9° WORKSHOP
IN EMATOLOGIA TRASLAZIONALE

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Mielofibrosi: biologia dell'emopoiesi extramidollare e implicazioni cliniche

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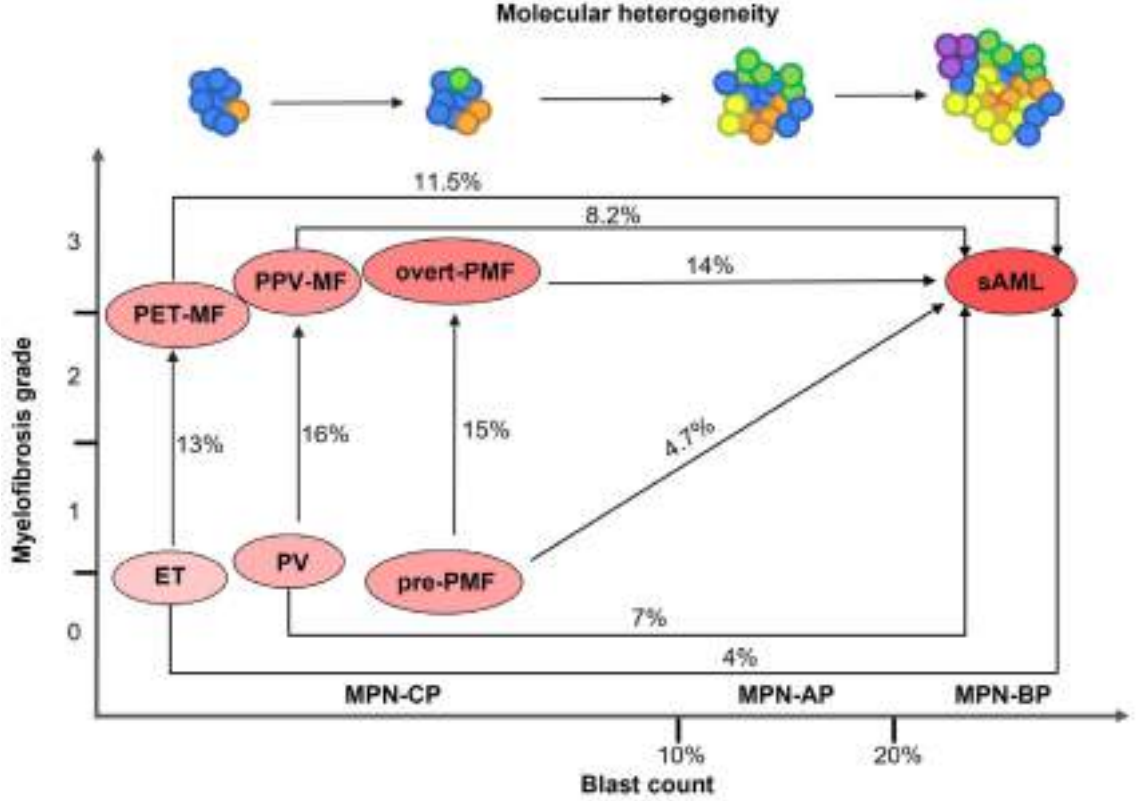
Disclosures di Giuseppe G. Loscocco

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

Disease progression and evolution in MPN



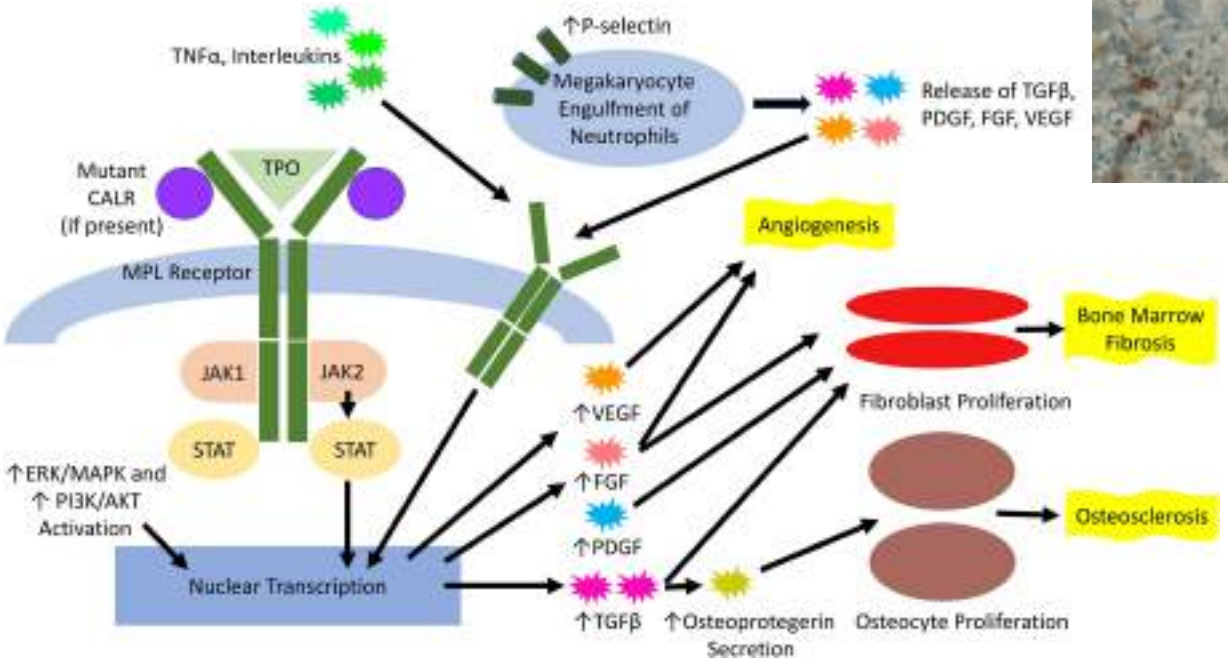
Rontauroli, S. et al *Int. J. Mol. Sci.* 2022

