GIORNATE EMATOLOGICHE VICENTINE

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Infiammazione e neoplasie mieloproliferative croniche

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Disclosures of Alessandro Lucchesi

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Novartis					x	x	
Pfizer					x		
Incyte			x		x		
Morphosys						x	
SOBI					x	x	
Grifols			x		x	x	
BMS					x	x	
Amgen					x	x	
Sanofi			x		x	x	
Protagonist					x	x	

Acute inflammation

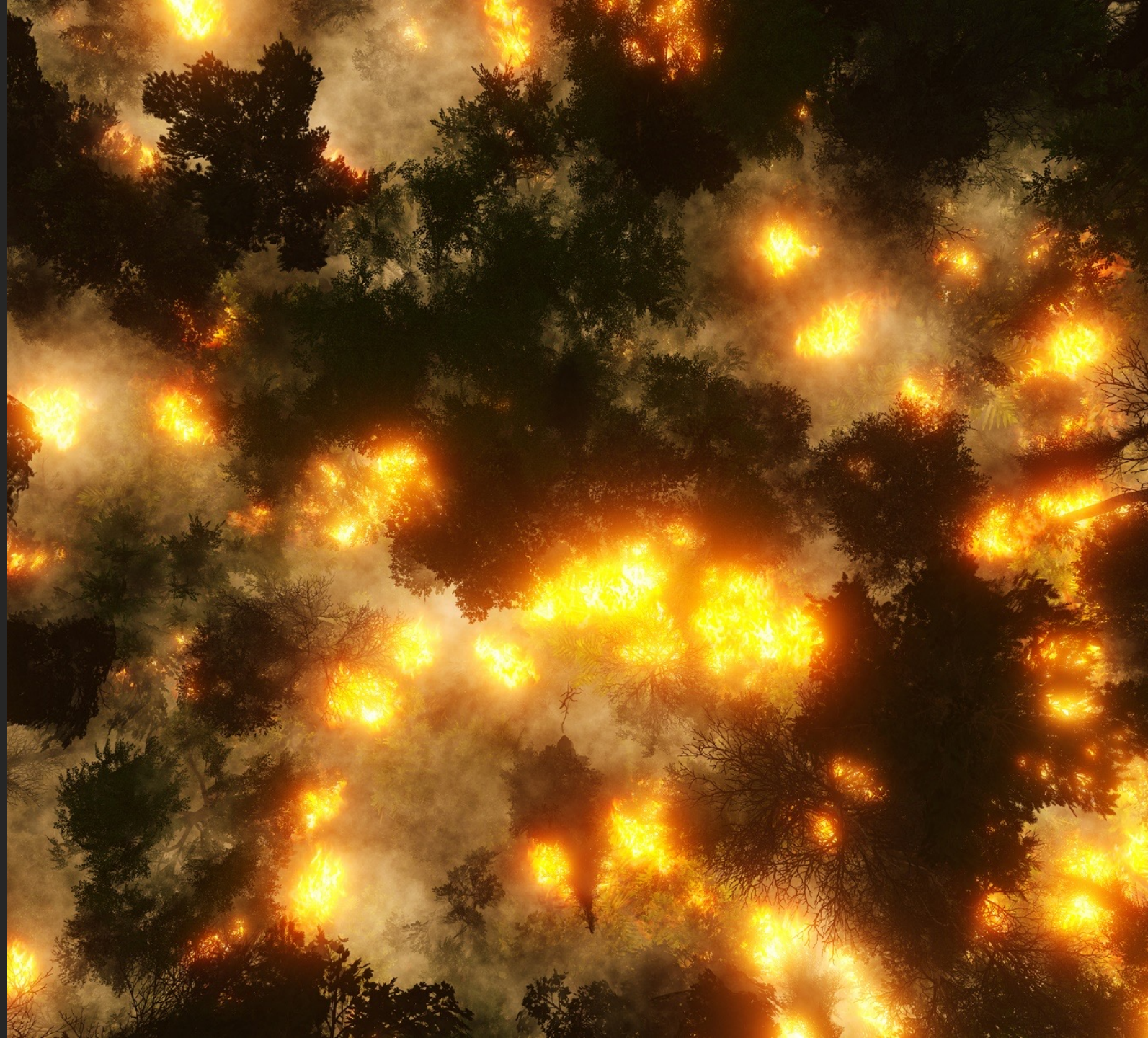
- *Credits: Patrick Emerson*

Chronic inflammation

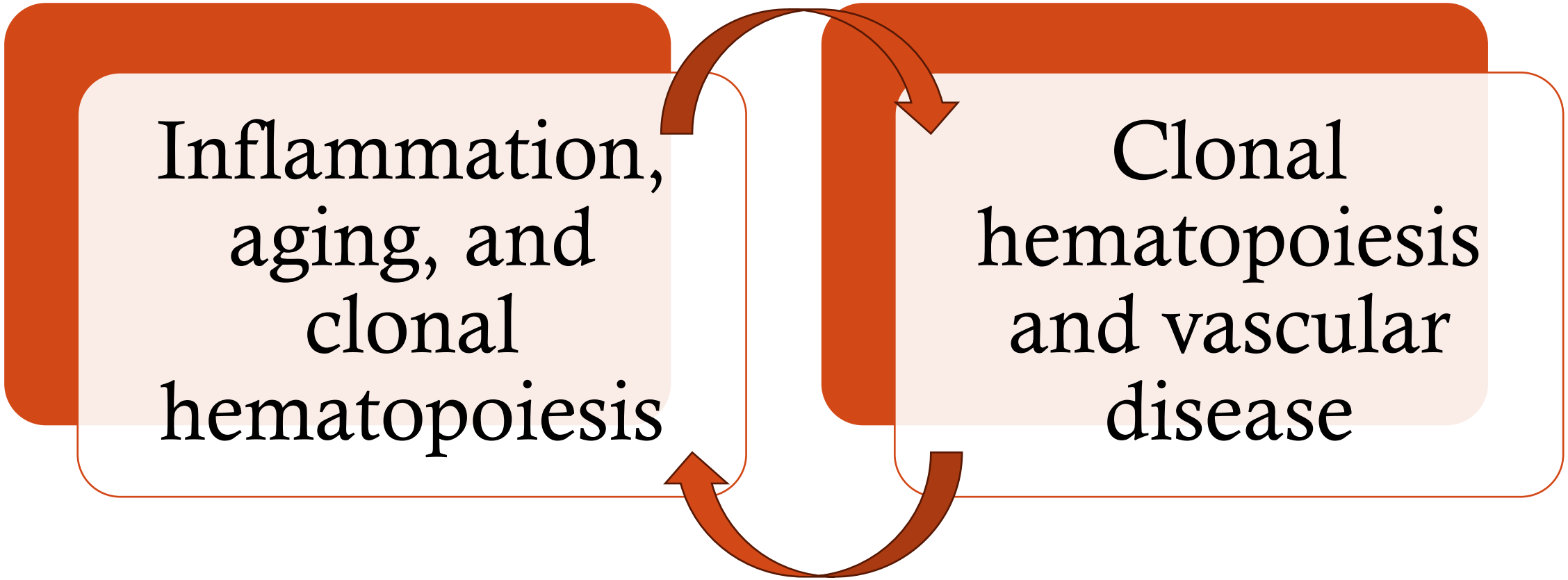
- *Credits: Dr.ssa Anna Ferrari*

1. INFLAMMATION, CLONAL HEMATOPOIESIS AND VASCULAR DISEASE

MPN MIGHT BE PRECEDED BY A CHIP-INDUCED VASCULAR DISEASE



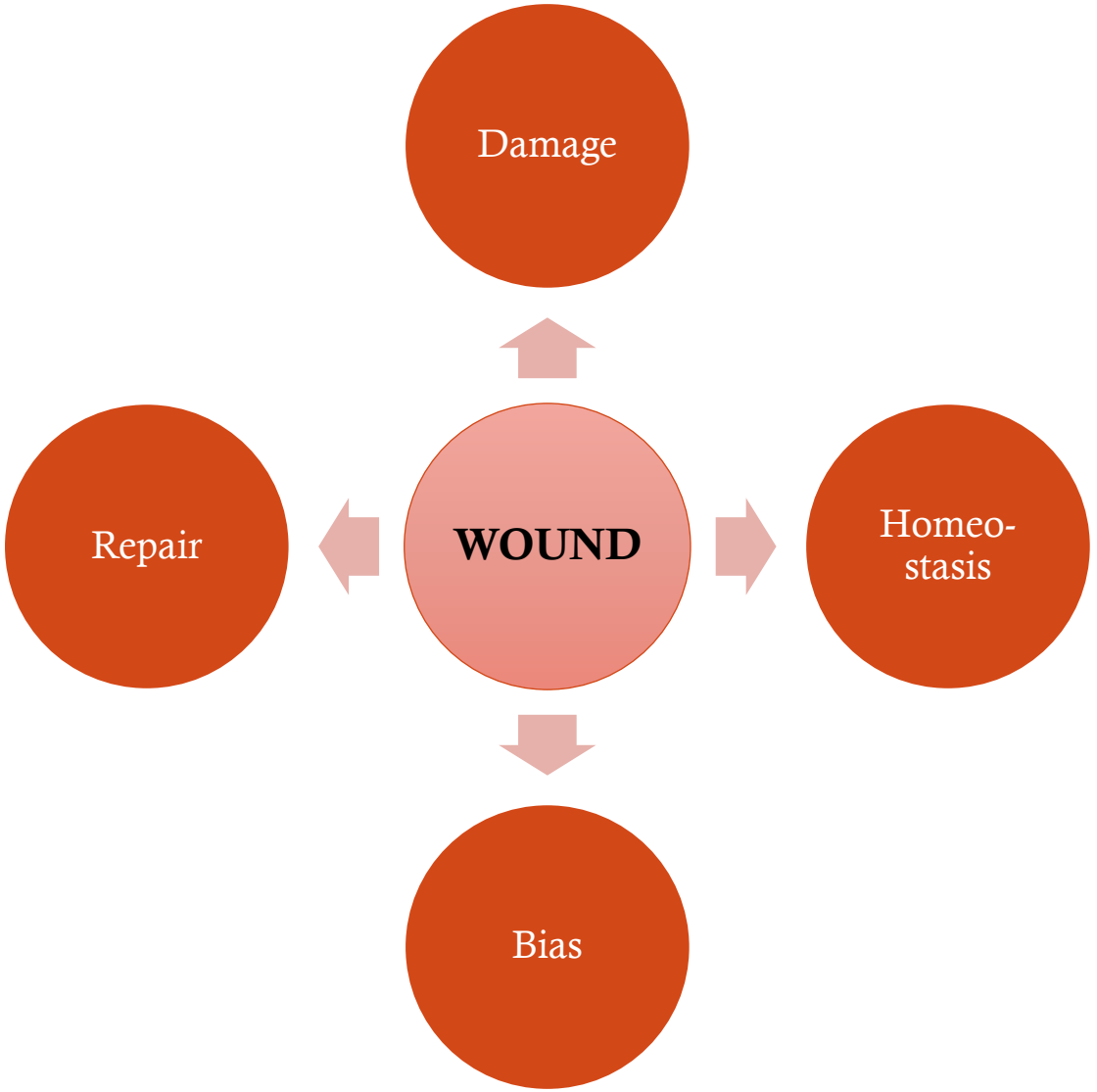
SUMMARY


Inflammation, aging, and clonal hematopoiesis
Clonal hematopoiesis and vascular disease

KEYWORDS

“Some pain has no relief, it can only be sealed
You can grasp the wound to feel the scar unhealed.”

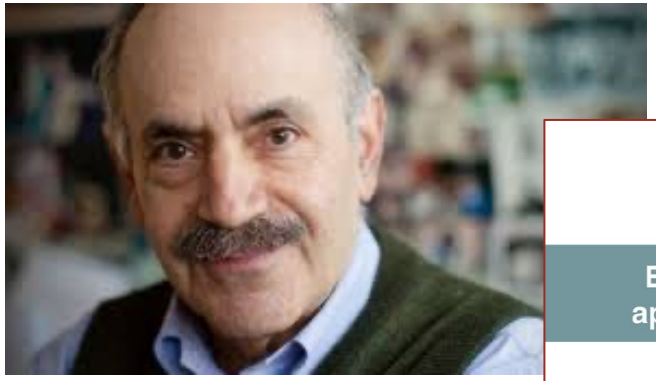
— **Munia Khan**



INFLAMMATION AND CANCER

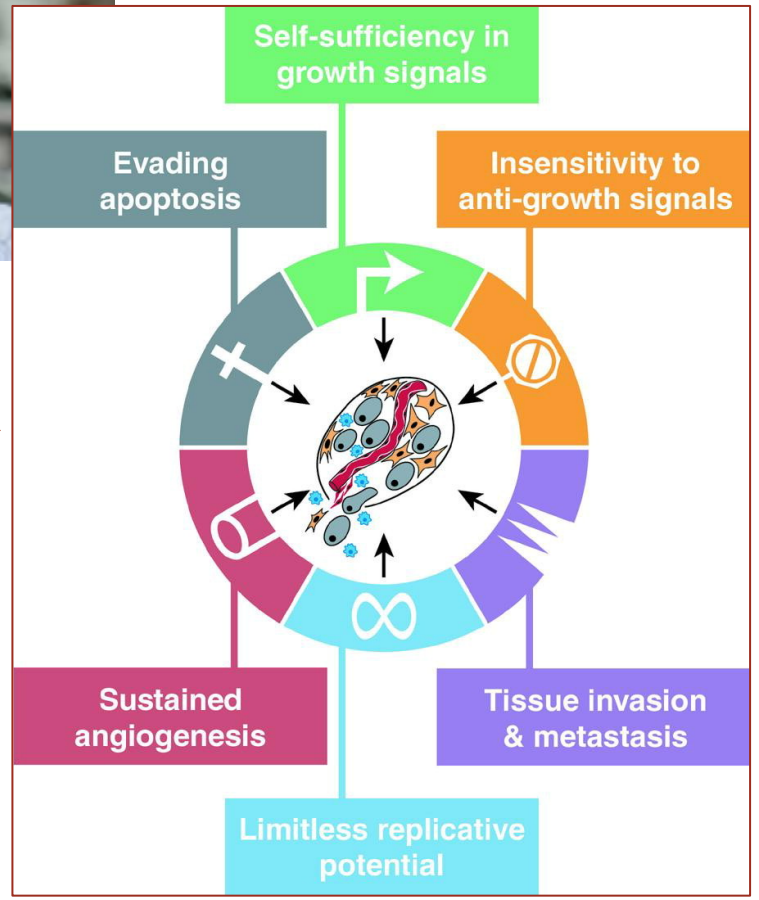
Harold F. Dvorak

Cancers as "wounds that do not heal" (1986)



Douglas Hanahan & Robert A. Weinberg

"The hallmarks of cancer" (2000)

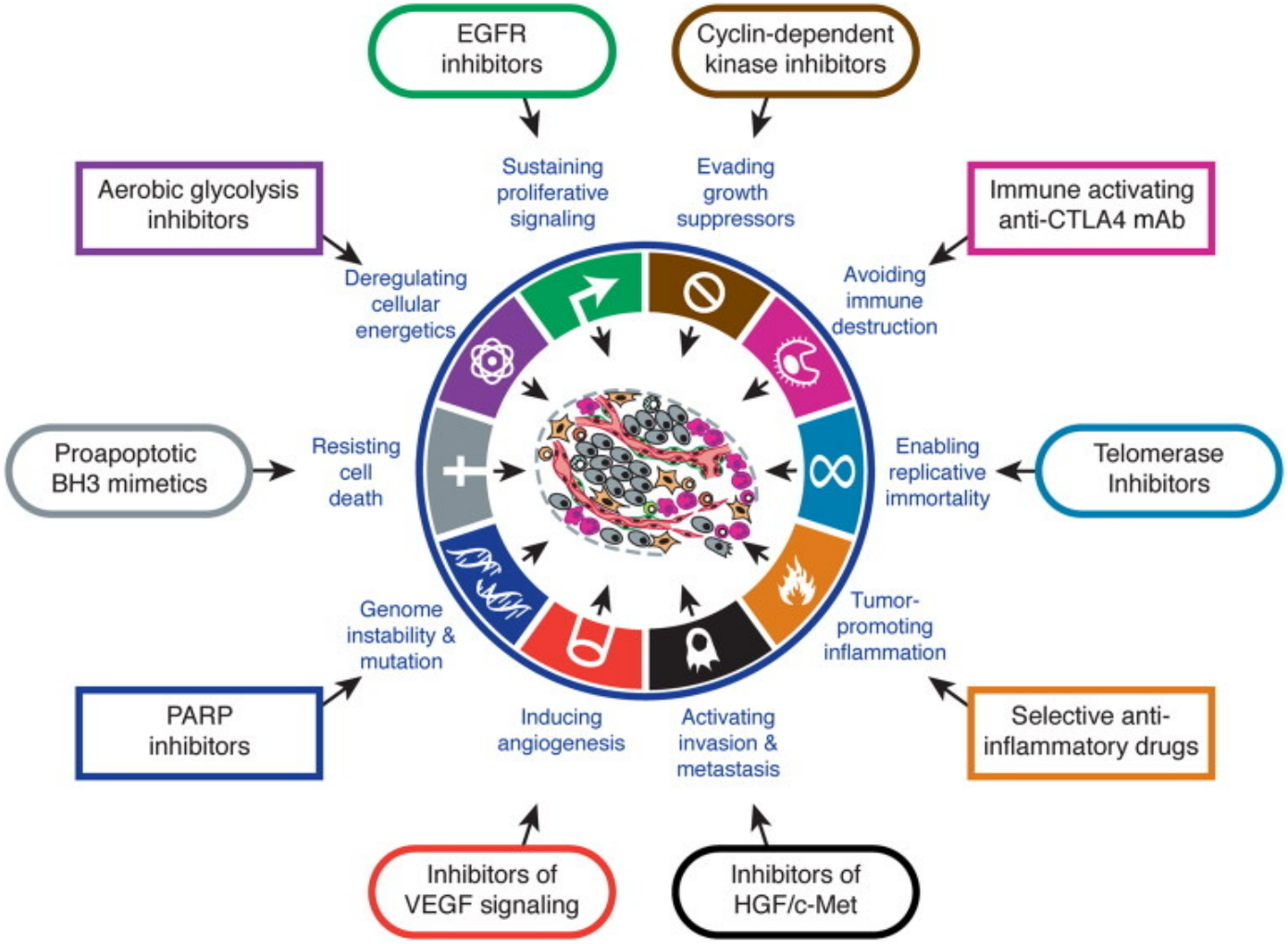


7th hallmark: chronic inflammation
(Colotta et al, 2009)

HALLMARKS OF CANCER (NEXT GEN)

Vascular disease is a potential consequence of each identified hallmark!

