1st Annual Florence Meeting on MPN
(April 2011)

Role of FL and p38 MAPK pathway in the control of megakaryopoiesis in PMF patients: Mediators of the dialogue between hematopoietic and stromal cells?

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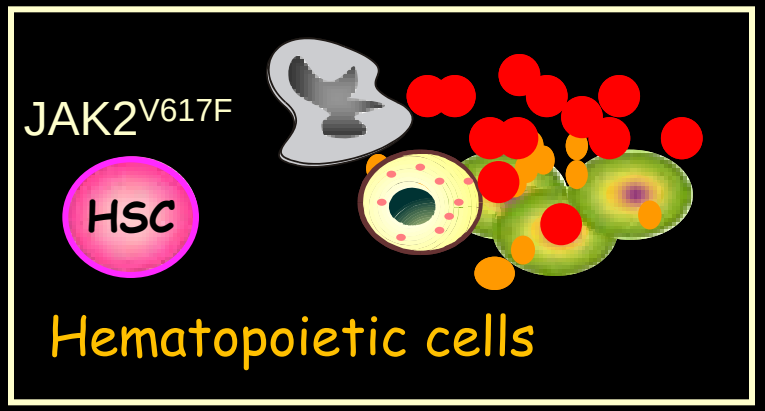
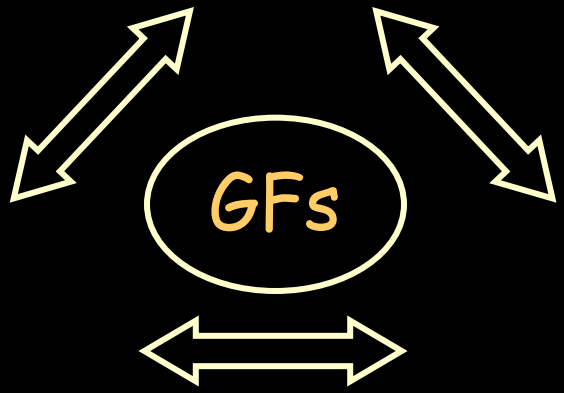
Primary myelofibrosis, a disease of hematopoietic stem cells and of their microenvironment

Clonal amplification of HSC and of dystrophic MK
Egress of HSC from BM to spleen/liver through PB

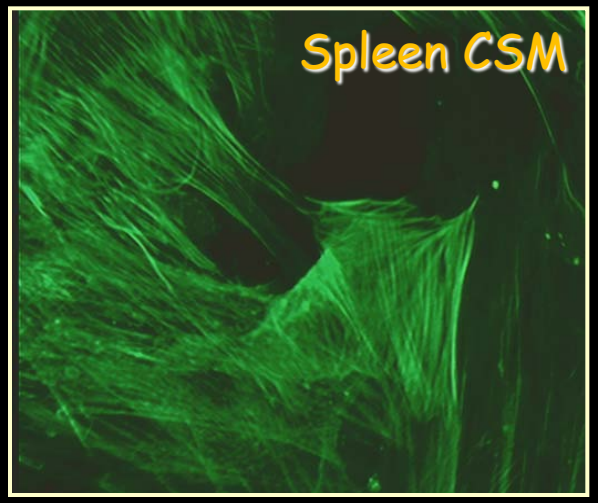


Myelofibrosis and osteosclerosis

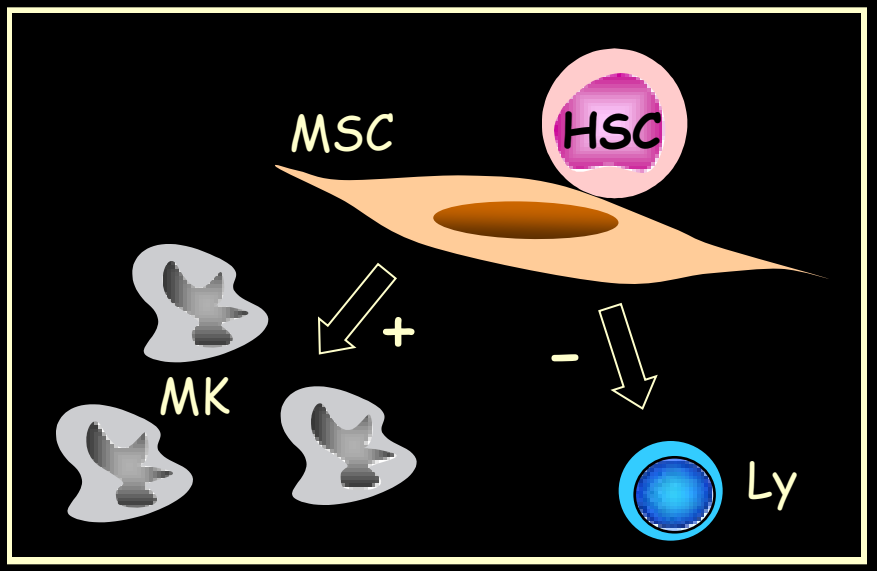


Neoangiogenesis

Role of stromal cells in PMF myeloproliferation and dysmegakaryopoiesis



Myofibroblasts (Alpha-SMA$^+$)
Tenascin, Collagens
Adhesion molecules (VLA-4, CD9…)
Chemokines (MCP-1, MIP-1, Rantes..)
Cytokines (GM-CSF, IL-15, FL..)