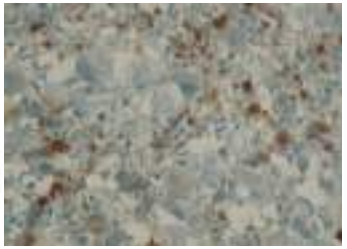
Myelofibrosis: types and diagnosis

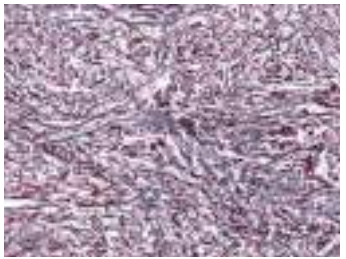
PMF, early/prefibrotic stage (pre-PMF)	PMF, overt fibrotic stage
Major criteria 1. Bone marrow biopsy showing megakaryocytic proliferation and atypia,* bone marrow fibrosis grade < 2, increased age-adjusted BM cellularity, granulocytic proliferation, and (often) decreased erythropoiesis 2. *JAK2*, *CALR*, or *MPL* mutation† or presence of another clonal marker‡ or absence of reactive bone marrow reticulin fibrosis§ 3. Diagnostic criteria for *BCR::ABL1*-positive CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms are not met	Major criteria 1. Bone marrow biopsy showing megakaryocytic proliferation and atypia,* accompanied by reticulin and/or collagen fibrosis grades 2 or 3 2. *JAK2*, *CALR*, or *MPL* mutation† or presence of another clonal marker‡ or absence of reactive myelofibrosis§ 3. Diagnostic criteria for ET, PV, *BCR::ABL1*-positive CML, myelodysplastic syndrome, or other myeloid neoplasms‖ are not met
Minor criteria • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 × 10^9/L • Palpable splenomegaly • Lactate dehydrogenase level above the above the reference range	Minor criteria • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 × 10^9/L • Palpable splenomegaly • Lactate dehydrogenase level above the above the reference range • Leukoerythroblastosis
The diagnosis of pre-PMF or overt PMF requires all 3 major criteria and at least 1 minor criterion confirmed in 2 consecutive determinations	

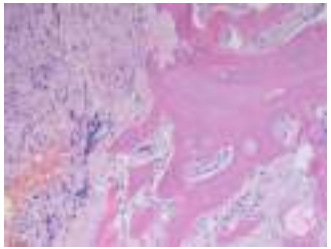
Arber D. *et al.*, 2022, Blood, 140(11) 1200-1228

Pathophysiology of BM Fibrosis



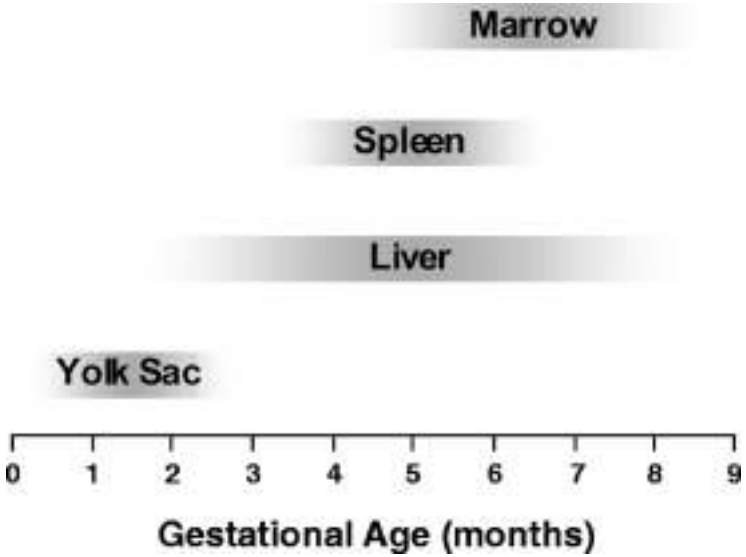




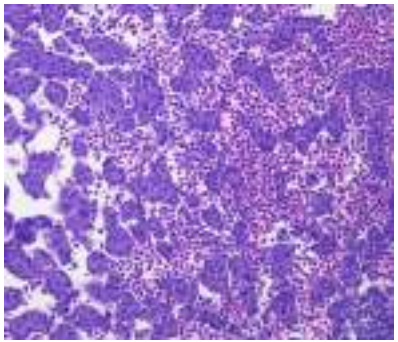


Adapted from Garmezy B Blood Rev. 2021;45:100691

Hematopoiesis



Hematopoiesis starts one week after fertilization from focal islets of extra **embryonic yolk sac**. At 5-6 weeks of gestation the progenitor cells from yolk sac enter **liver** for hematopoiesis. At seventh week the progenitor cells colonize **spleen**. After birth in normal situations **bone marrow** becomes the primary and only site of hematopoiesis.



Fetal liver showing intrasinusoidal development of hematopoietic cells (mainly erythroid).

Definition and causes of extramedullary hematopoiesis

Definition: extramedullary hematopoiesis (EMH) is a rare entity, in which blood cells production occurs outside the of bone marrow due to inadequate bone marrow function. It was first described by Guizetti in 1912 during autopsy.

Reduced marrow production or peripheral destruction of blood cells	*Displacement of marrow stem cells into peripheral circulation*
Thalassemia **Sickle cell disease** Hereditary spherocytosis Autoimmune haemolytic anaemia Iron-deficiency anaemia Megaloblastic anaemia (B12 and folate deficiency) Infections	**MPN (primary myelofibrosis)** Osteopetrosis Leukaemia Lymphoma Granulomatous diseases BM metastasis Storage disorders

Mechanisms of extramedullary hematopoiesis

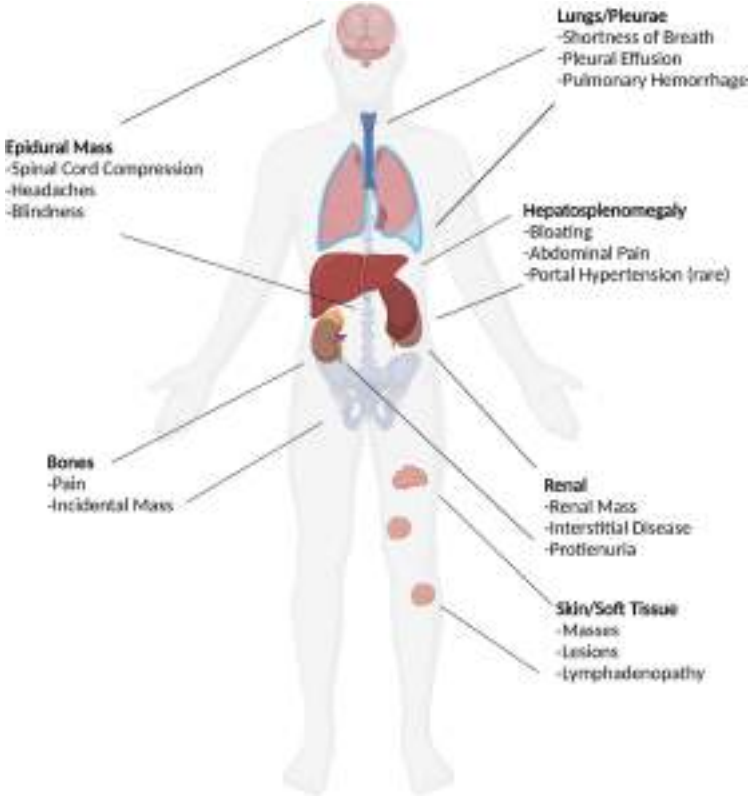
Filtration theory of spleen: Immature hematopoietic cells from the bone marrow move into the spleen or other sites where they tend to thrive.

Compensatory theory: EMH compensates for the diminished erythropoiesis and when bone marrow does not provide sustenance, hematopoietic cells proliferate where they get adequate space and ideal microenvironment.