Hanahan & Weinberg, Cell 2011



HSC AND INFLAMMATORY RESPONSES

01

HSCs are key players in systemic inflammation

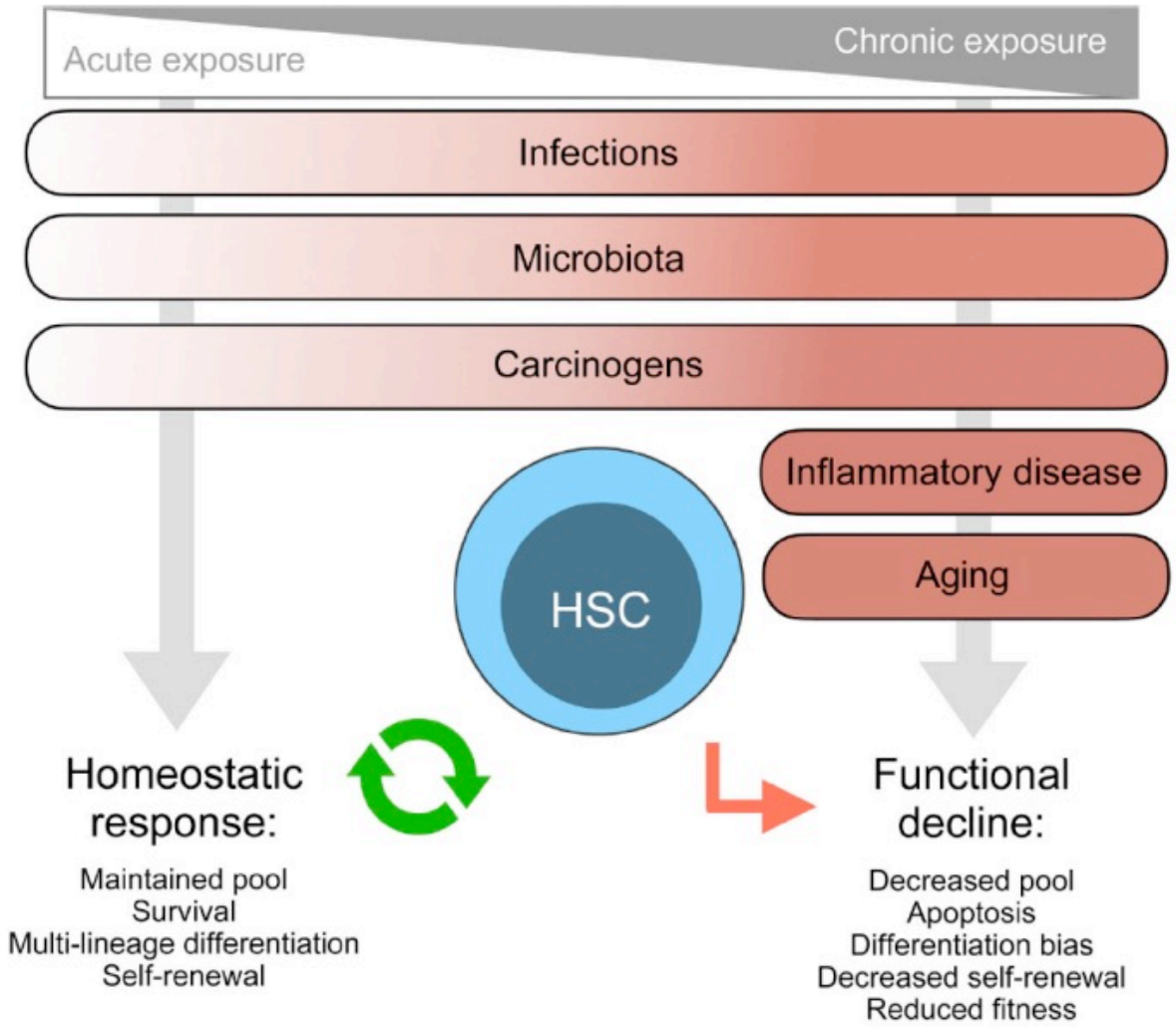
02

External inflammatory cues → cellular responses

03

Demand-adapted axis between peripheral stress and BM function

Tikizawa et al, Blood 2012

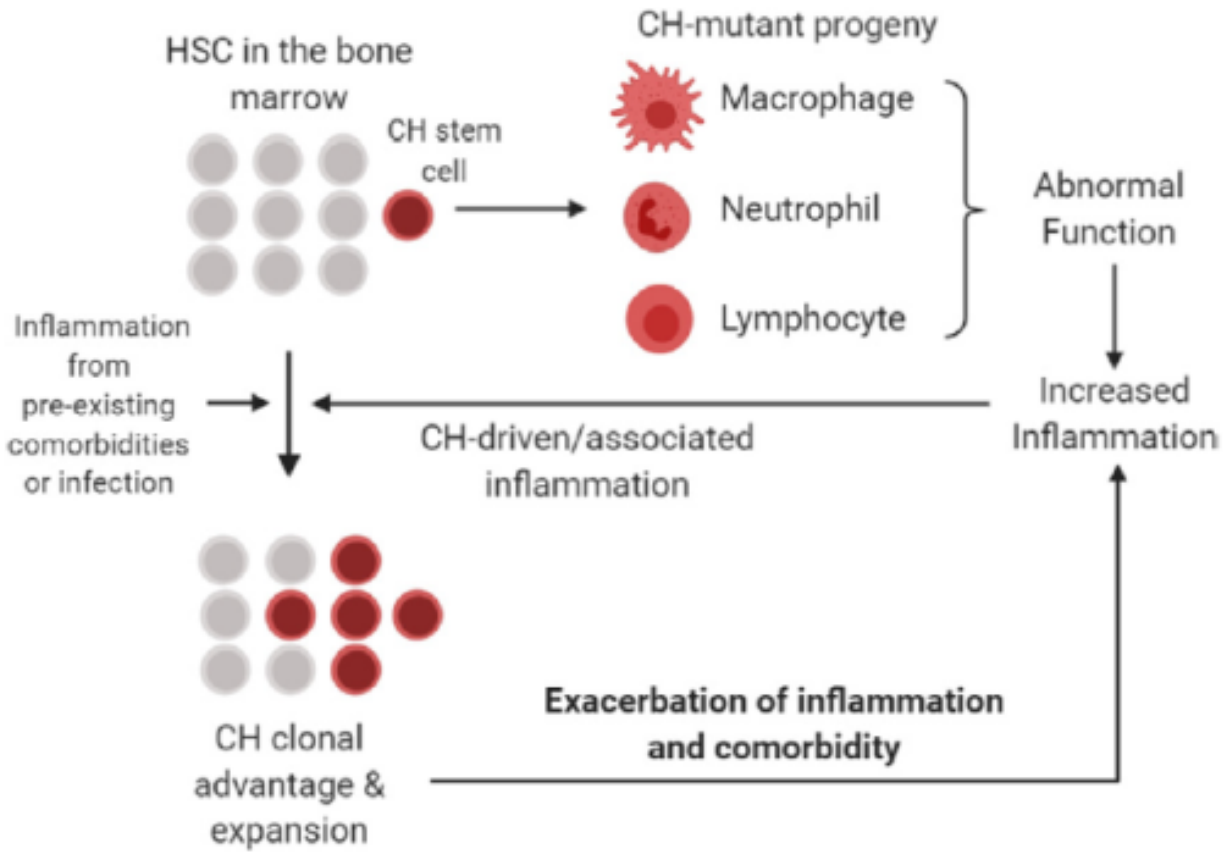


INFLAMMATION AND HSC: CAUSES AND CONSEQUENCES

Caiado et al, JEM 2021

CLONAL HEMATOPOIESIS OF UNDETERMINED POTENTIAL

- CH-mutant HSC → progeny with dysregulated function → more inflammation
- Inflammation from other sources (infections, comorbidities, carcinogens) → clone expansion



Cook et al, J Exp Hematol 2020

EFFECT OF DTA/CHIP MUTATIONS ON DIFFERENT LEUKOCYTES

Cook et al, J Exp Hematol 2020

Macrophages

Mutation	Effect	Related Comorbidities	Key References
Tet2	Inflammasome activation and IL-1β secretion	Atherosclerosis and heart failure	21, 52, 53
Dnmt3a or Tet2	Increased chemokine and cytokine production	Atherosclerosis	56
Jak2	Increased cytokine production, RBC phagocytosis	Atherosclerosis	55

Colour coding key

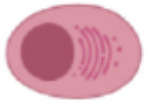
- Evidence in mouse model
- Evidence in humans

T cells

Mutation	Effect	Related Comorbidities	Key References
Tet1, Tet2, Tet3	Inhibit T-regs	Inflammatory and autoimmune diseases	74, 77
Tet2	Increased proliferation	Lymphoma	67, 75
Dnmt3a	Increased IL-13 production	Lung inflammation	76

Neutrophils and other granulocytes

Mutation	Effect	Related Comorbidities	Key References
Dnmt3a	Inappropriate degranulation	Pulmonary diseases	58
Jak2	Primed for NETosis	Thrombosis	61

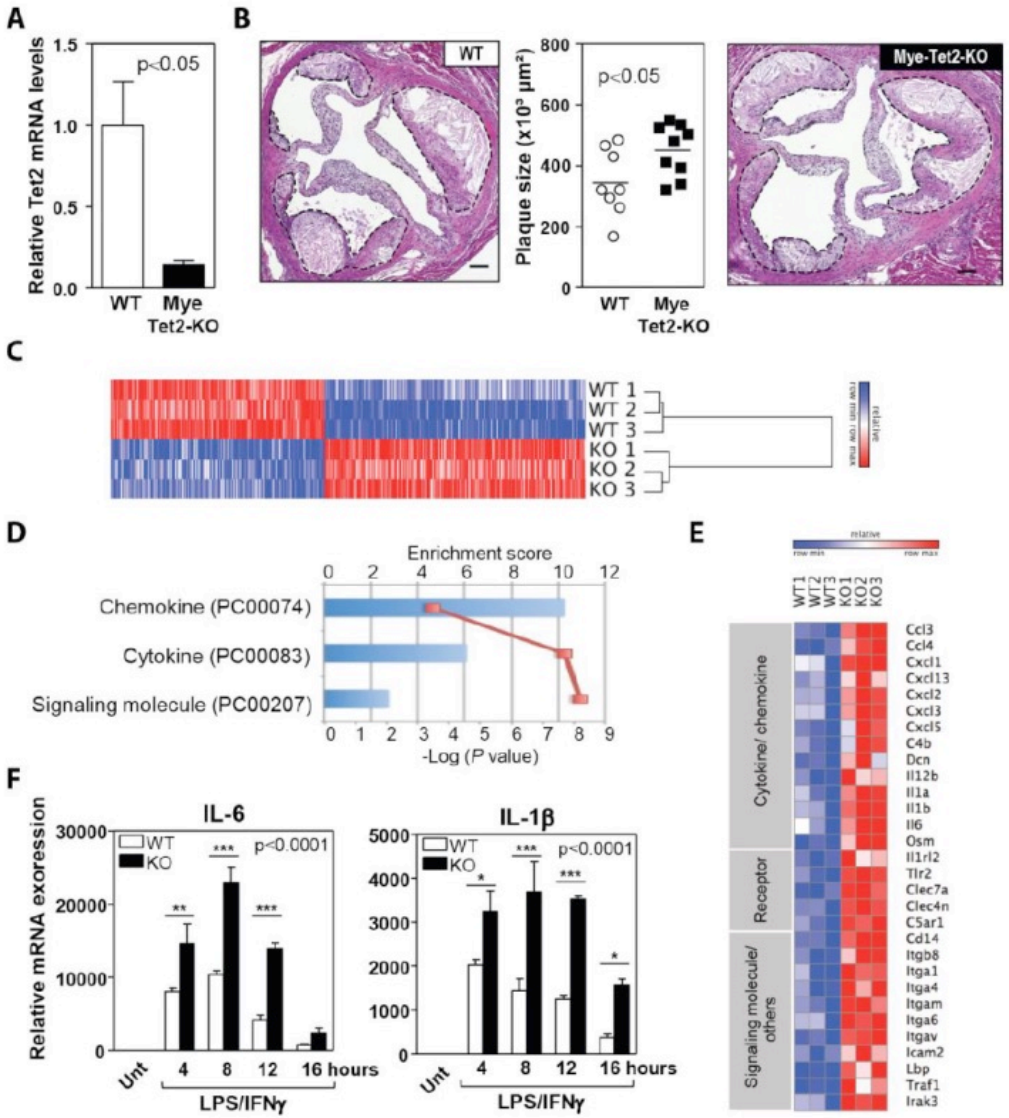


B cells and plasma cells

Mutation	Effect	Related Comorbidities	Key References
Tet2	Impaired plasma cell differentiation	Vulnerability to infection and lymphoma	80

Natural killer cells

Mutation	Effect	Related Comorbidities	Key References
Tet2, Tet3	Abnormal development and function	Infiltration of organs and lymphoma	82



TET2 AND ATHEROSCLEROSIS IN MICE MODELS

Fuster JJ et al, Science 2017

A CHIP and Coronary Heart Disease, According to Mutated Gene

Subgroup	No. of Participants with Myocardial Infarction/ No. at Risk	Hazard Ratio (95% CI)	P Value
No mutation			
BioImage	94/326		
MDC	299/607		
JHS/FUSION/FHS	169/3505		
DNMT3A			
BioImage	5/14	1.7 (0.7–4.1)	0.27
MDC	11/15	2.5 (1.4–4.7)	0.003
JHS/FUSION/FHS	8/99	1.1 (0.5–2.2)	0.90
Fixed-effects meta-analysis		**1.7 (1.1–2.6)**	**0.01**
TET2			
BioImage	3/7	1.6 (0.5–5.0)	0.46
MDC	2/6	0.8 (0.2–3.3)	0.76
JHS/FUSION/FHS	4/16	3.5 (1.3–9.6)	0.01
Fixed-effects meta-analysis		**1.9 (1.0–3.7)**	**0.06**
ASXL1			
BioImage	4/6	2.1 (0.7–5.8)	0.16
MDC	3/6	1.4 (0.5–4.6)	0.53
JHS/FUSION/FHS	2/10	2.8 (0.7–11.4)	0.15
Fixed-effects meta-analysis		**2.0 (1.0–3.9)**	**0.05**
JAK2			
BioImage	0/0		
MDC	2/2	10.0 (2.4–41.5)	0.001
JHS/FUSION/FHS	1/3	17.4 (2.4–127.6)	0.005
Fixed-effects meta-analysis		**12.0 (3.8–38.4)**	**<0.001**
Other			
BioImage	7/17	1.8 (0.8–3.9)	0.16
MDC	3/4	1.9 (0.6–6.0)	0.28
JHS/FUSION/FHS	6/35	3.0 (1.3–6.9)	0.009
Fixed-effects meta-analysis		**2.2 (1.3–3.7)**	**0.002**

B CHIP and Myocardial Infarction, According to Mutated Gene

ATVB and PROMIS	No. of Participants with Myocardial Infarction/ No. at Risk	Odds Ratio (95% CI)	P Value
DNMT3A	31/46	1.4 (0.7–2.8)	0.29
TET2	12/13	8.3 (1.2–357.5)	0.02
ASXL1	8/8	Undefined	0.02
JAK2	16/16	Undefined	<0.001
Other	20/22	6.9 (1.7–61.6)	0.001

C Myocardial Infarction and Coronary Heart Disease, According to Mutated Gene

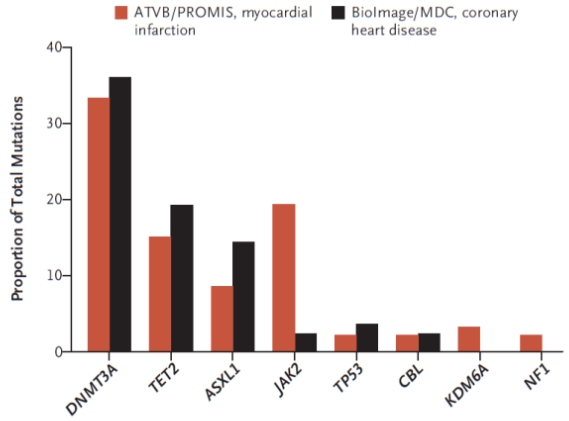


Figure 2. Association between Coronary Heart Disease and Early-Onset Myocardial Infarction among CHIP Carriers, According to Genetic Mutation.