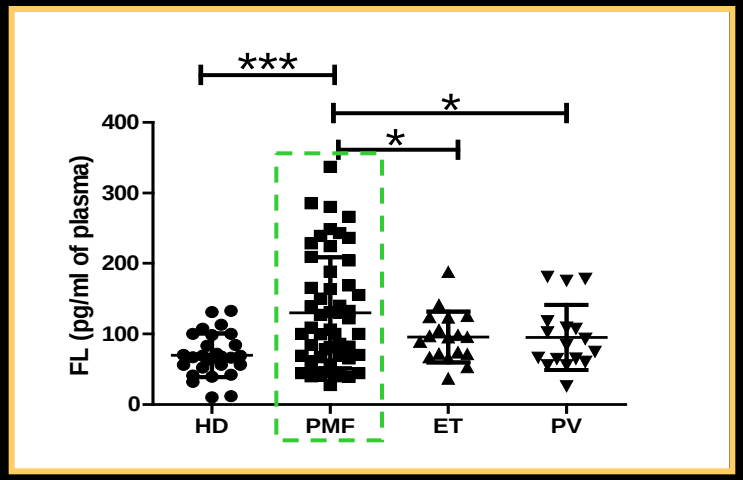


Stromal cells participate in the proliferation of CD34$^+$ and MK cells and in their altered differentiation

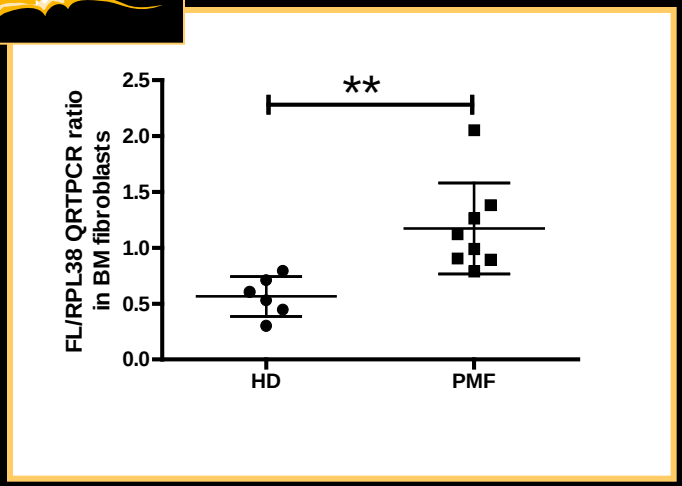
Inter-dependency of the CD34$^+$/fibroblast couple

Hasselbalch., Dan Med Bull. 1993
Brouty-Boyé et al., Int. J. Cancer, 1998 & 2001
Brouty-Boyé et al., Clin. Immunol., 2003
Briard et al., Clin. Immunol., 2003
Bock et al., Br. J. Haematol., 2009

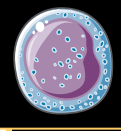
FL level is increased in the plasma of PMF patients and is produced by stromal and CD34+ cells