Myelostimulatory theory: Stimulation of EMH in bone marrow and other fetal hematopoietic sites due to some factors.

Redirected differentiation theory: some unknown factor, which aids in differentiation of adult stem cell into hematopoietic lineage cells by aberrantly secreted cytokines or secondary to chronic anemia. Stem cells are present in various body tissues which have the capability to repair damaged tissue and proliferate and differentiate into cell types. This theory explains occurrence of EMH in various tissues and organs.

Locations and symptoms of EMH in myelofibrosis



EMH in MPN commonly presents as organomegaly; up to 60–90% of patients with MF have **splenomegaly**.

This can lead to abdominal pain, bloating, and in some cases portal hypertension.

Concerning complications are often most notable when EMH occurs in or near the central nervous system, which can lead to spinal cord compression, headaches, blindness, or other devastating consequences.

Chernak BJ, Int Rev Cell Mol Biol. 2021;365:97-116

Extramedullary hematopoiesis in the absence of MPN

Associated conditions	Involved sites									
	Spleen	Liver	Lymph nodes	Para-spinal region	Retroperitoneal region	Pre-sacral region	Lung	Heart	Mediastinal region	Other sites
All patients (n = 309)	164	78	20	16	5	7	8	3	2	34
Myelodysplastic syndromes (n = 41)	23	8	4	1	1	0	2	0	0	6
Acute myeloid leukemia (n = 28)	12	8	3	1	0	0	1	1	0	6
Hemolytic anemia (n = 24)	22	2	0	0	1	0	0	0	0	0
Thalassemia (n = 22)	11	4	0	4	1	1	1	0	1	4
Non-Hodgkin's lymphoma (n = 19)	15	2	0	0	0	0	0	0	0	2
Immune thrombocytopenic purpura (n = 17)	15	1	2	0	0	0	0	0	0	0
Metastatic cancer (n = 17)	3	7	3	1	0	0	1	0	0	3
Plasma cell neoplasms (n = 12)	4	3	1	0	0	1	0	0	0	2
Hereditary spherocytosis (n = 8)	5	0	0	2	0	1	0	0	0	0
Cirrhosis (n = 7)	2	3	0	0	0	0	0	0	0	0
Acute lymphoblastic leukemia (n = 6)	3	2	1	0	0	0	0	0	0	0
Chronic lymphocytic leukemia (n = 6)	2	1	1	2	0	0	0	0	0	0
Hodgkin's lymphoma (n = 5)	2	3	1	0	0	0	1	0	0	1
"Idiopathic" PNH (n = 12)	2	0	0	1	1	4	2	0	0	0
Others (n = 93)	43	30	4	2	1	0	0	2	1	10

Fan, N. Blood Cancer Journal 8, 119 (2018)

Biology of EMH in MF: CXCL12/CXCR4 axis

Proteolytic environment - neutrophil elastase (NE), total and active-matrix metalloproteinase 9 (MMP-9), and soluble vascular cell adhesion molecule-1 (sVCAM-1) - exists in MF which might result in the sustained mobilization of $CD34^+$ cells.

Xu M, Blood. 2005;105(11):4508-4515

Reduced expression of CXCR4 by CD34+ cells is a characteristic of MF which is associated with the constitutive mobilization of CD34+ cells and occurs in patients with advanced forms of the disease.

Rosti V, Blood Cells Mol Dis. 2007;38(3):280-286

Proteolytic degradation of CXCL12 is characteristic of PMF and that the resulting **truncated forms of CXCL12**, in addition to the reduced expression of CXCR4 by CD34(+) cells, lead to a profound mobilization of HSC/HPC in PMF.

Cho SY, *Cancer Res*. 2010;70(8):3402-3410

MF **splenic microenvironment is characterized by increased levels of intact, functional CXCL12**, which contributes to the localization of MF CD34(+) cells to the spleen and the establishment of extramedullary hematopoiesis.

Wang X, Exp Hematol. 2015;43(2):100-9.e1

Biology of EMH in MF: CXCL12/CXCR4 axis

Similar abnormalities in the SDF-1/CXCR4 axis are observed in **PMF patients** and in the **Gata1 low** mice model of myelofibrosis.

Migliaccio AR, Exp Hematol. 2008;36(2):158-171

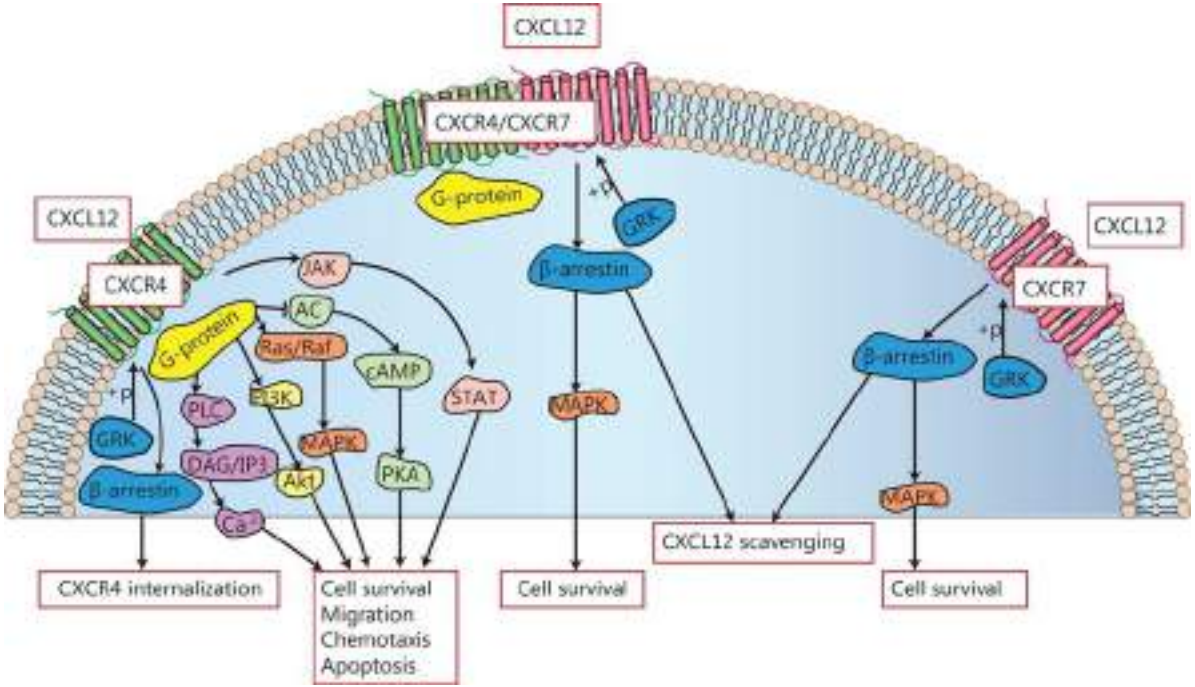
CD34+ cells from PMF patients, unlike those from normal subjects, presented **hypermethylation of CXCR4 promoter** CpG island 1.