Panel A shows a forest plot of hazard ratios for the risk of coronary heart disease in BioImage, MDC, and three prospective cohorts that were unselected for coronary events: the Jackson Heart Study (JHS), Finland–United States Investigation of NIDDM [Non–Insulin-Dependent Diabetes Mellitus] Genetics (FUSION), and the Framingham Heart Study (FHS), according to mutated gene. Hazard ratios for the listed mutations were obtained by a fixed-effects meta-analysis of Cox proportional-hazards models after adjustment for age, sex, type 2 diabetes status, total cholesterol, HDL cholesterol, triglycerides, smoking status, and hypertension. Panel B shows the risk of early-onset myocardial infarction among the participants in ATVB and PROMIS (combined analysis), according to the mutated gene. The odds ratios for myocardial infarction were obtained with the use of Fisher's exact test; P values were not adjusted for multiple hypothesis testing. Panel C shows the proportion of total mutations (according to gene) among participants with myocardial infarction in the ATVB and PROMIS studies, as compared with those with coronary heart disease in the BioImage and MDC studies.

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 13, 2017 VOL. 377 NO. 2

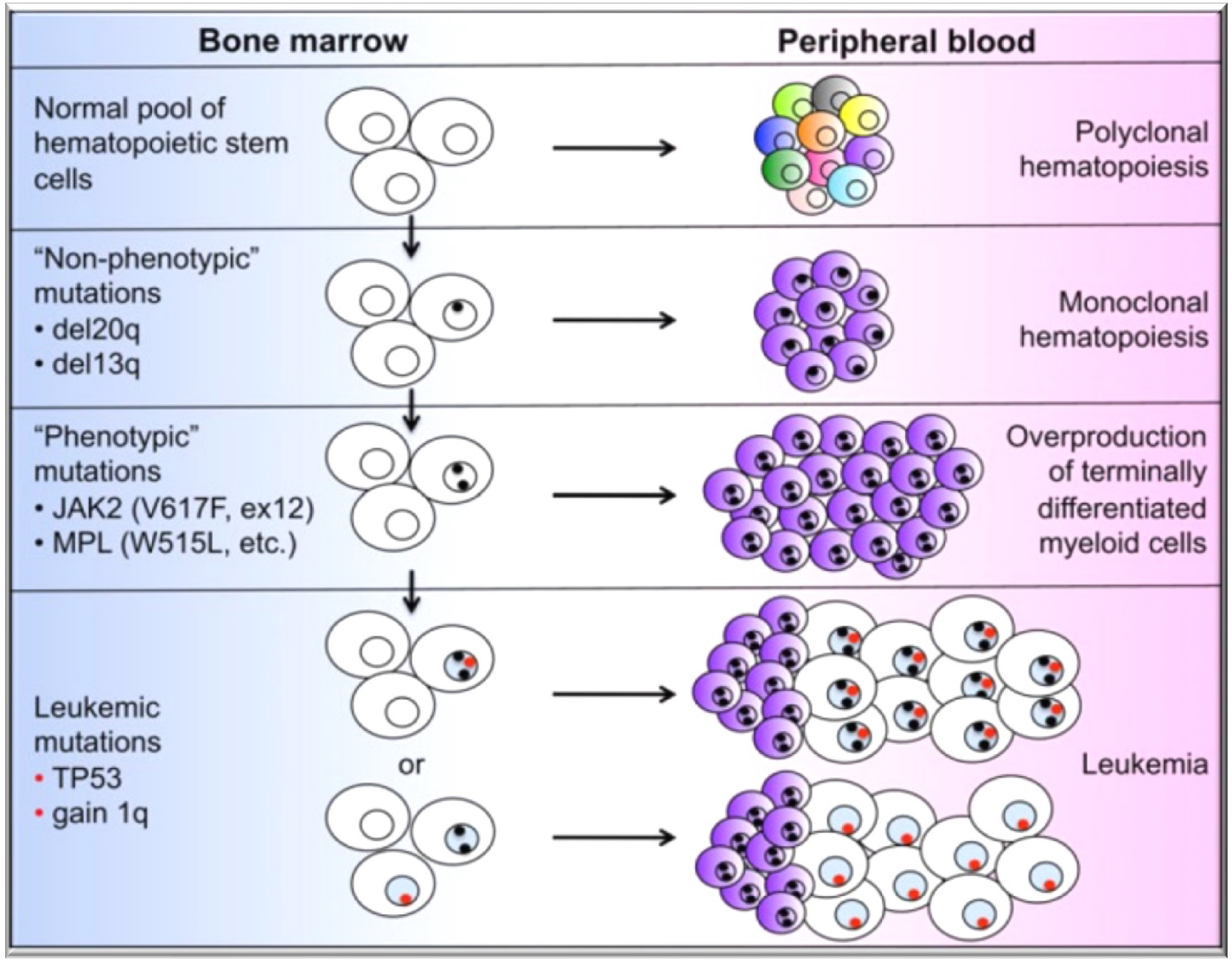
Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease

S. Jaiswal, P. Natarajan, A.J. Silver, C.J. Gibson, A.G. Bick, E. Shvartz, M. McConkey, N. Gupta, S. Gabriel, D. Ardissino, U. Baber, R. Mehran, V. Fuster, J. Danesh, P. Frossard, D. Saleheen, O. Melander, G.K. Sukhova, D. Neuberg, P. Libby, S. Kathiresan, and B.L. Ebert

The meta-analysis shows unequivocally that DTA mutations (ASXL1, TET2, DNMT3A) are associated with coronary artery disease.

THE «MECHANICISTIC» ERA

Clonal evolution is intended as the progressive accumulation of genetic lesions



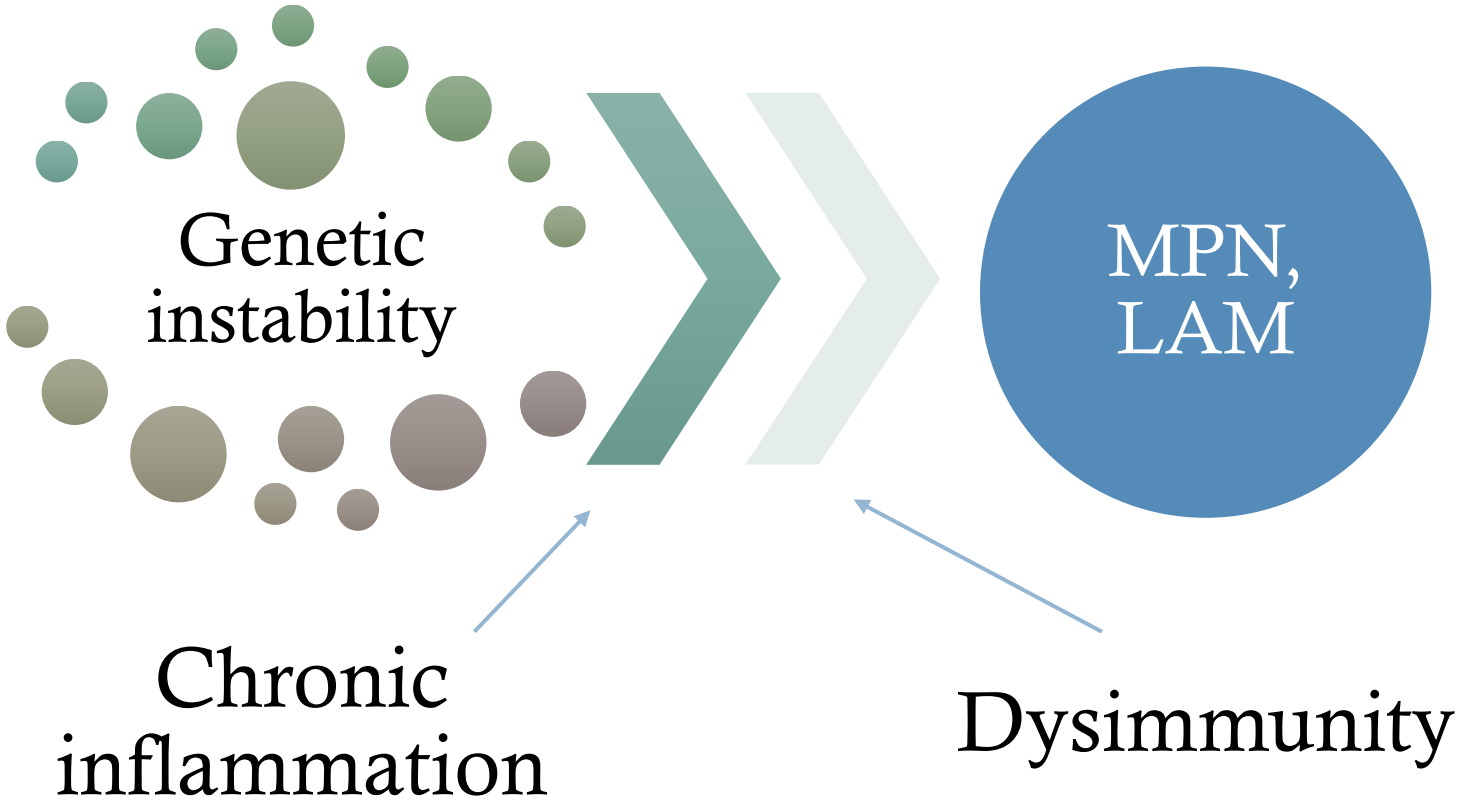
Milosevic & Kralovics, Int J Hematol 2013

THE DORMANT INTELLIGENCE

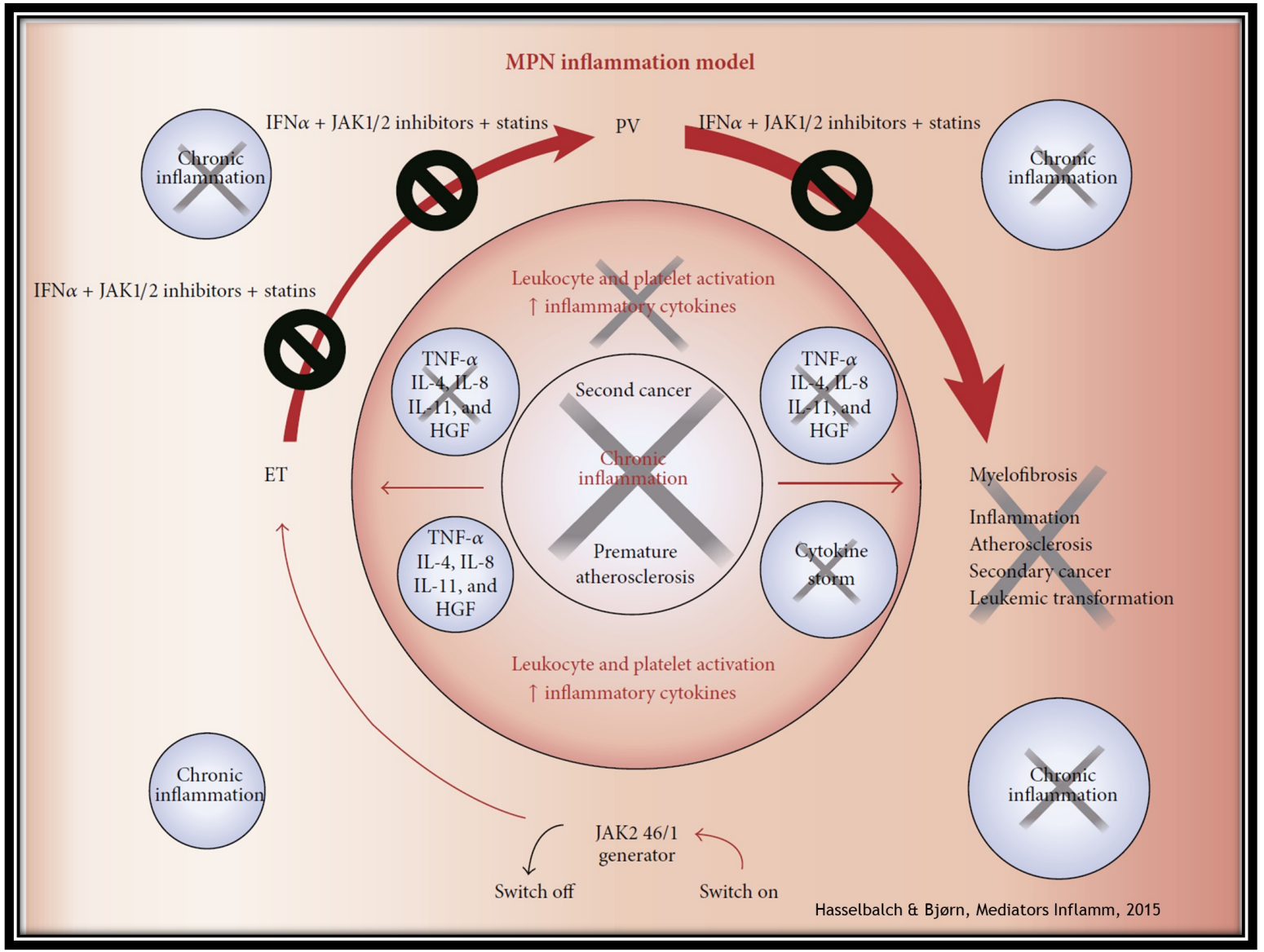
- Polarity: each degree of nature consists of an antithetical but complementary pair
- Cohesion: the interaction of forces that aim to balance each other
- Metamorphosis: the transformability of elements into one another
- Power: each degree of the evolutionary scale is the result of transformation from its predecessor
- Analogy: the affinity of phenomena

