 1 patient had a bleeding event consisting of macroscopic hematuria that required red cell transfusions
- 1 patient had a cerebral vascular accident

HU - 1 patient developed bilateral vertebral artery blockage noted on imaging but without clinical consequences.
- 1 patient progressed to MF after 46 months
- 1 patient treated with HU died of lung cancer at 9 months

Cumulative incidence of thrombosis was 2% (95% CI, 0.3-13) for HU and 2% (95% CI, 0.3-15) for PEG at 24 months

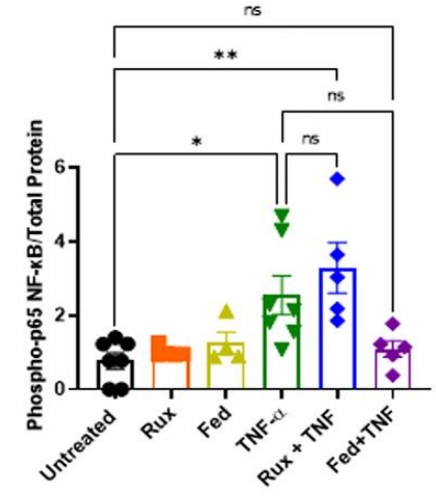
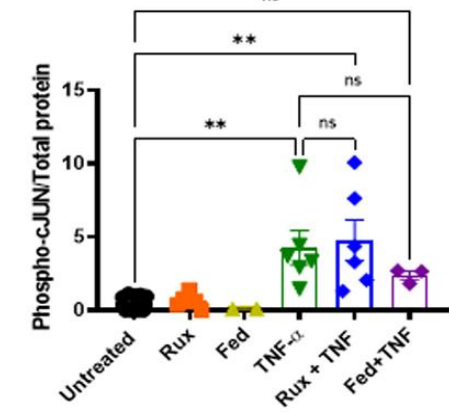
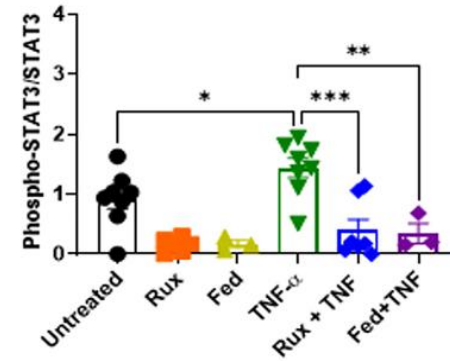
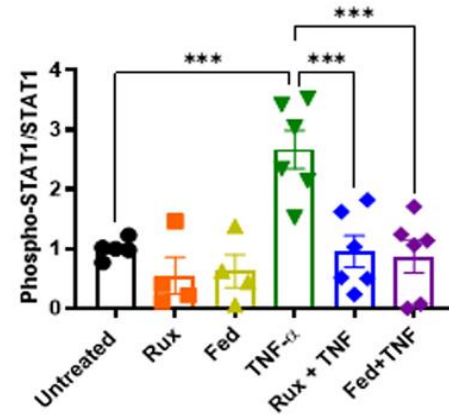
Low-dose aspirin treatment reversed accelerated arterial thrombosis and increased platelet activity in Jak2VF CH