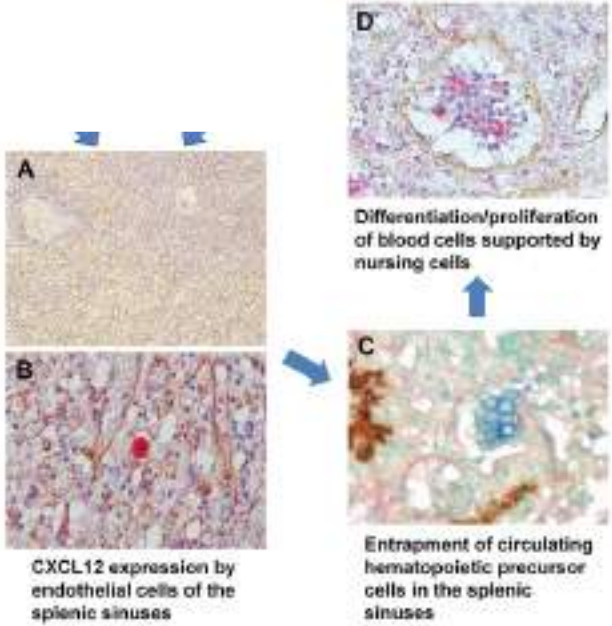
Bogani C, Stem Cells. 2008;26(8):1920-1930

CXCL12/CXCR4 pathway is over activated in MF patients by oncogenic JAK2 that maintains high **PI3K signaling** over the threshold required for CXCR4 activation.

Abdelouahab H, Oncotarget. 2016;8(33):54082-54095

Overview of CXCL12/CXCR4 signaling in EMH

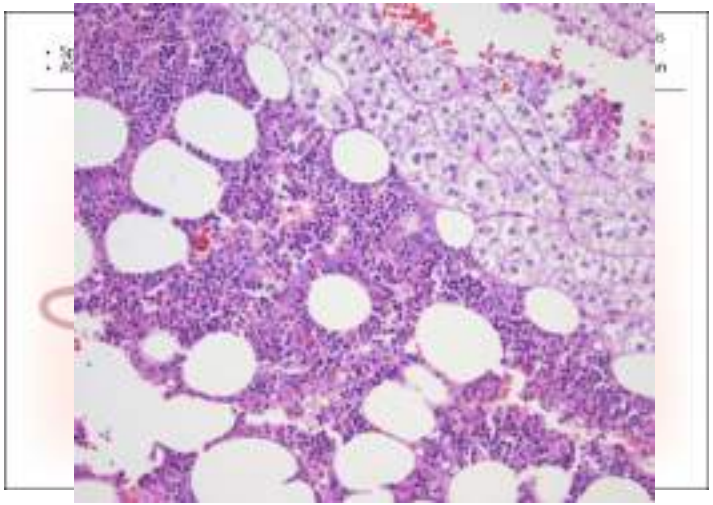




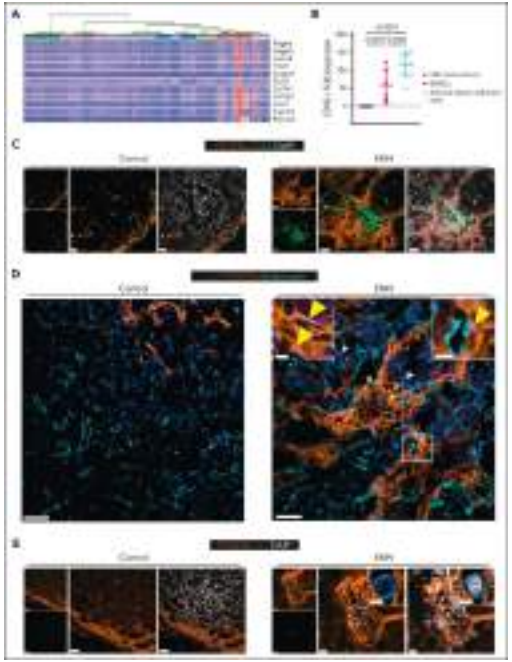
Yamamoto K, Mol Med Rep. 2016;13(1):587-591
Wu X, Cancer Biol Med. March 12, 2021

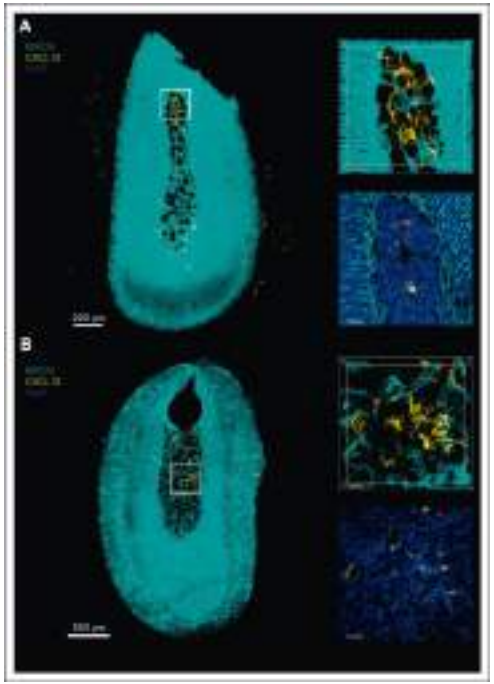
A recent experimental model of EMH

CXCL12/CXCR4–dependent extramedullary hematopoietic niches



Inducible EMH in adrenal glands was induced by **splenectomy,** followed 10 days later by daily injection of hormonals (the cocktail of G-CSF, testosterone, and ACTH) for 20 to 21 days.