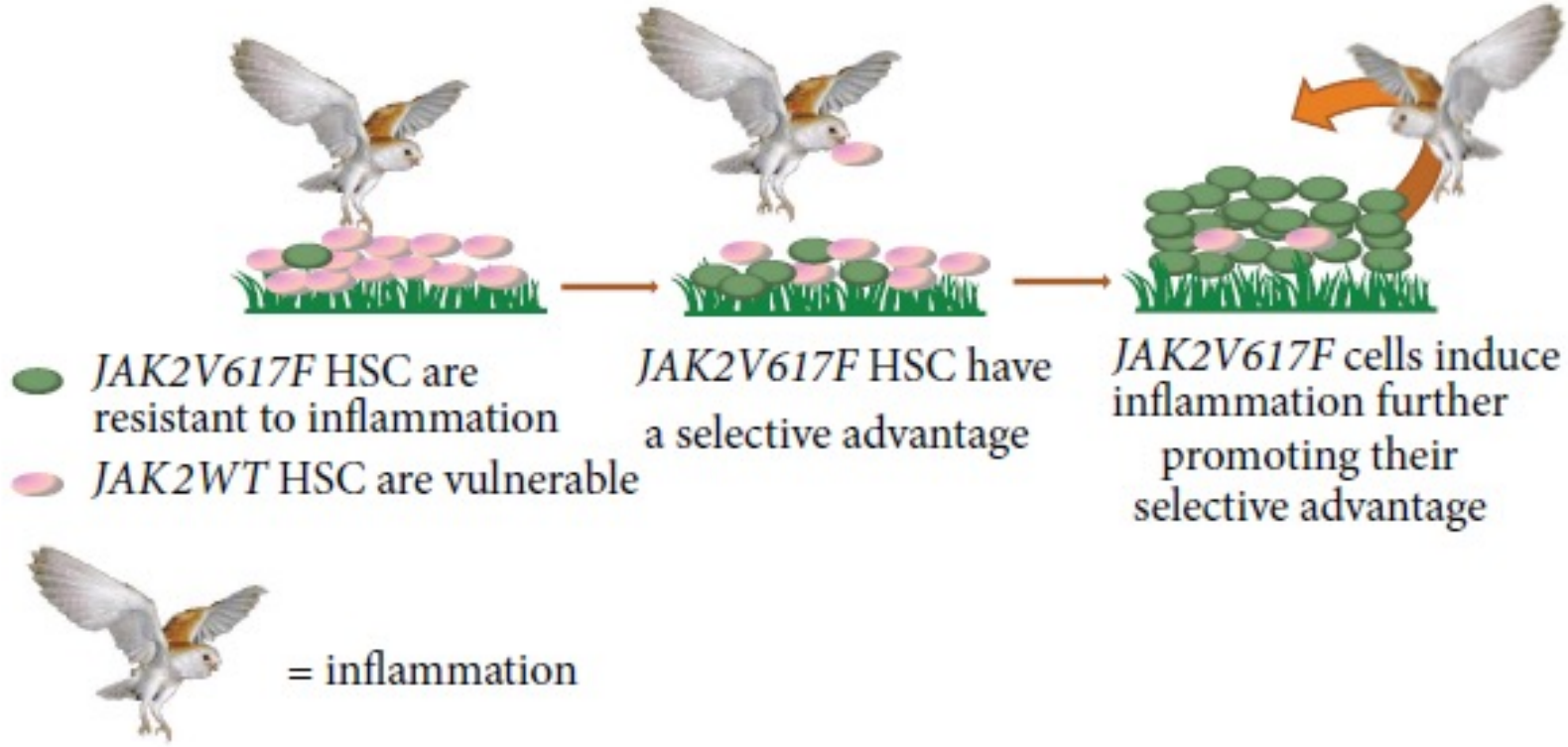
Friedrich Schelling 1775 - 1854

MODEL COMPLETION



THE «VICIOUS CIRCLE» MODEL



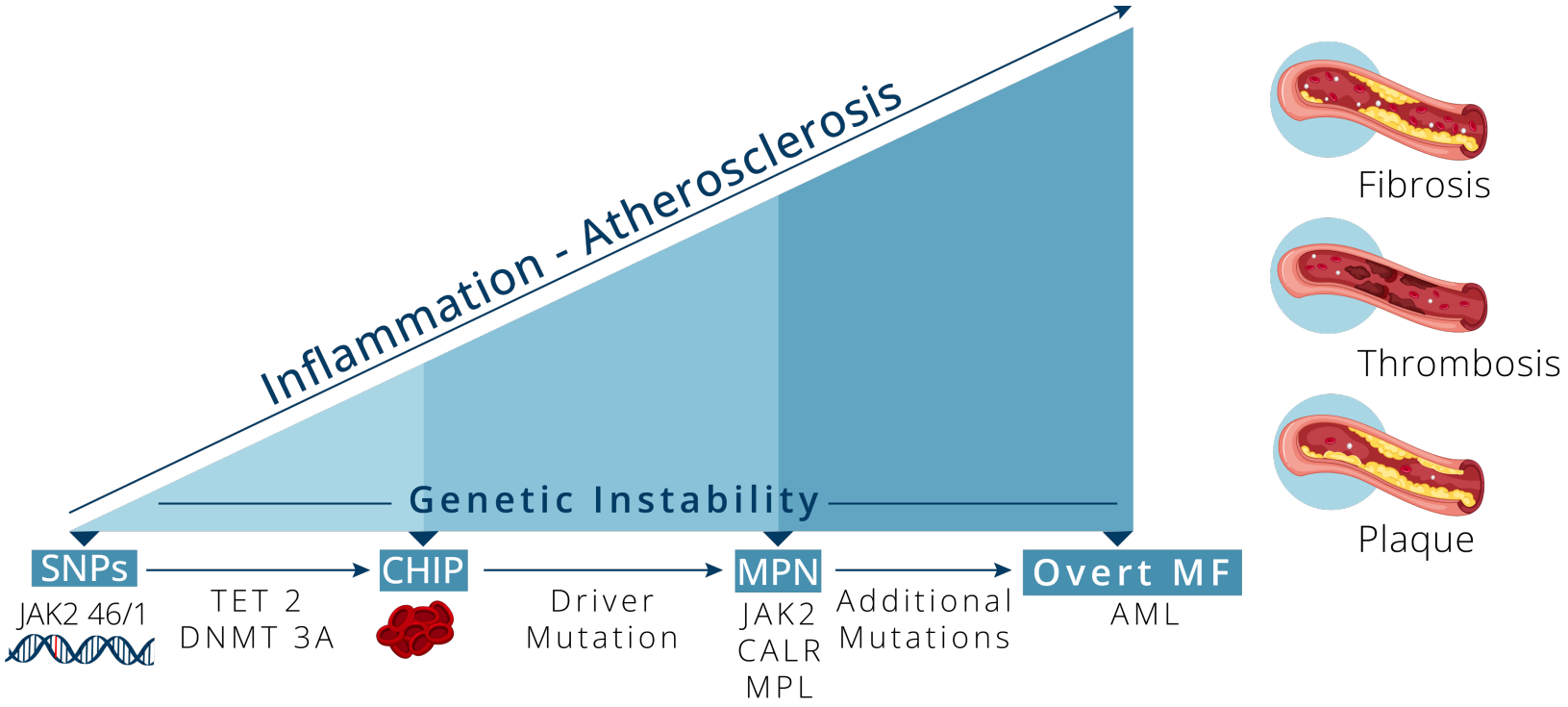


CLONAL SELECTION AND ADVANTAGE

Fleischman, Mediators inflamm 2015

* Courtesy of Dr. Giulio Giordano

- JAK2V617F HSCs
  - induce inflammation
  - are resistant to it
- A vascular, inflammatory disease may precede JAK2 mutation



MODEL OF PROGRESSION

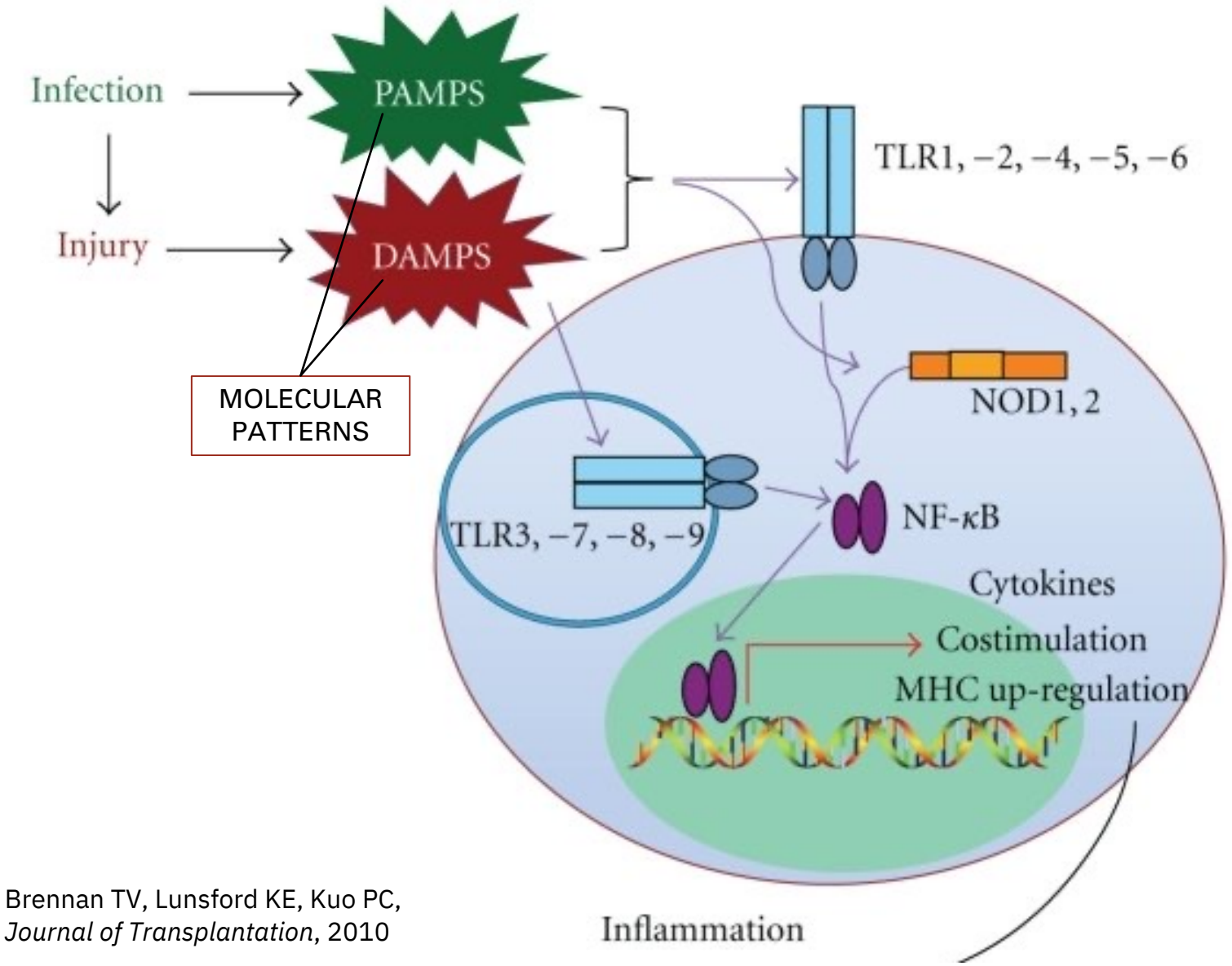
Lucchesi et al [unpublished]

2. INFLAMMATION AND CANCER, INFLAMMASOME ACTIVATION IN MPNS

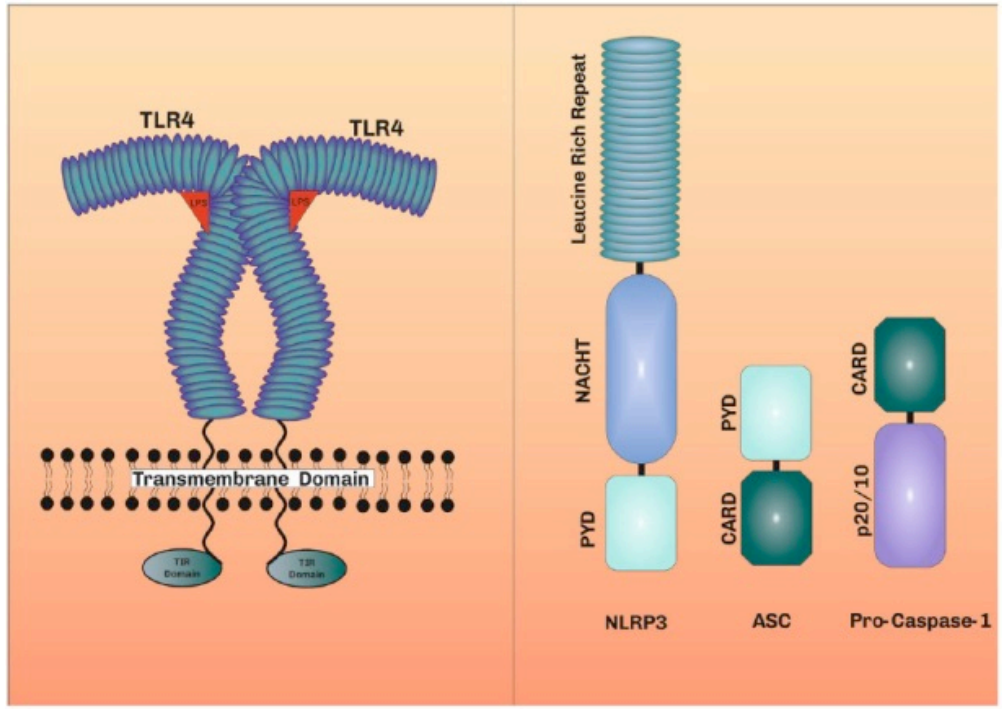
A MECHANISM AS FUNDAMENTAL AS IT IS DELETERIOUS

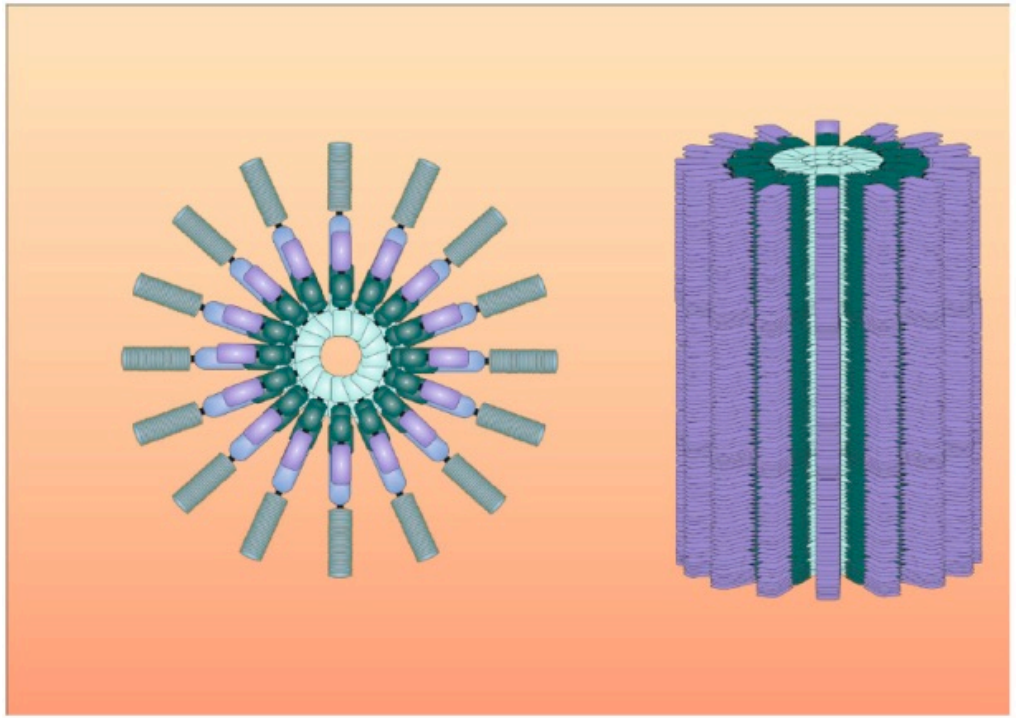
PATTERN RECOGNITION RECEPTORS

- Cells must respond to tissue insults caused by endogenous and/or exogenous stimuli
- To cope with this duty, innate immunity is always at the forefront.
- Cells of innate immunity recognise 'danger signals' through a specialised set of membrane, Toll-like receptors, and intracytoplasmic NOD-like receptors.