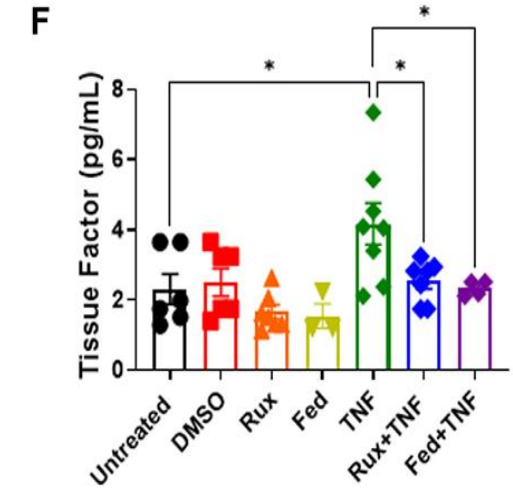
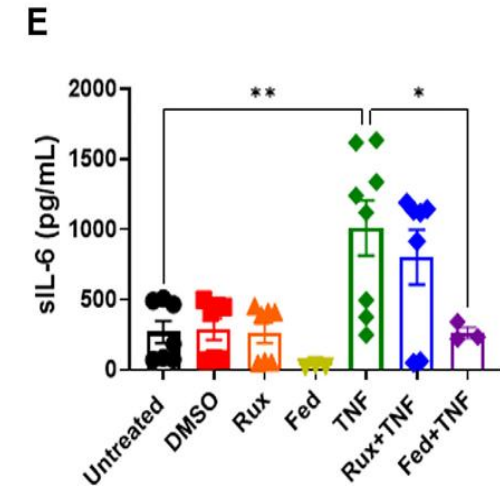
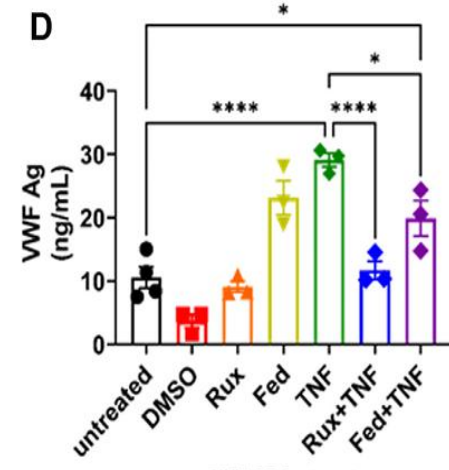
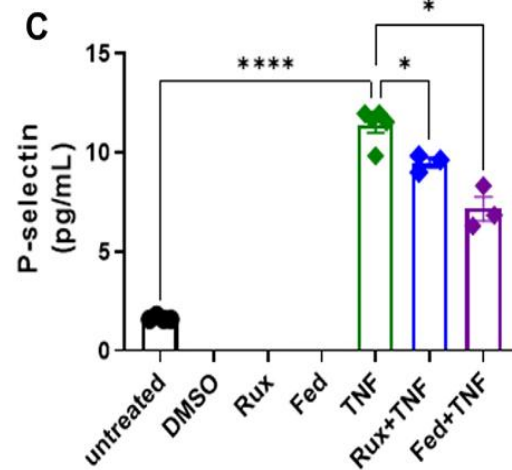
JAK-STAT inhibition reduces endothelial prothrombotic activation and leukocyte-endothelial proadhesive interactions



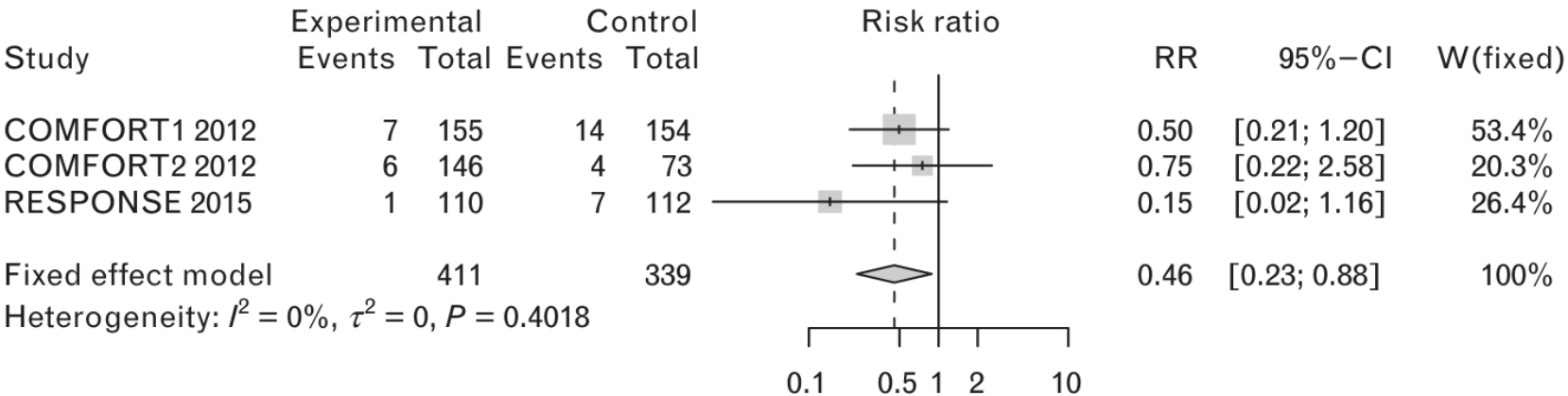
HUVEC treatment
Cell lysate



HUVEC treatment
Conditioned media



Forest plot for relative risk of all thrombotic events comparing ruxolitinib to control.¹



Does Ruxolitinib prevent thrombosis in patients with Polycythemia Vera?