Schyrr F, Blood 2024; 144 (9): 964–976
Kurata M, Blood. 2024;144(9):921-922

Endothelial and mesenchymal cells in MF

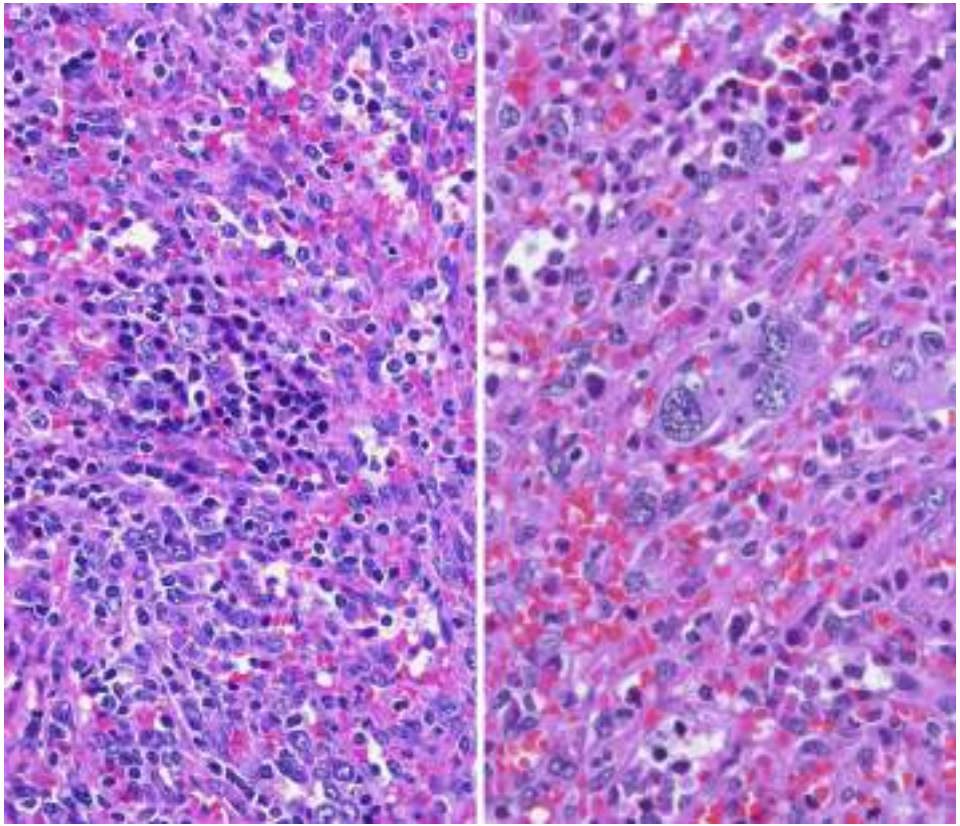
BM marrow nestin$^+$ mesenchymal stem cells (MSCs) innervated by sympathetic nerve fibres regulate normal HSC. Abrogation of this regulatory circuit is essential for MPN pathogenesis. **Sympathetic nerve fibres, supporting Schwann cells and nestin$^+$ MSCs are consistently reduced in the bone marrow of MPN patients** and mice expressing *JAK2(V617F)* mutation in HSCs. MSC reduction is not due to differentiation but is caused by bone marrow neural damage and Schwann cell death triggered by interleukin-1β produced by mutant HSCs. In turn, ***in vivo*** **depletion of nestin$^+$ cells or their production of CXCL12 expanded mutant HSC number and accelerated MPN progression**.

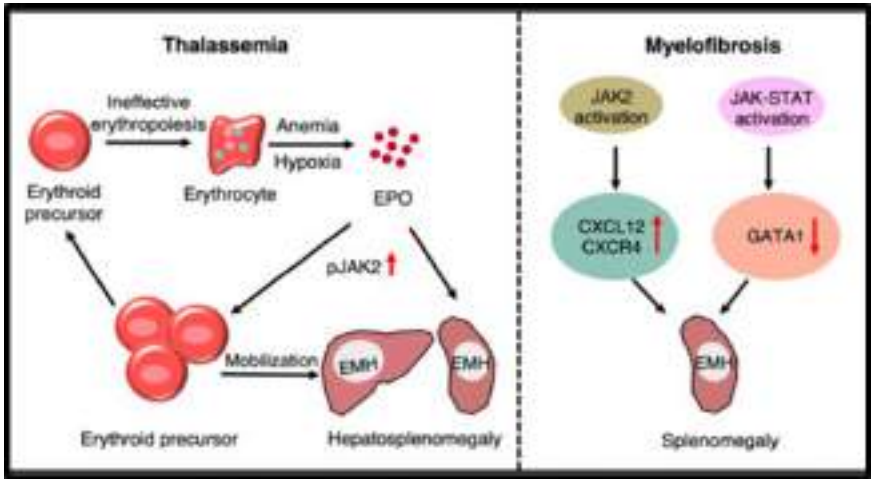
Arranz L, Nature. 2014;512(7512):78-81

>30% of the endothelial cells in the small vessels of the **bone marrow and spleen** of patients with primary myelofibrosis have a mesenchymal phenotype, which is suggestive of the process known **as endothelial-to-mesenchymal transition** (EndMT). EndMT can be reproduced in vitro by incubation of cultured endothelial progenitor cells or spleen-derived endothelial cells with inflammatory cytokines.