Brennan TV, Lunsford KE, Kuo PC, *Journal of Transplantation*, 2010





INFLAMMASOME ASSEMBLY

Kinra M, Scand J Immunol, 2021

- Dimerised TLR after pathogen contact
- NLR (NOD-like receptors):
  - There are 4 types
  - NLRP3 is the most studied in autoinflammatory conditions and chronic disorders
  - LRR activates downstream signalling

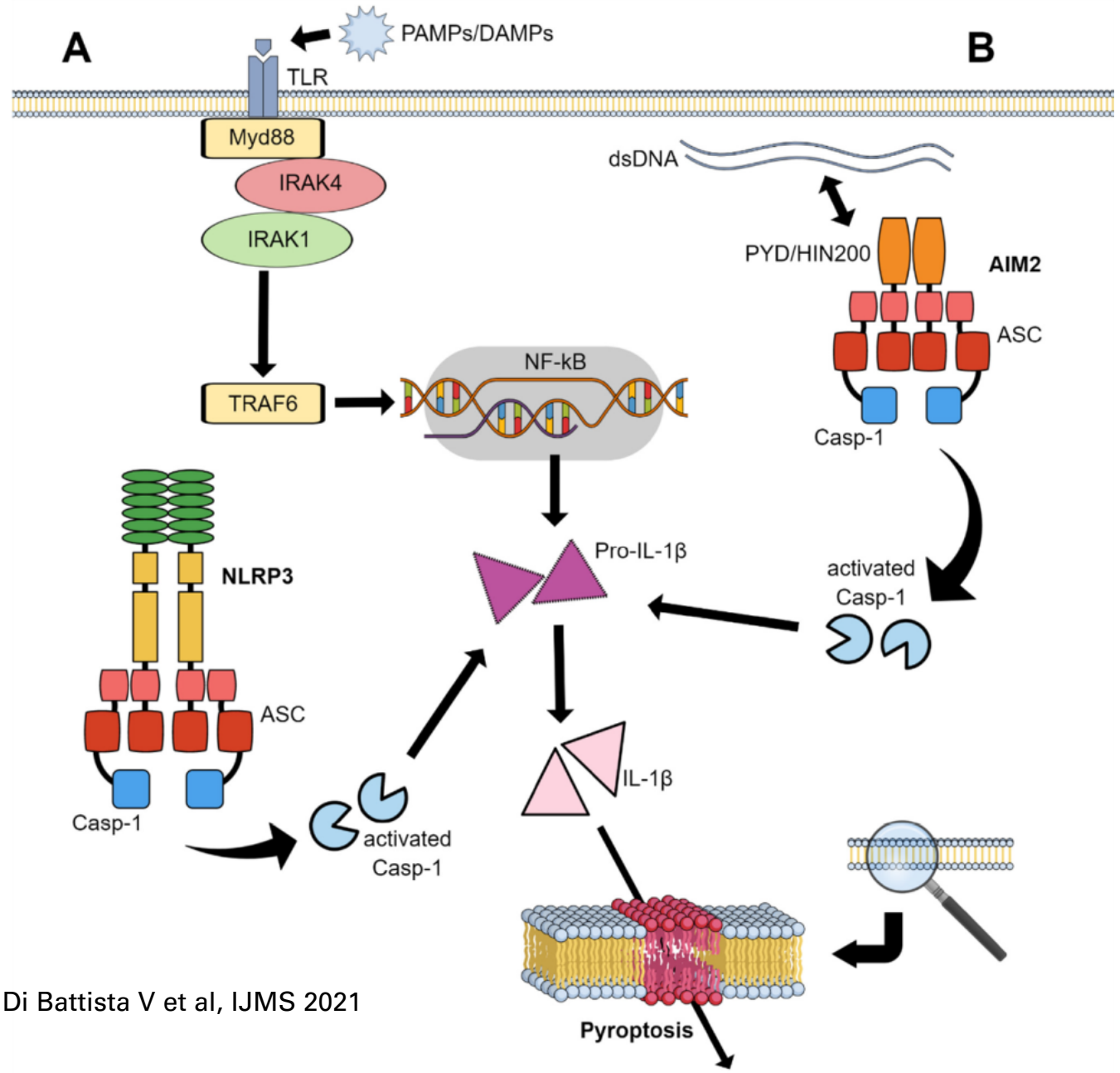
INFLAMMASOMES AND PYROPTOSIS

NLRP3 triggered by

- TLR agonists
- Pathogens and damage-associated signals

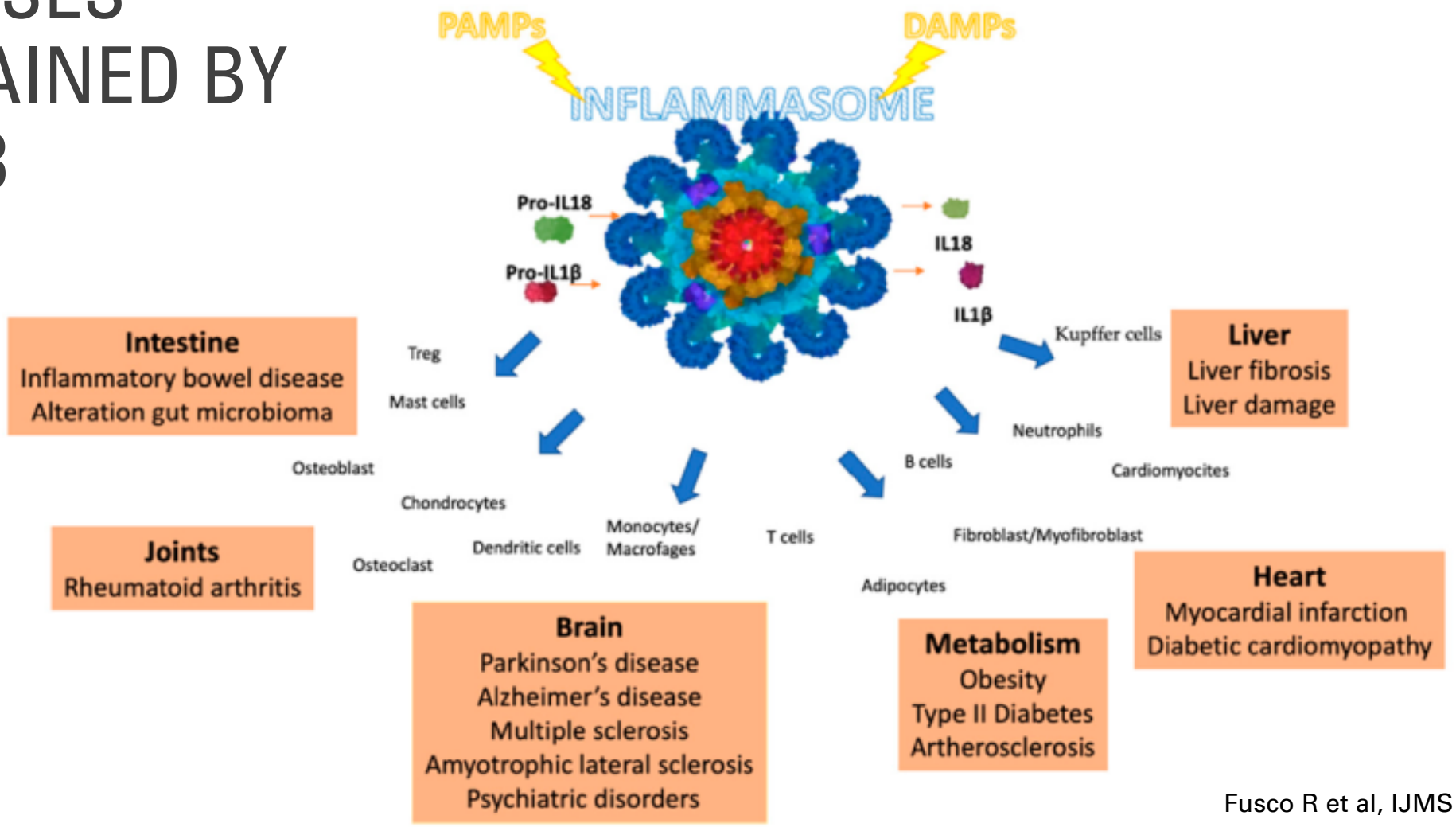
Pyroptosis

- Form of programmed cell death (cytoplasmic pores from gasdermine D)
- Associated with atherogenic vascular damage
- Associated with tumour promotion



Di Battista V et al, IJMS 2021

DISEASES SUSTAINED BY NLRP3



Fusco R et al, IJMS 2021

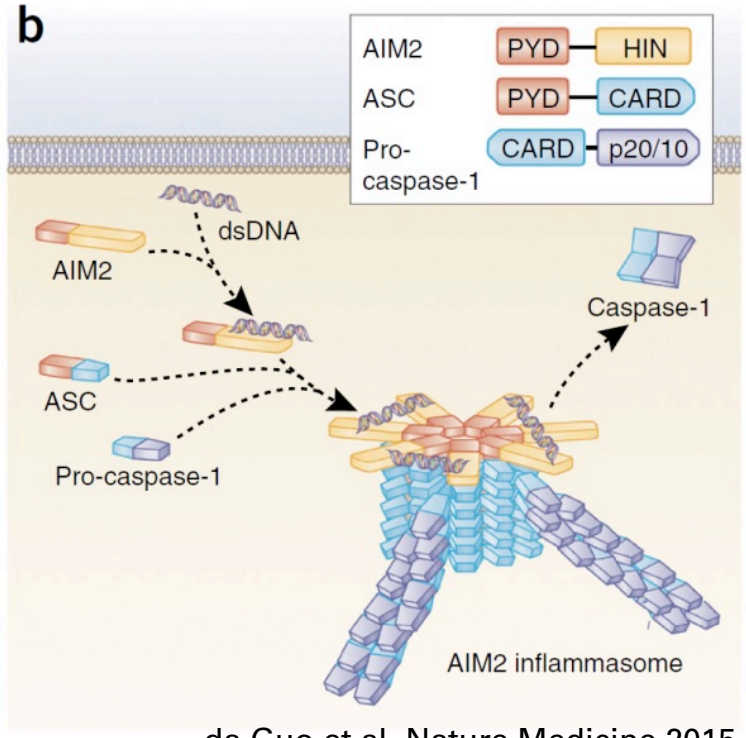
Liew et al. Exp Hematol Oncol (2016) 5:2
DOI 10.1186/s40164-016-0032-7

Experimental Hematology & Oncology

RESEARCH Open Access

Identification of AIM2 as a downstream target of JAK2V617F

Ei Leen Liew[1,2], Marito Araki[3], Yumi Hironaka[1], Seiichi Mori[4], Tuan Zea Tan[5], Soji Morishita[3], Yoko Edahiro[1], Akimichi Ohsaka[3] and Norio Komatsu[1*]



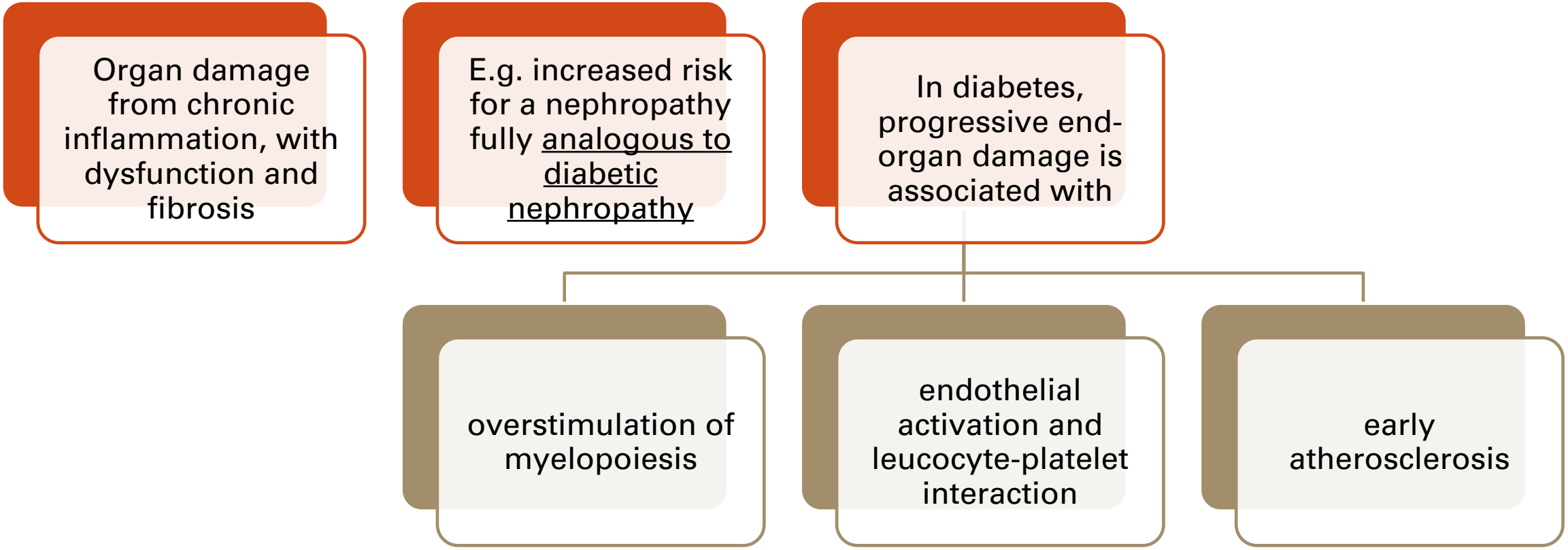
da Guo et al, Nature Medicine 2015

Apoptotic-DNA sensor

Functional macrophagic maturation

MPNs ≈ Lupus!