Systematic Review
Articles/EHA/ASH/Additional sources



80 records screened

Metanalysis



15 records included

N= 4
Randomized Clinical Trials
included

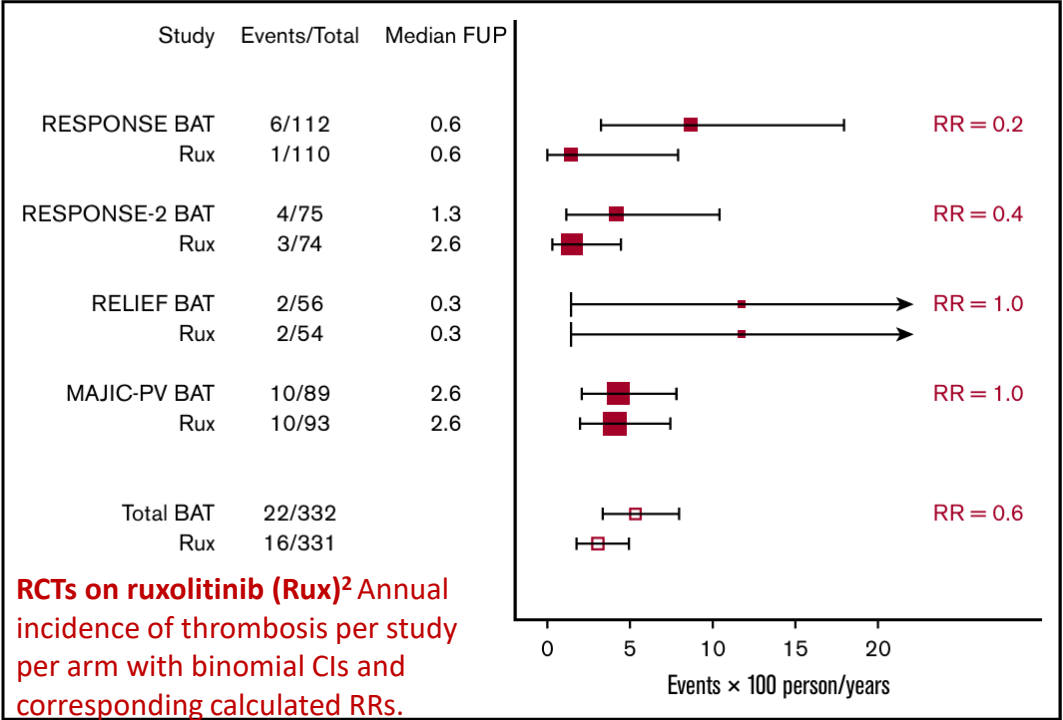
663 patients		
	vs	
Ruxolitinib		Best Available Therapy
331		332
Median F-up (min-max): 1 year (0.3 -2.6)		
Endpoint: thrombosis		
N (%)	16 (4.8%)	22 (6.6%)
Incidence pts-year (95% CI)	3.09% (1.22-4.96)	5. 51 % (3.72-7.30)
RR	0.56 (p=0.098)	

Even though not formally confirmed, results suggest an efficacy of Ruxolitinib to prevent thrombosis in patients with PV.

Limitations of this study:

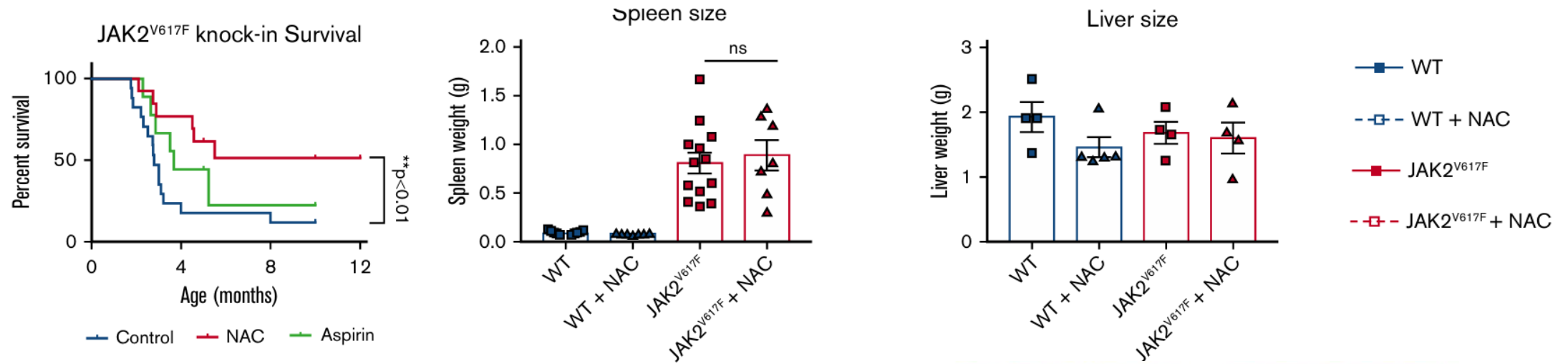
- scarcity of high quality data
- low number of events
- short follow-up duration.