Erba BG *Am J Pathol*. 2017;187(8):1879-1892

Splenic EMH

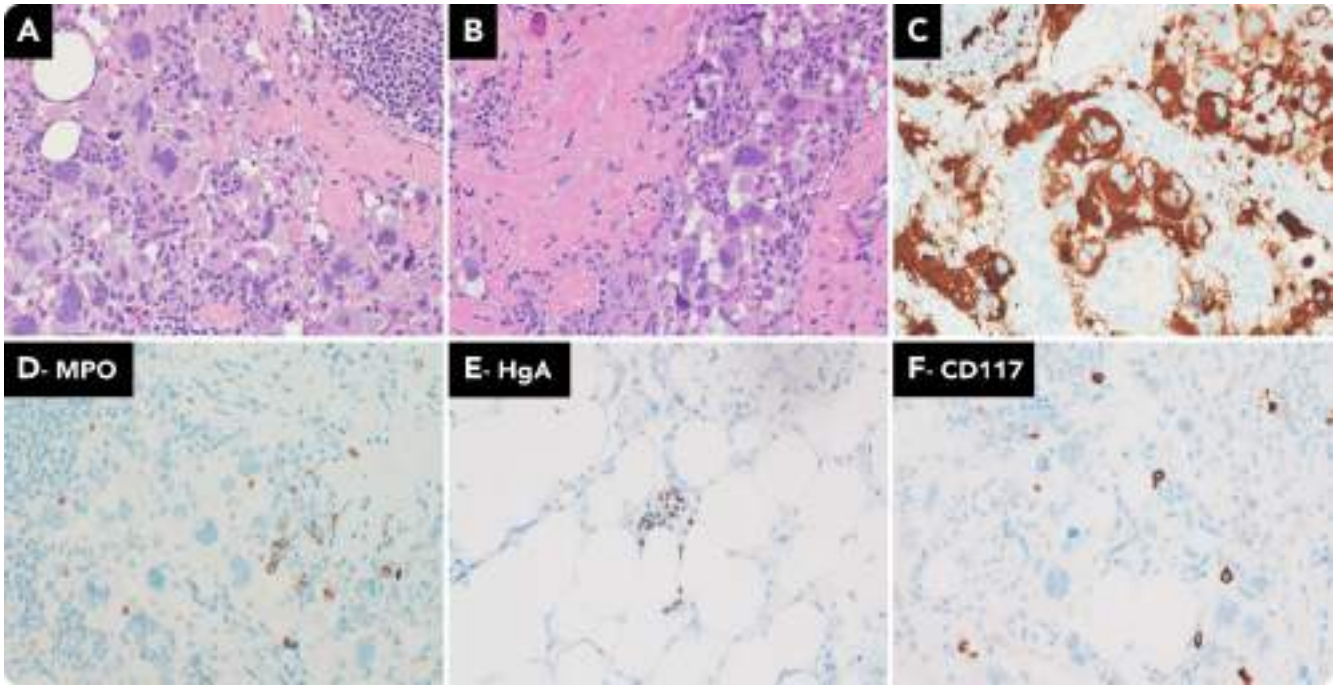




O'Malley DP. *Mod Pathol*. 2007;20(4):405-415

Differential diagnosis of EMH in MF

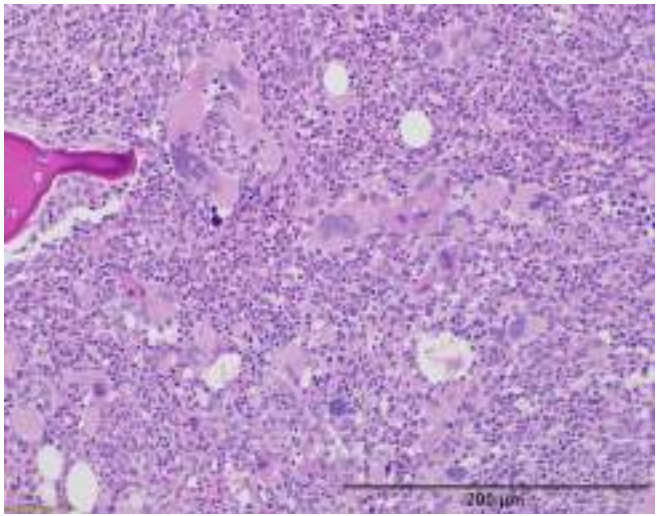
- **Sclerosing extramedullary hematopoietic tumor**

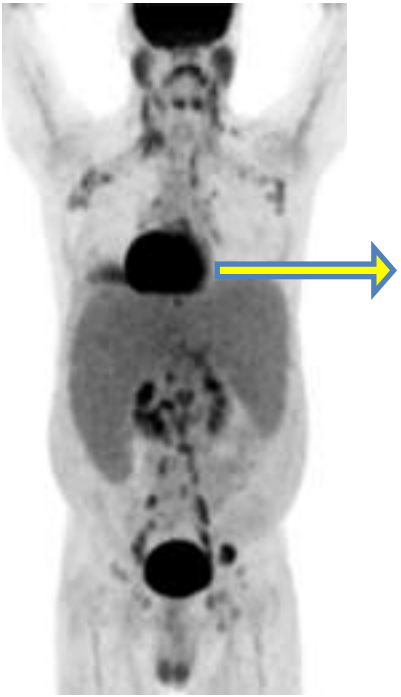


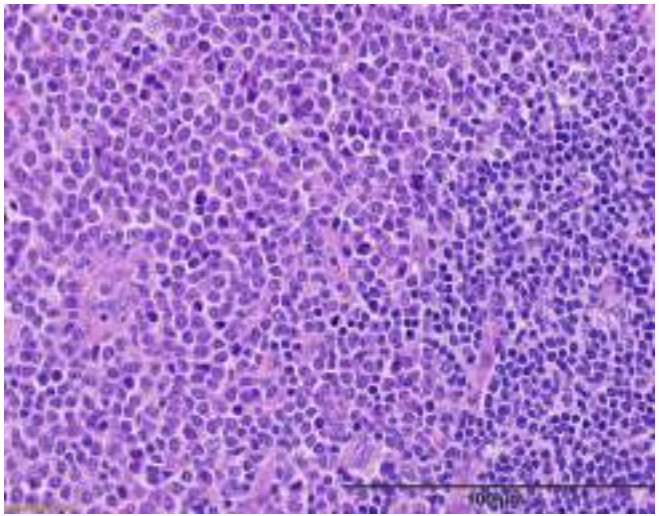
Barouqa M, Blood. 2022;139(22):3345

Differential diagnosis of EMH in MF

- **Myeloid sarcoma**





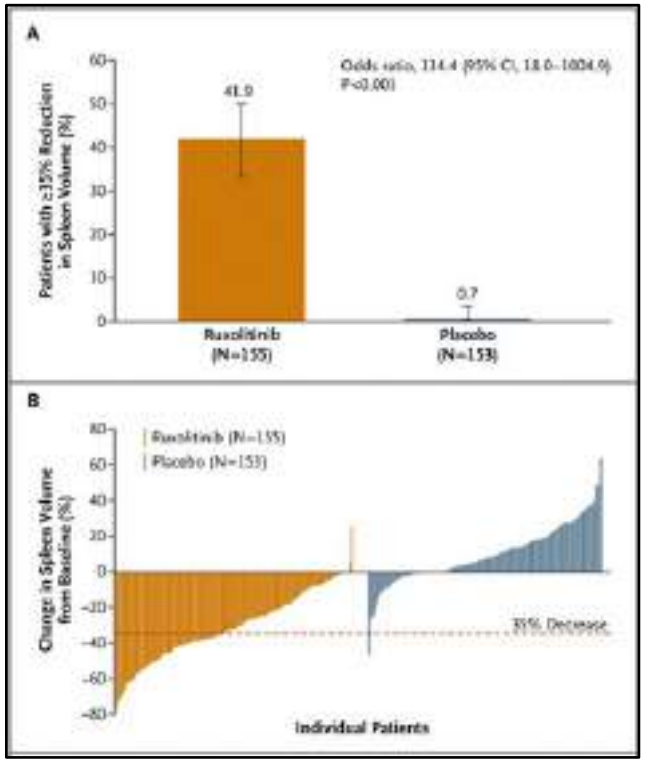


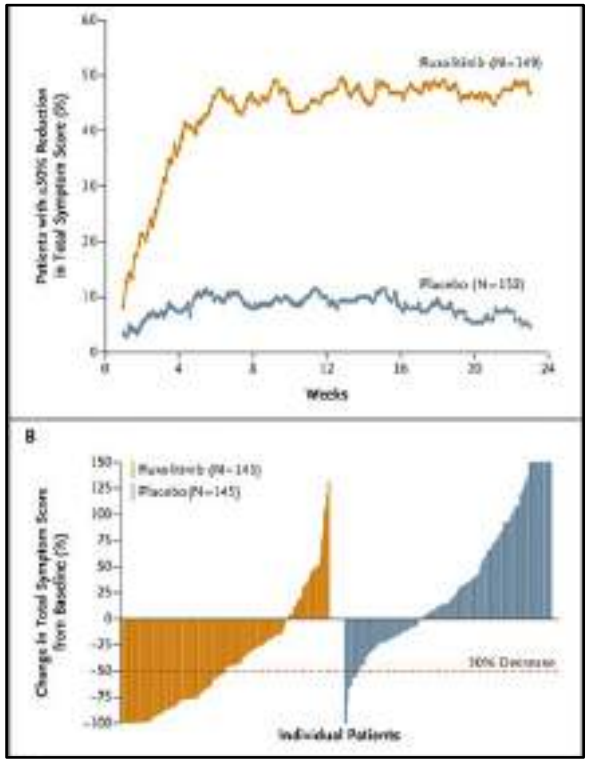
Trennepohl C, Clin Case Rep. 2019;7(11):2274-2276