MPN: OTHER ANALOGIES



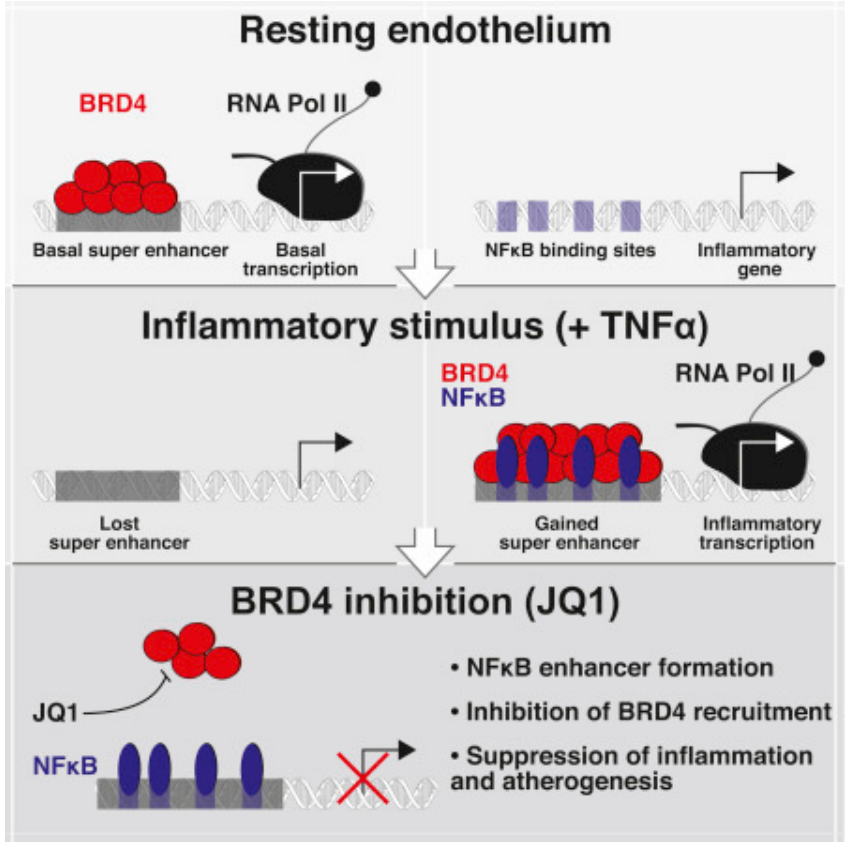
Hasselbalch HC, Leukemia Research, 2014

Key transcription factor in innate immunity

It is constitutively active in MPN mouse models irrespective of driver mutations

It is modulated by BET proteins

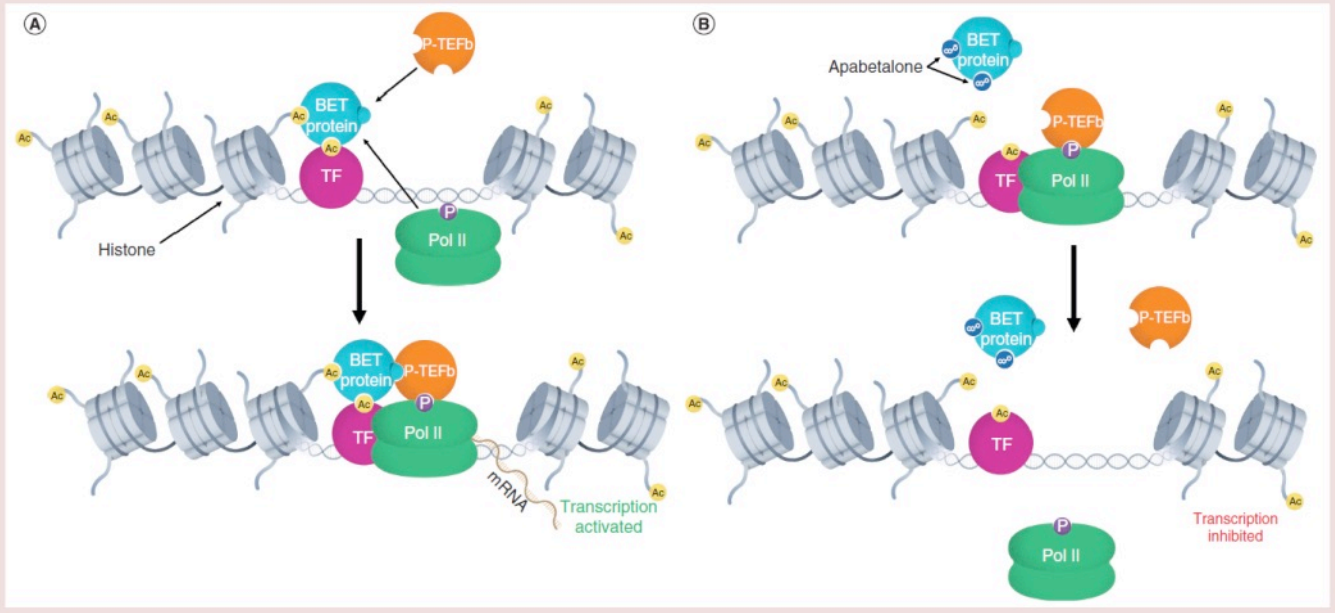
NF-KB ACTIVATION

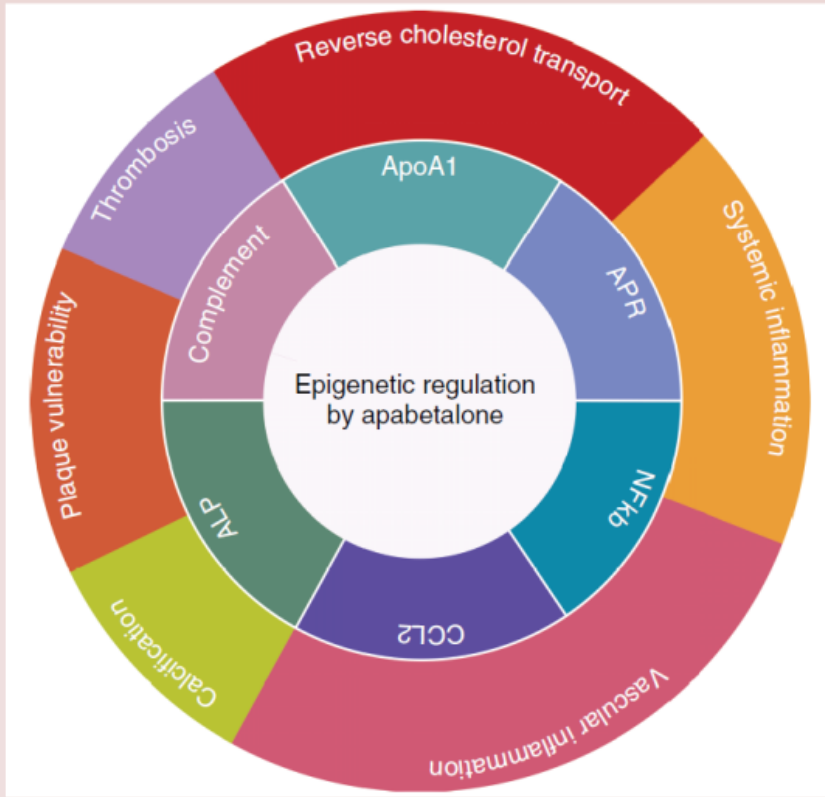


Kleppe M et al, Cancer Cell 2018
Brown JD, Molecular Cell 2014

BET INHIBITION IN CARDIOVASCULAR DISEASE AND DIABETES

BRANDS J & RAY KK, FUTURE CARDIOLOGY, 2021





3. INFLAMMASOME, PLATELETS, NETS AND THROMBOSIS

PLATELETS AS PLAYERS IN INNATE IMMUNITY; THROMBO-INFLAMMATION

THROMBO-INFLAMMATION

Tentative definition: «the pathological responses within the vasculature following inflammatory triggers and leading to organ damage»

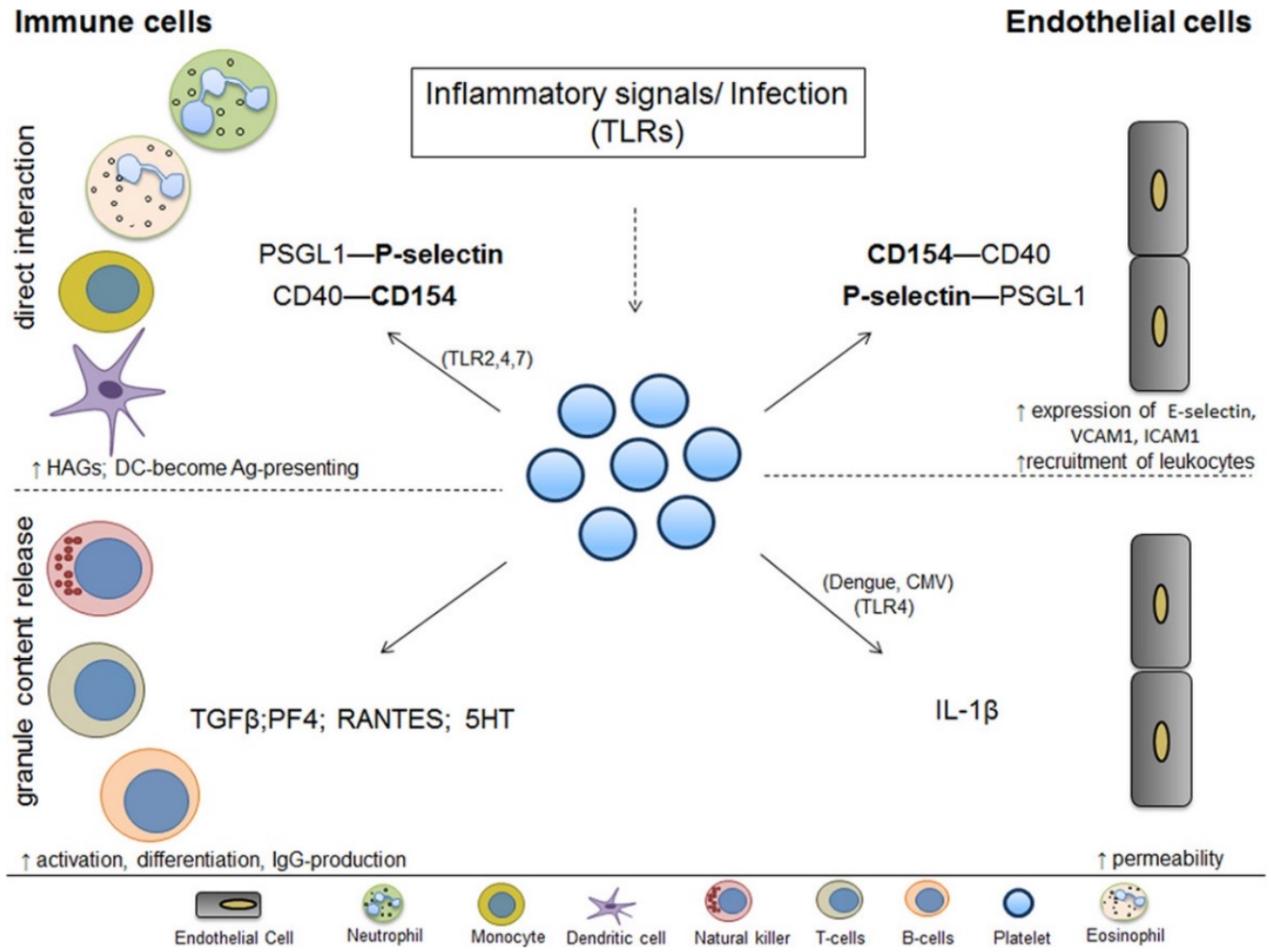
The triggers:		
Blood vessel injury	Invasion by pathogens	Non infectious (sterile inflammation)

Gao L et al, Front Immunol 2019; Bhuria V et al, IJMS 2022

KEY POINTS

The cells of the innate immune system play a key function in the pathogenesis of sterile thrombosis

Thrombo-inflammation is a critical therapeutic target for many diseases

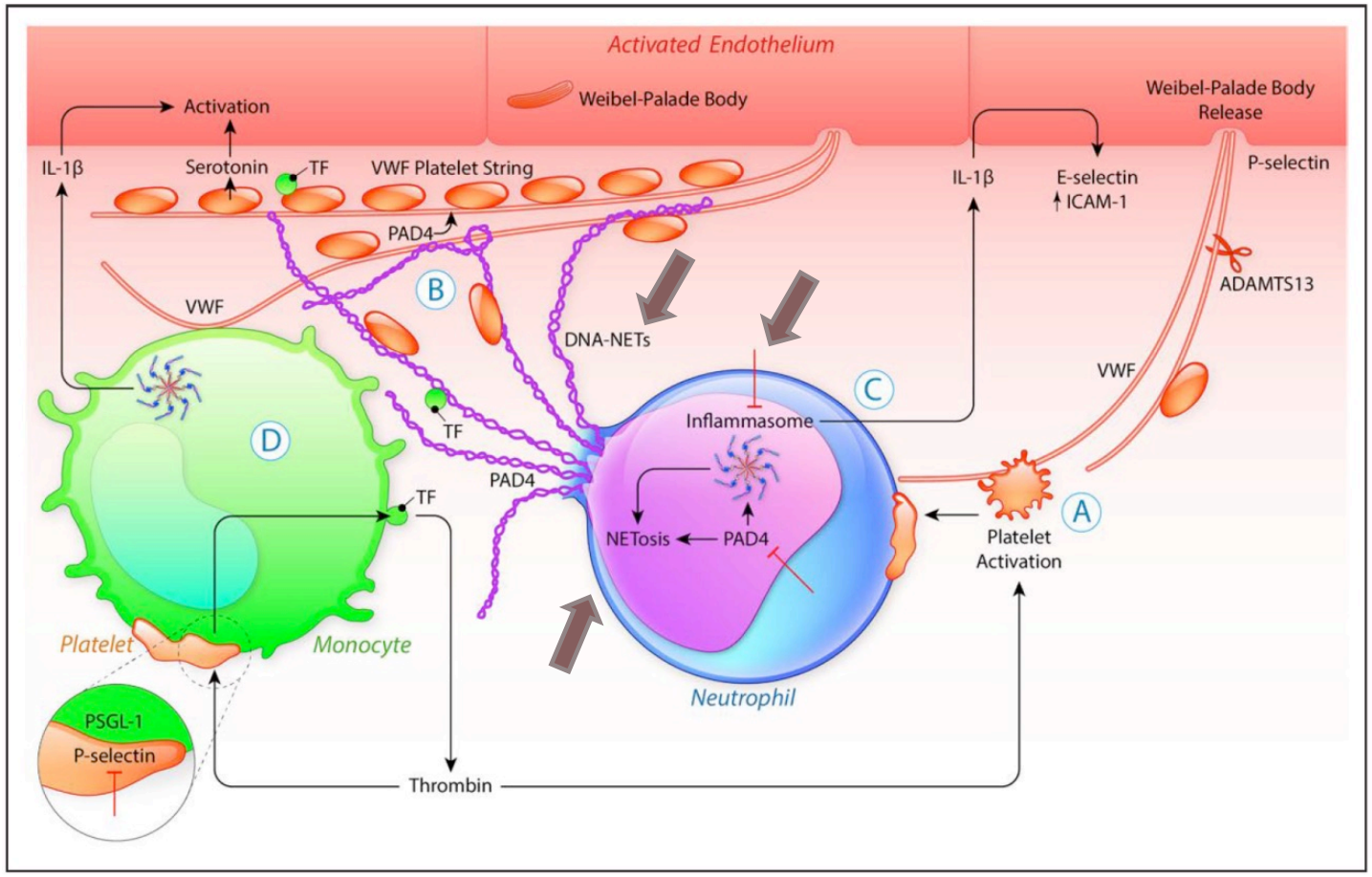


PLATELETS AS MEDIATORS OF IMMUNITY AND INFLAMMATION

KOUPENOVA M ET AL, CIRC RES. 2018

THROMBO-INFLAMMATION: KEY PLAYERS

- Interaction between platelets, macrophages, neutrophils and endothelium is mediated by:
- P-selectin
- vWF / ADAMTS-13
- NETs
- inflammasome



Wagner DD & Heger LA, Ateriosclr Thromb Vasc Biol 2022

INFLAMMASOME AND NETS

Inflammasome

- Pores on the cell membrane (pyroptosis) TF release
- IL-1β release → increases integrin expression and cellular interactions
- Supports the formation of NETs

NETosis

- It is a process of cell death with the formation of 'spider web' like chromatin structures
- It is designed to stop infectious processes, but induces tissue damage and thrombo-inflammation

PAD4 (protein arginine deiminase 4)

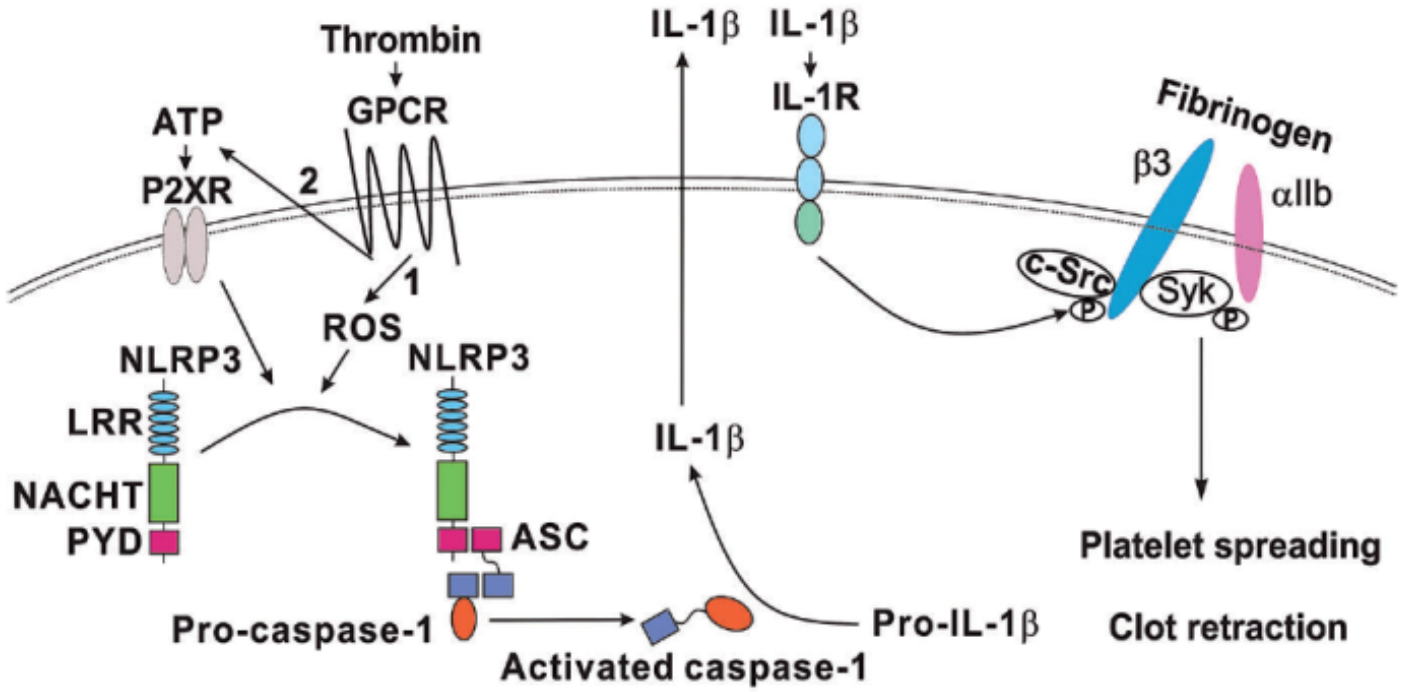
- Intra-cytoplasmic enzyme that supports both processes
- It adds citrulline by promoting chromatin unfolding (positively charges histones H3 and H4)

Wagner DD & Heger LA, Aterioscler Thromb Vasc Biol 2022
Wu C et al, Immunity 2019

NLRP3 AND INTEGRINS

- The platelet NLRP3 inflammasome promotes IL-1β secretion and is upregulated during platelet activation and thrombus formation in vitro