Future clinical trials to confirm that Ruxolitinib has an antithrombotic action are warranted.

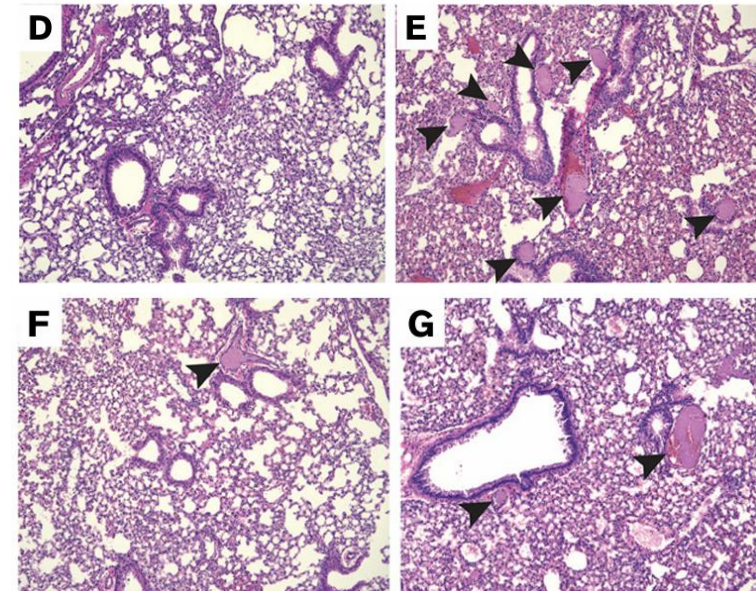
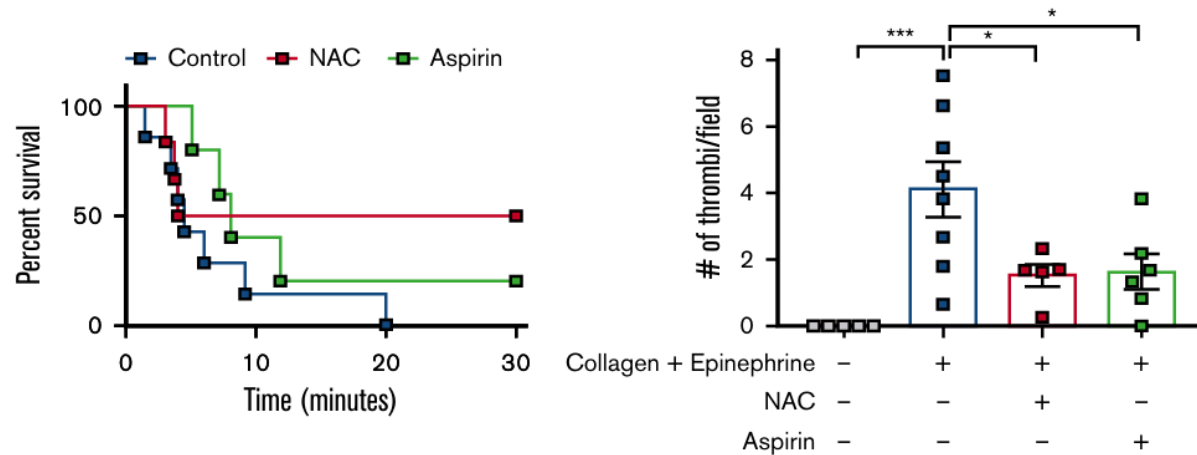


1. Blood Coagulation and Fibrinolysis 2016, 27:648–652. 2. Blood Adv. 2020 Jan 28;4(2):380-386.

N-acetylcysteine inhibits thrombosis in a murine model of MPNs



NAC reduces pulmonary thrombus formation in vivo



D. control
E. coll/epi-treated
F. NAC+coll/epi
G. aspirin+coll/epi



Conclusions

Role of JAK2 Mutations

JAK2 mutations are significant in thrombosis, impacting various treatment strategies and patient outcomes.

Circulating blood cells are both increased in quantity and are qualitatively abnormal in MPN, resulting in increased thrombotic risk.

Endothelial cells and non myeloid cells are both involved in pathogenesis of thrombosis in MPNs

Treatment Approaches

Understanding JAK2 mutations can guide clinicians in developing effective treatment plans for patients.

Cytoreductions : HU, IFN

JAK inhibitor

Antithrombotic drug

New treatments : anti-integrin monoclonal antibodies, bromodomain and extraterminal domain (BET) and histone deacetylase inhibitors, rizamlizumab and selectin inhibitors