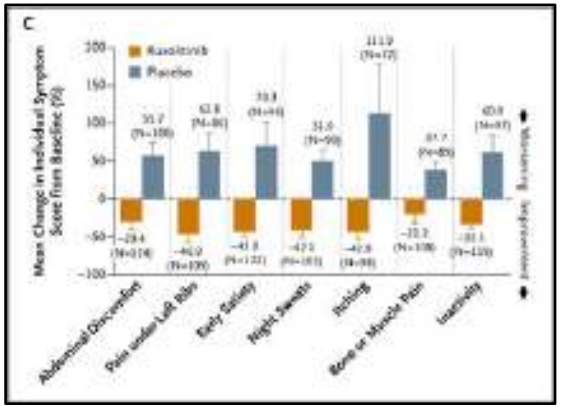
Treatment strategies available for EMH in MF

- EMH is a complication of ineffective hematopoiesis and **does not necessarily require treatment**.
- A retrospective review of **non-hepatosplenic EMH** found that 40% of patients required no treatment upon diagnosis (Koch et al., 2003); however nearly half of the 27 patients had MF, and of this subgroup, 71% required therapy.
- **Hepatosplenomegaly** can manifest as abdominal fullness, discomfort, portal hypertension and ascites; in the case of massive splenomegaly, ischemia and splenic infarction are possible complications as well (Mesa et al., 2006). Splenic sequestration can also occur, further worsening a patient's cytopenias.
- **JAK inhibitors** (ruxolitinib, fedratinib, momelotinib)
- Other modalities, such as **myelosuppressive agents, radiation or surgery**.

Ruxolitinib in MF: splenomegaly and symptoms







Verstovsek S, N Engl J Med. 2012;366(9):799-807

Other JAK inhibitors in MF

	PERSIST-1[8] PAC 400 mg QD N = 220	PERSIST-2[9] PAC 200 mg BID N = 43 ruxolitinib naïve	COMFORT-1[3] RUX N = 155	COMFORT-2[4] RUX N = 146	JAKARTA-1[10] FED 400 mg QD N = 96	SIMPLIFY-1[11]	
						MMB N = 215	RUX N = 217
PLT ($x10^9$/L) exclusion	None	Greater than 100	Less than 100	Less than 100	Less than 50	Less than 50	Less than 50
Baseline PLT ($x10^9$/L), median	166[d]	51	262	244	221	301 (mean)	301.5 (mean)
SVR35, (%)	19%	28%	42%	32%	36%	27%	29%
TSS50[b], (%)	NR	37%	46%	N/A	36%	28%	42%

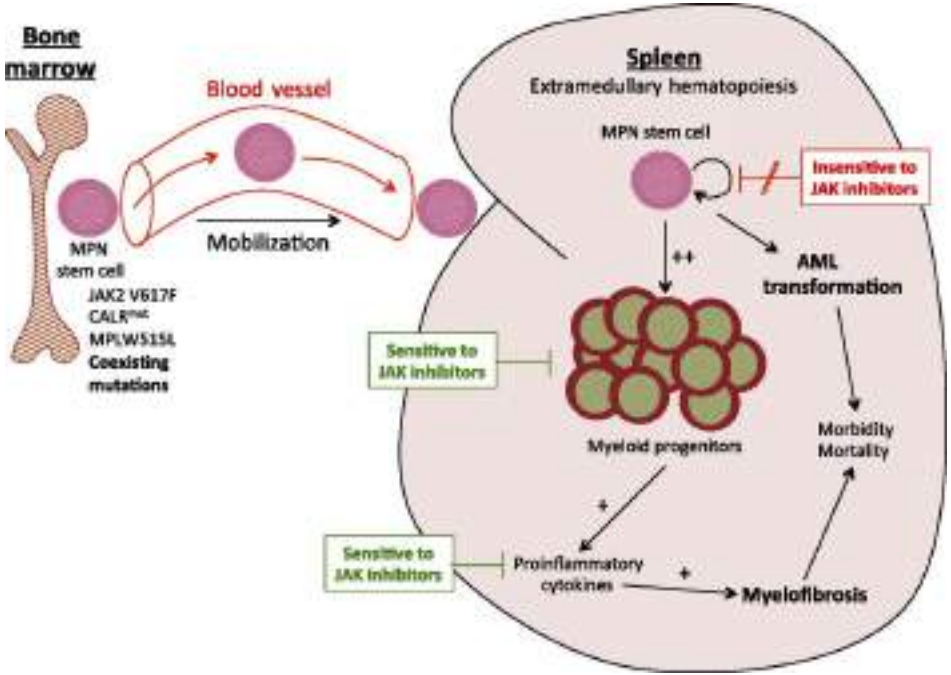
Abbreviations: BID = twice daily; FED = fedratinib; MMB = momelotinib; PAC = pacritinib; PLT = platelets; QD = once daily; RUX = ruxolitinib; SVR35 = spleen volume reduction of ≥35% from baseline; TSS50 = total symptom score response of ≥50% from baseline.

Median response to JAKi is 3.5 years; loss of response is often associated with clonal progression.

Alternative JAKi after JAKi failure is associated with **very poor responses**. Better results are available in the subset of JAK intolerance (i.e., cytopenia).

Mascarenhas J, Clin Lymphoma Myeloma Leuk. 2023;23(10):714-718.

JAK2 inhibitors do not affect MF stem cells



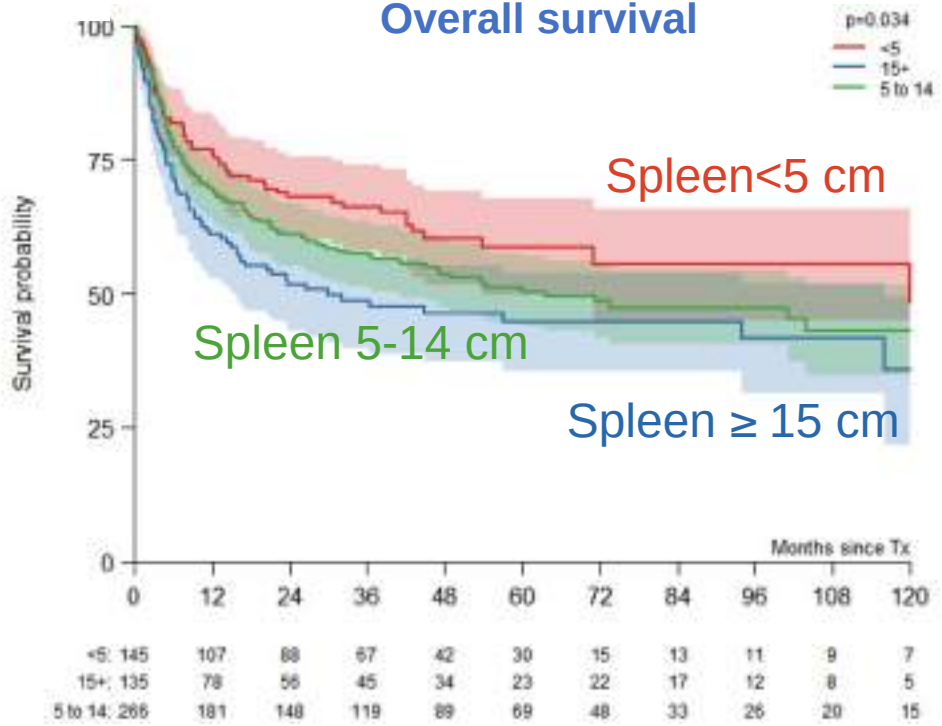
- JAK2-mutant MPN stem cells are not effectively targeted by JAK inhibition
- MPN progenitor cells in the spleens of patients with MF undergo apoptosis resulting in a reduction in splenomegaly.
- Suppressing the inflammatory milieu through JAK inhibition could potentially delay progression or even reverse MF within the bone marrow.