- NLRP3-/- platelets transfused into wild-type mice resulted in prolonged bleeding time, delayed arterial thrombus formation, defects in fibrinogen and thrombin function



↑TGF-ß

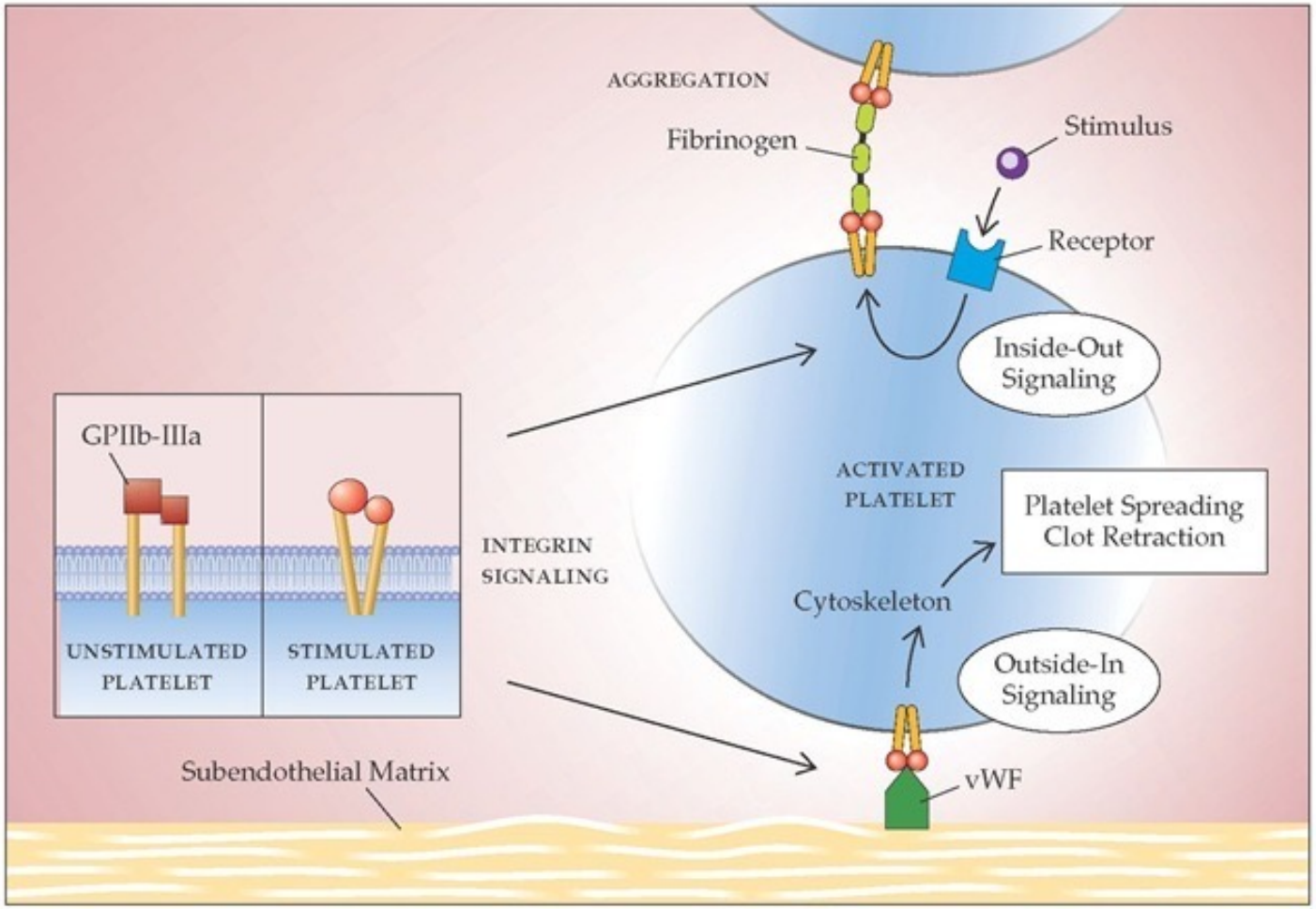
Quiao J, Haematologica 2018

4. DEVISING MODELS OF THROMBO-INFLAMMATION IN MPNS

FROM PLATELET FIBRINOGEN RECEPTOR TO THE CIRCULATING WOUND

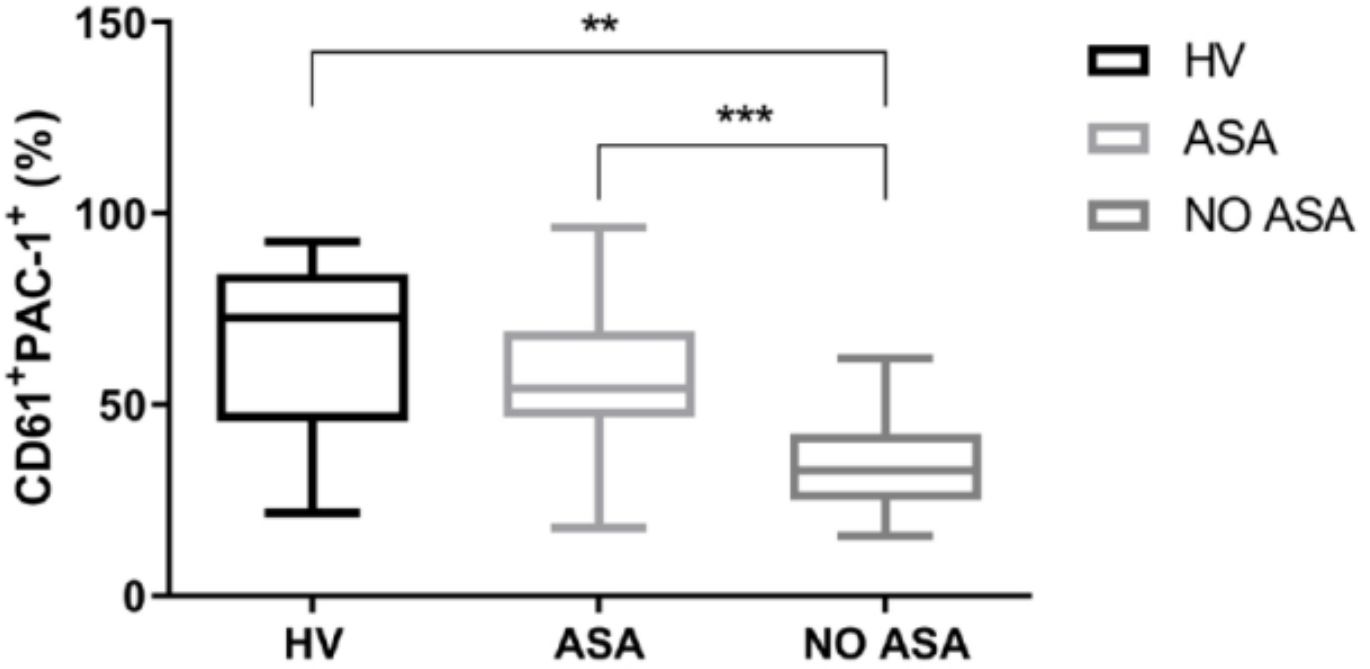
PLATELET FIBRINOGEN RECEPTORS

- A refined method for the determination of platelet activation appears to be the use of platelet PAC-1 antibody, able to identify the expression of the fibrinogen receptor of platelet glycoprotein IIb/IIIa *(Lu et al. Artif Organs, 2011).*
- This expression is indeed unique in the process of platelet activation, and yet rarely analyzed.



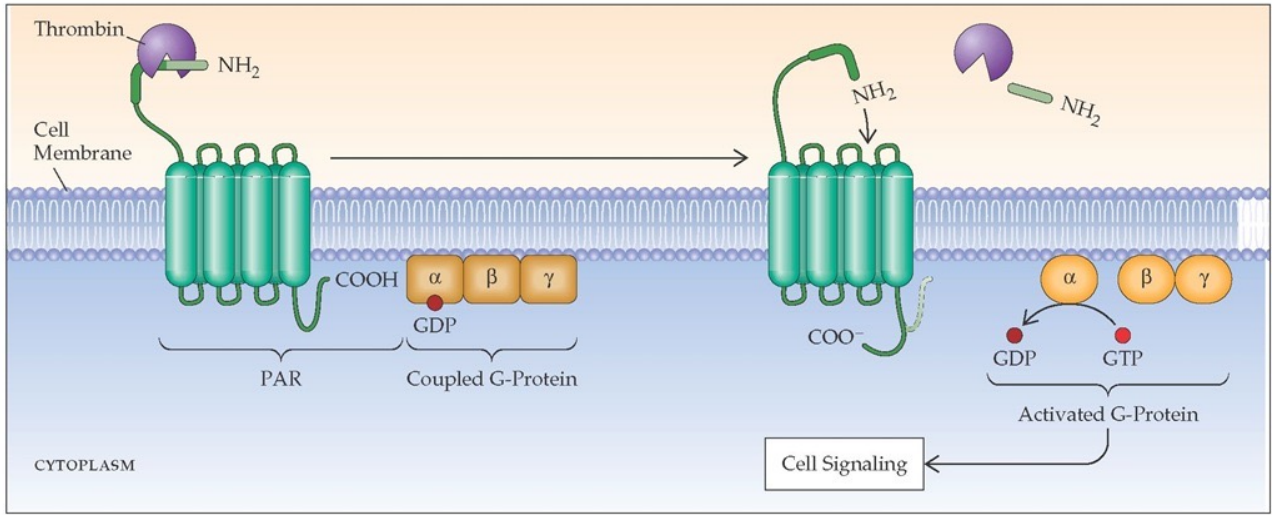
MPN: PLATELET FIBRINOGEN RECEPTOR

- Surprisingly, we have been able to verify a very low PAC-1 binding to platelets in patients with MPN not receiving cytoreduction nor antiplatelet agents if compared to that observed in healthy subjects (35.3 ± 12.9 vs 65.3 ± 24.2 respectively, *P*=0.008).
- The use of aspirin seems conversely to restore the expression of platelet fibrinogen receptor, as PAC-1 binding capacity is comparable to that of healthy volunteers (56.7 ± 18.7).



Lucchesi A et al, BJH 2020

ET: THROMBIN AND THE PAR1 RECEPTOR

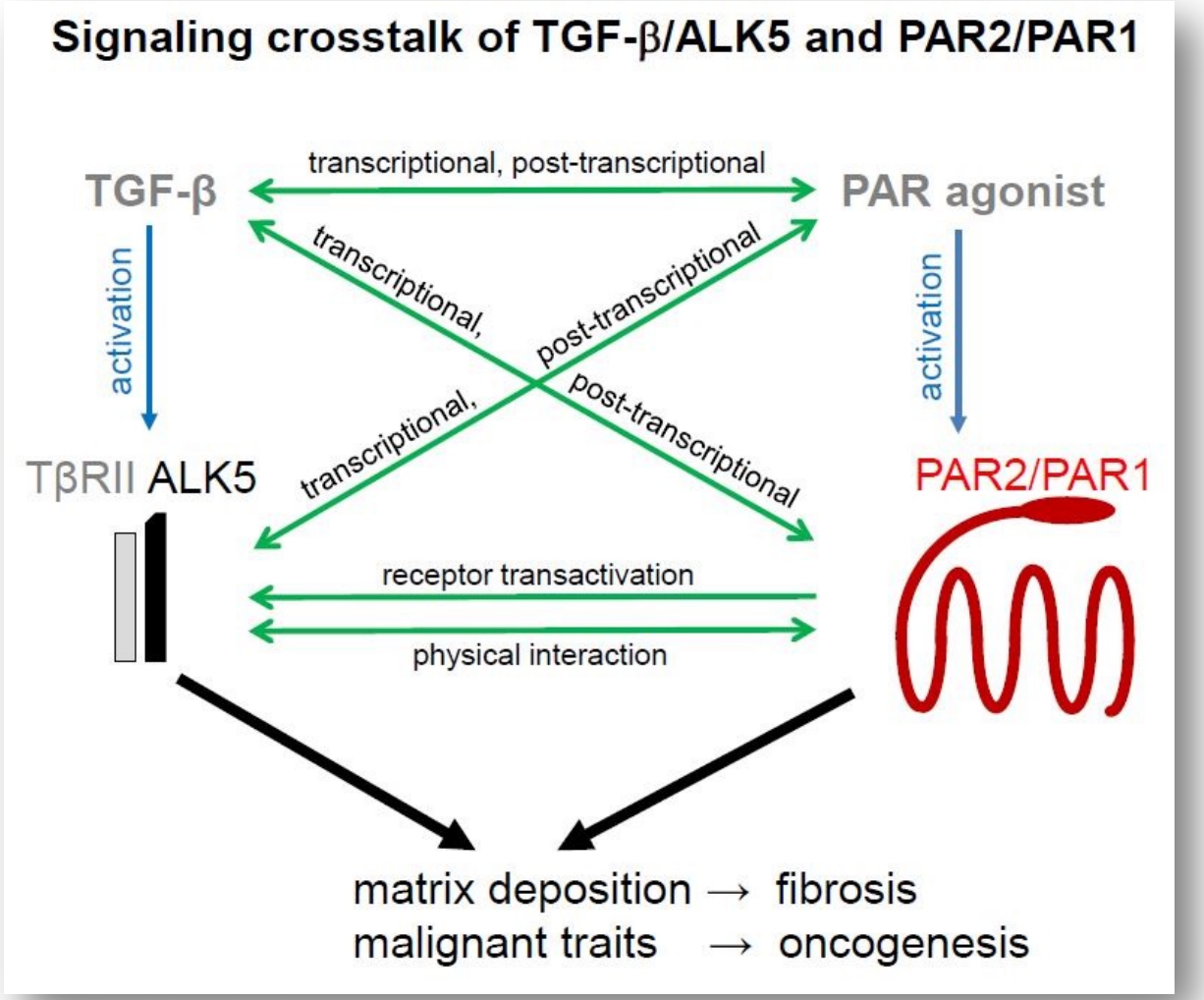


Moore et al. Blood, 2013

An enhanced Protease-Acivated Receptor-1 (PAR1) mediated expression of GPIIb-IIIa after thrombopoietin stimulation, followed by the disappearance of fibrinogen binding sites.

An increased thrombin generation could secondly lead to PAR1 activation, determining both a major conversion of fibrinogen into fibrin and a disappearance of PAC-1 expression.

PAR receptors are expressed in platelets, endothelium, and smooth muscle, contributing to both normal and pathological hemostasis.



International Journal of *Molecular Sciences*

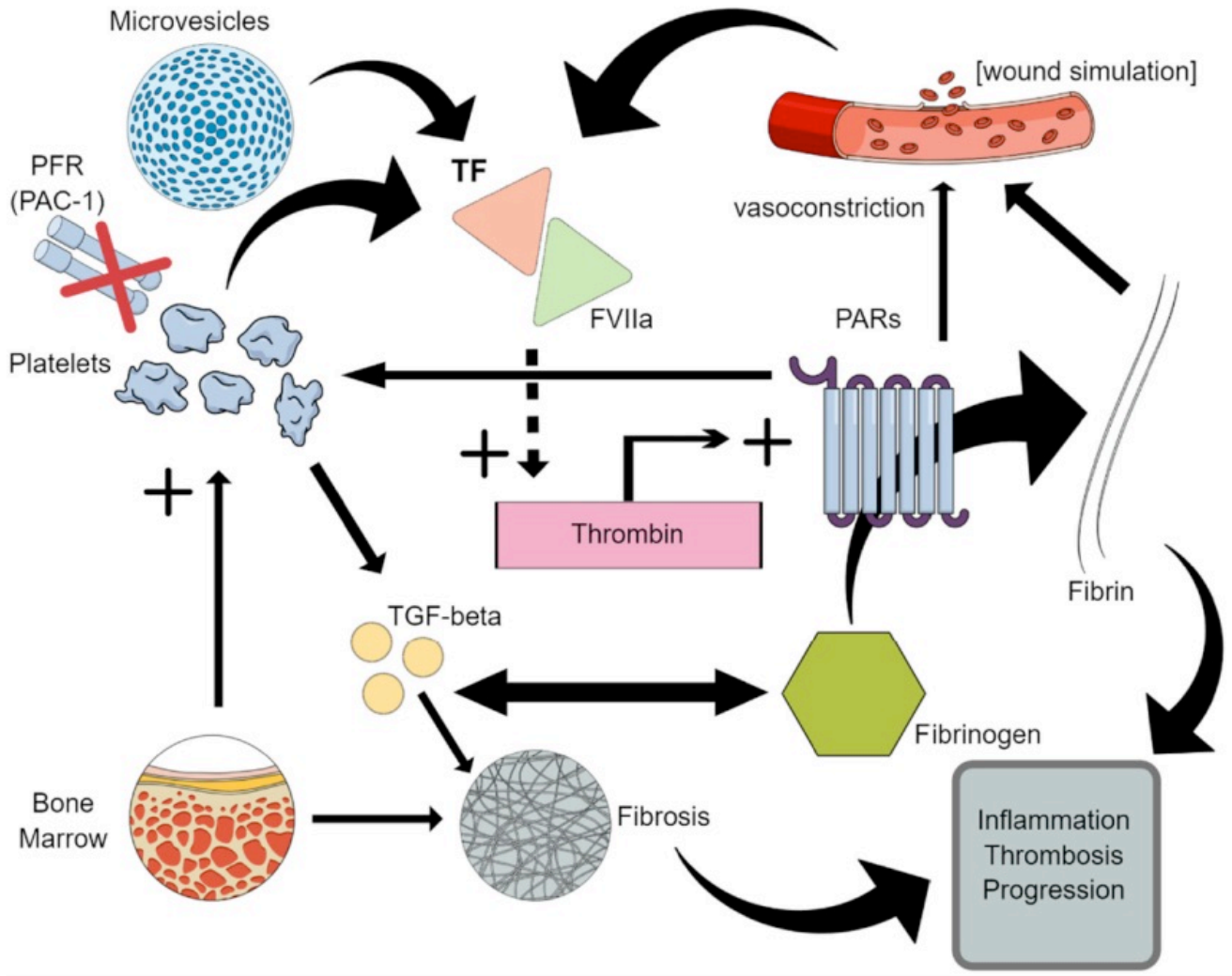
MDPI

Review

Signaling Crosstalk of TGF-β/ALK5 and PAR2/PAR1: A Complex Regulatory Network Controlling Fibrosis and Cancer

Hendrik Ungefroren [1,2,*], Frank Gieseler [1], Roland Kaufmann [3], Utz Settmacher [3], Hendrik Lehnert [1] and Bernhard H. Rauch [4]

- Platelet activation with a PAR1 agonist triggers TGF-beta secretion.
- Activation of PAR1 and PAR2 with PAR1-AP and PAR2-AP, respectively, led to activation of adventitial fibroblasts from rat aorta, including their proliferation and differentiation, ECM synthesis, as well as production of TGF-beta, IL-6 and MCP-1.



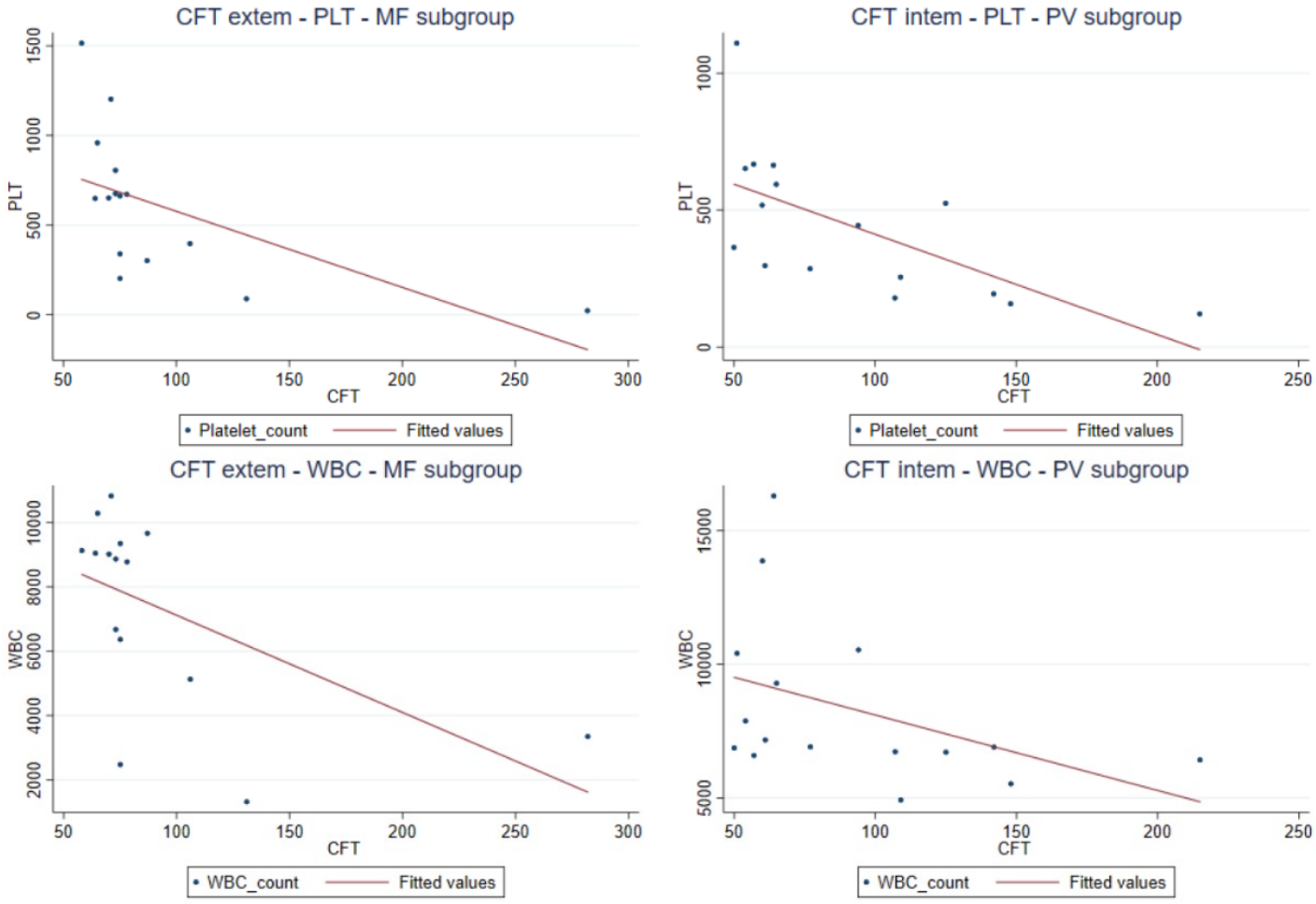
THE «CIRCULATING WOUND» (MPN)

LUCCHESI A ET AL, IJMS 2021

ROTATIONAL THROMBOELASTOMETRY (ROTEM) FOR THE ASSESSMENT OF HYPERCOAGULABLE STATES IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS, WITH RESPECT TO DIFFERENT GENETIC DETERMINANTS OF DISEASE

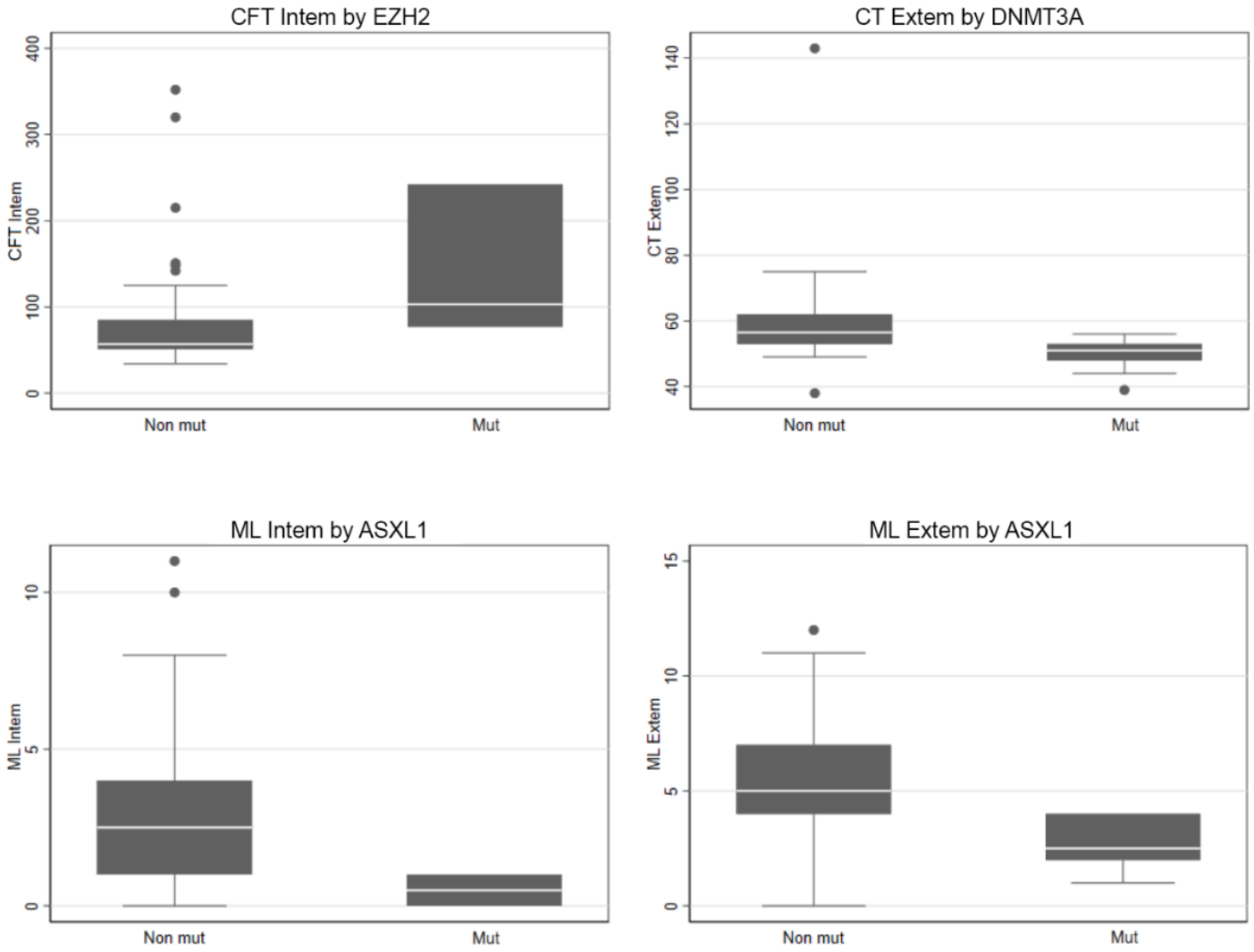
ROTEM PARAMETERS AND CELL COUNTS

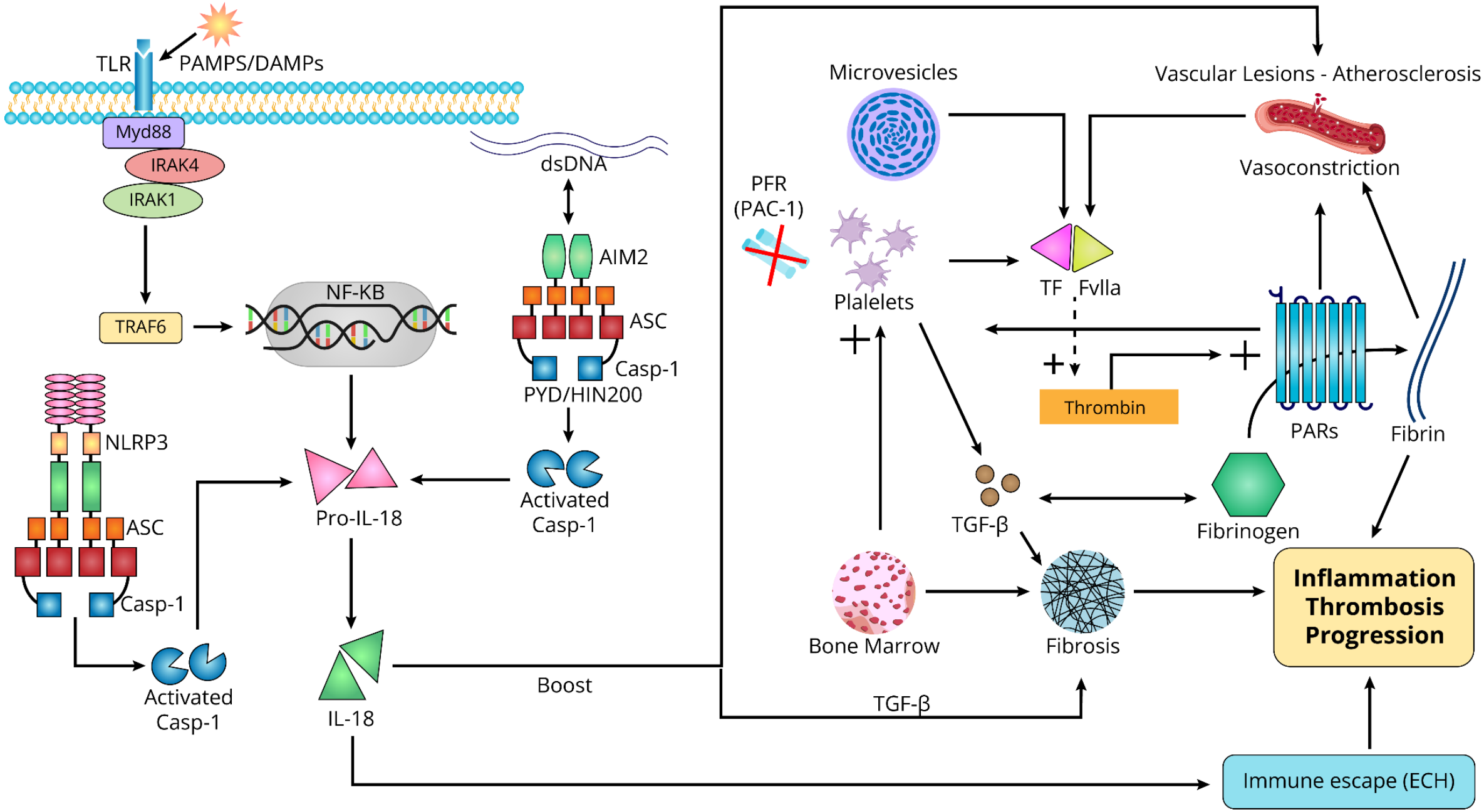
- WBC: INTEM was involved in PV (rho= -0.61, **p= 0.038**), while in PMF the modified value was that of EXTEM (rho= -0.52, **p= 0.016**)
- PLT: CFT values on EXTEM were affected for all conditions, but particularly for PMF (rho= -0.75, **p=0.001**), the CFT values on INTEM only for PV (rho= -0.73, **p= 0.001**)
- <u>Minimal impact on CALR mutated TE</u>



DTA/HMR MUTATIONS

- Among the DTA mutations, the presence of DNMT3A shows a significant reduction in clotting time (CT) in EXTEM, while ASXL1 is associated with reduced maximum lysis (ML).
- EZH2 could be responsible for CFT elongations in the INTEM assay.




TLR
PAMPS/DAMPs
Myd88
IRAK4
IRAK1
TRAF6
NF-KB
dsDNA
AIM2
ASC
Casp-1
PYD/HIN200
NLRP3
ASC
Casp-1
Pro-IL-18
Activated Casp-1
Activated Casp-1
IL-18
Boost
Microvesicles
Vascular Lesions - Atherosclerosis
Vasoconstriction
PFR (PAC-1)
Plalelets
TF FvIIa
Thrombin
PARs
Fibrin
TGF-β
Fibrinogen
Bone Marrow
Fibrosis
TGF-β
Inflammation Thrombosis Progression
Immune escape (ECH)

01

MPNs represent a model of perpetual vascular damage from sterile inflammation

02

Not only haemostasis: platelets as leading players in innate immunity

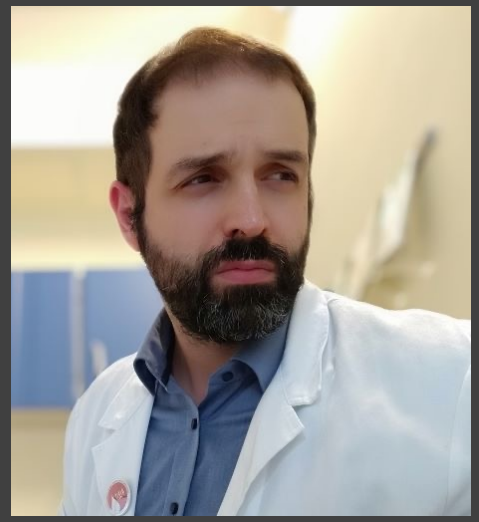
03

The prolonged action of inflammasomes is responsible for thromboinflammation, fibrosis and organ damage

04

The downstream signalling of NLRP3 represents a potential therapeutic target in MPNs

CONCLUSIONS



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