After 10 years we know that it is not so, since there is no clear disease modifying activity from JAK inhibitors alone.

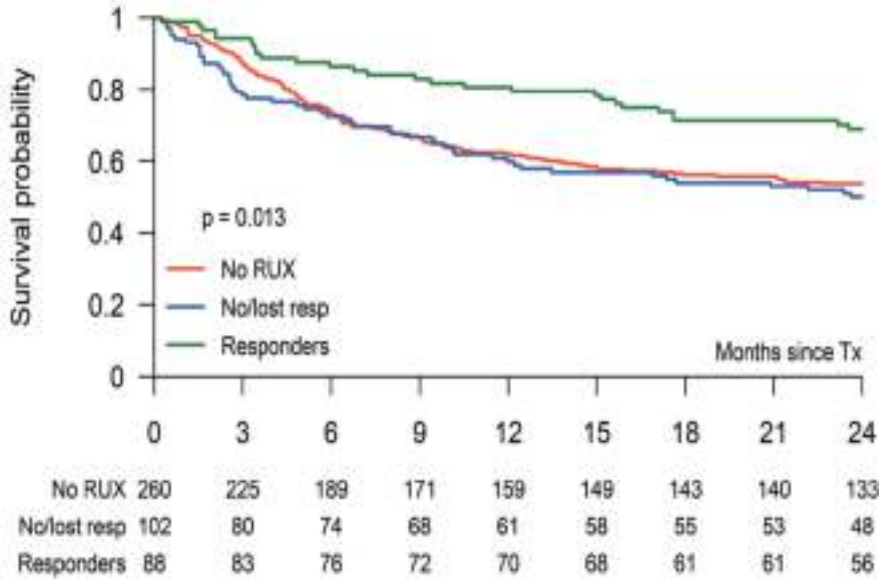
Lane SW Blood. 2014;124(19):2898-2900
Wang X, Blood. 2014;124(19):2987-2995

Splenomegaly and allo-HSCT in MF

Overall survival

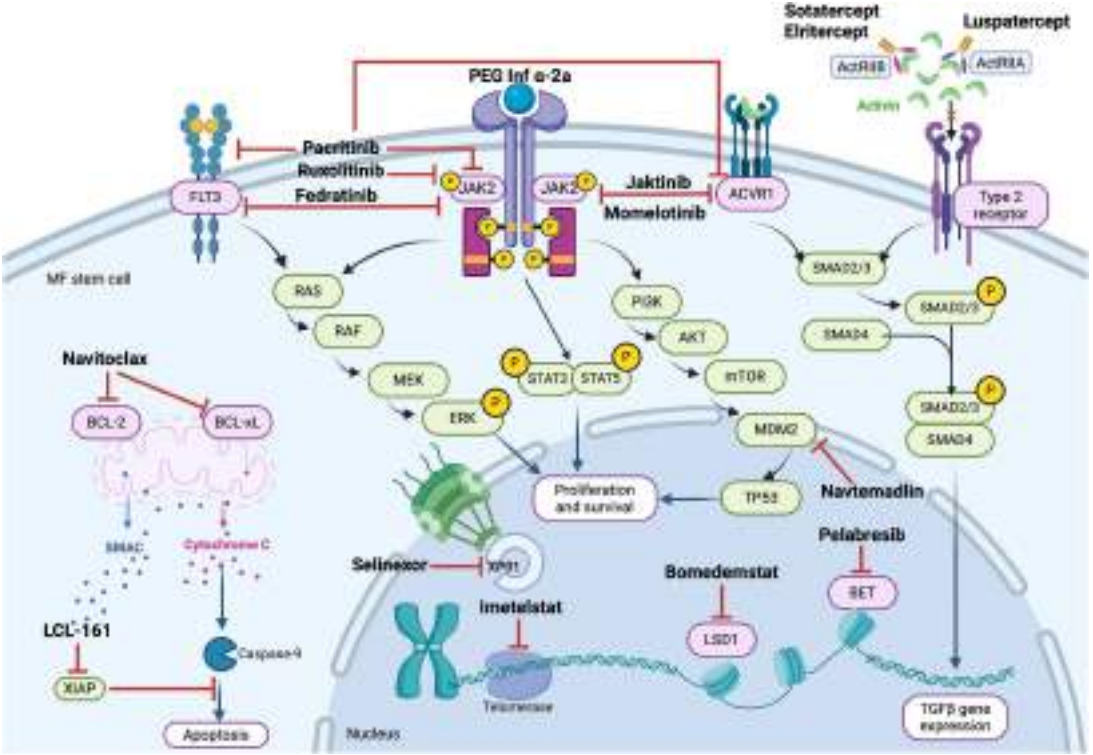


Event-free survival



Polverelli N et al. Am J Hematol 2021; 96(1): 69-79 ; Kroger N et al. Leukemia 2021; 35:3551–3560

New therapies in myelofibrosis: which targets?



Conclusions

- Extramedullary hematopoiesis (EMH) is often a response to **ineffective marrow production** of hematologic cells in various diseases including MF.
- Filtration theory – through **leukoerythroblastosis-** is the most accredited mechanisms of EMH in MF.
- The burden of EMH can lead to symptomatic discomfort and mechanical obstructive complications, most commonly in the **spleen and liver**. Splenomegaly has an adverse impact on allo-HSCT outcome.
- The exact mechanisms of EMH in MF are still poorly understood, however the role of **CXCL12-CXCR4** axis, through activation of JAK-STAT and **PI3K signaling** seems to be crucial.
- **JAK inhibitors** (ruxolitinib, fedratinib, momelotinib), although not disease modifying agents, are effective on splenomegaly and symptoms in most cases.
- Alternative drugs, particularly for JAKi R/R or intolerant patients are under investigations.