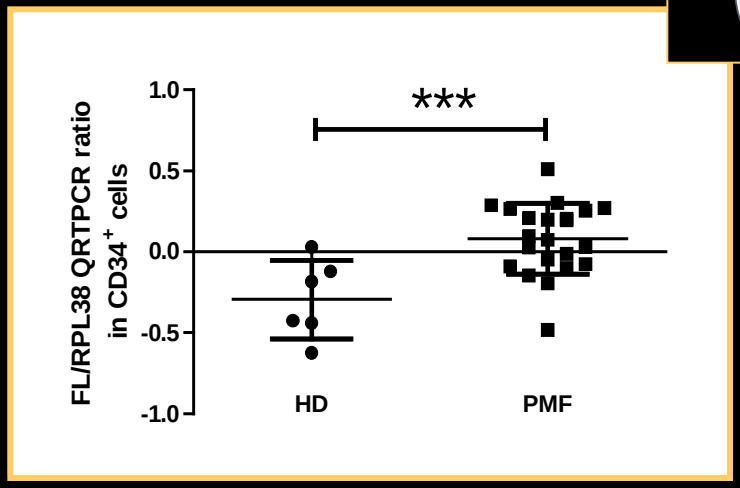


BM CSM



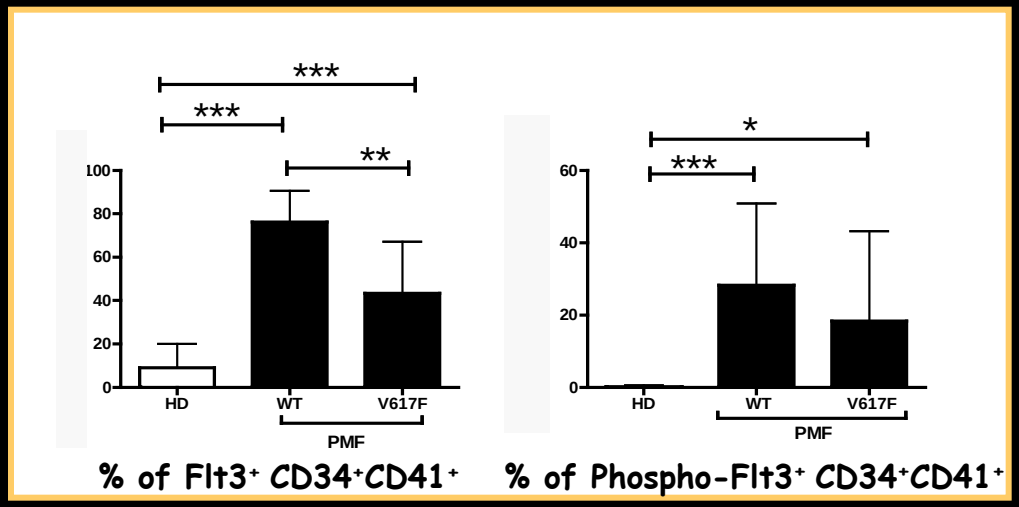
CD34+

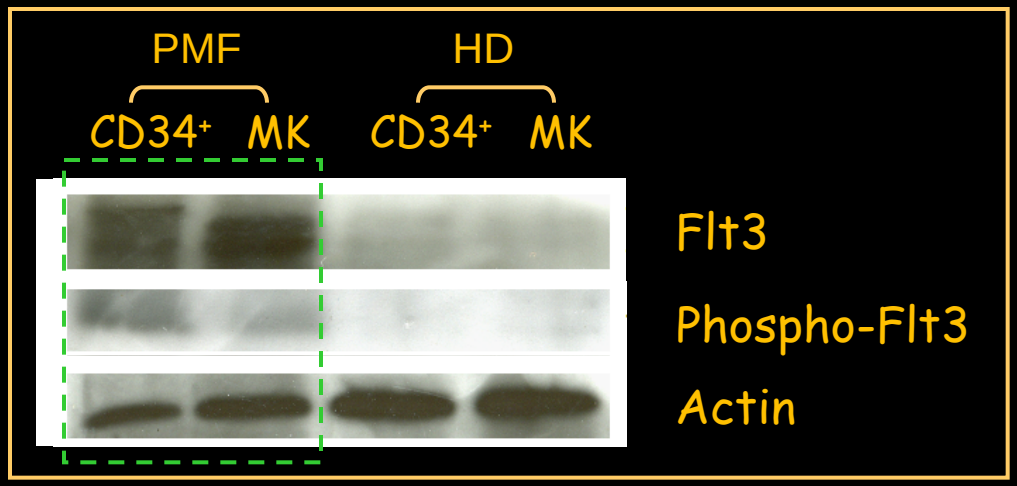


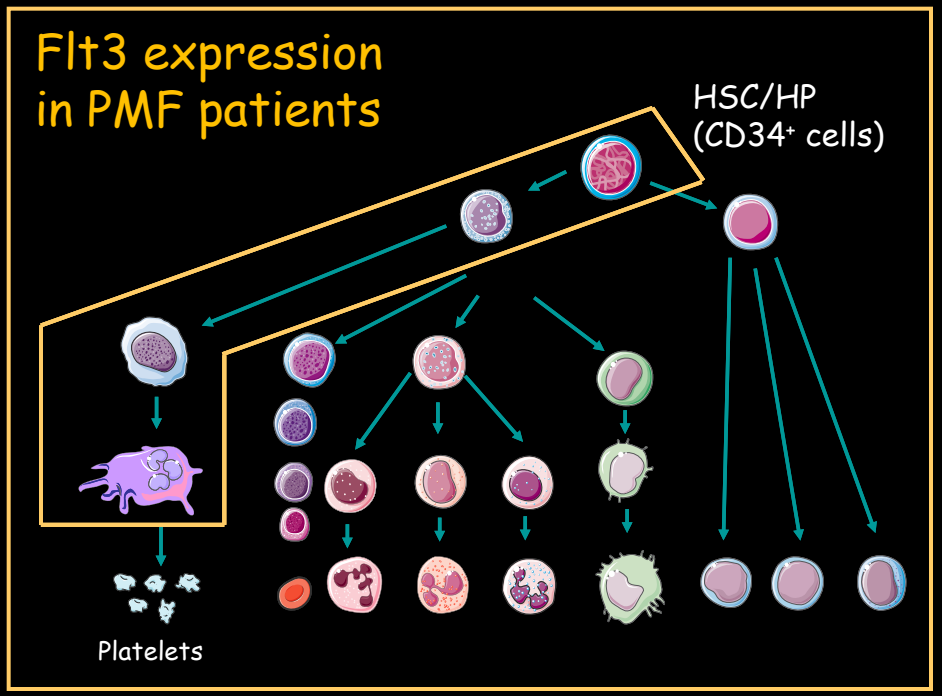


(Desterke et al; Cancer Research, April 2011)

Flt3 is overexpressed and activated in CD34+ and MK cells from PMF patients, independently of JAK2^{V617F}

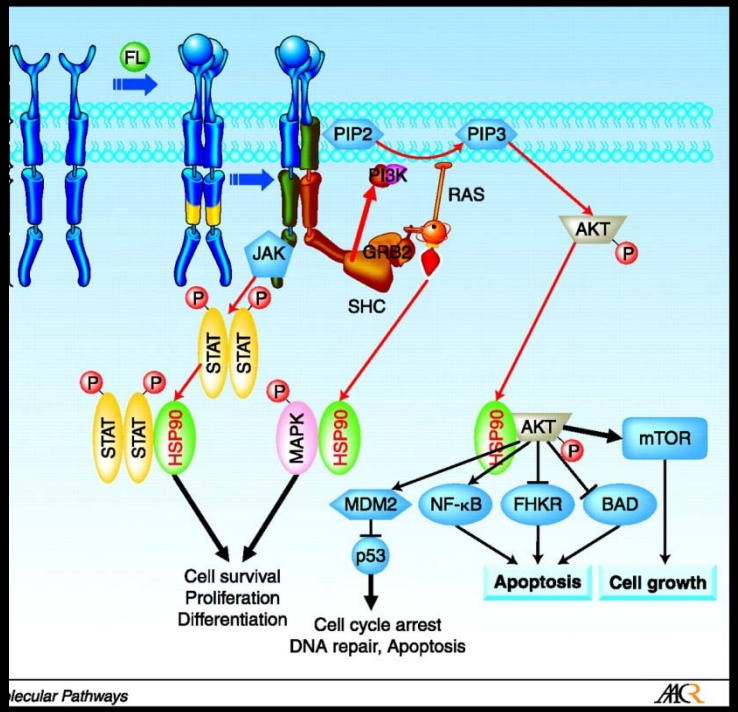




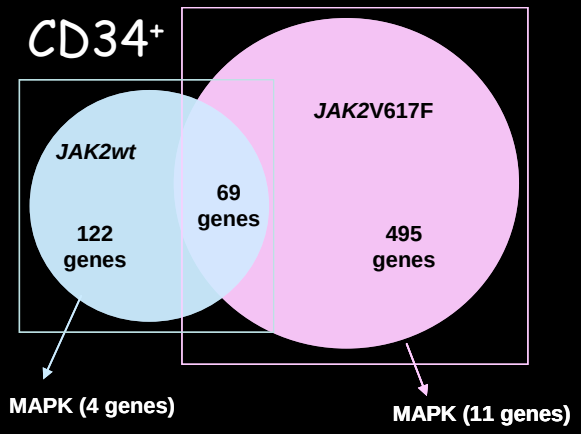


(Desterke et al; Cancer Research, April 2011)

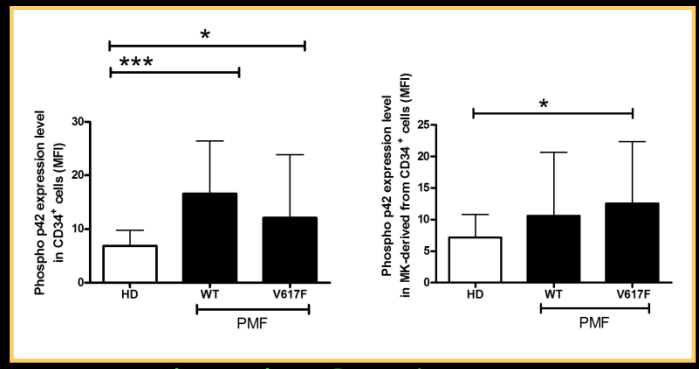
Phosphorylation of MAPKs is increased in PMF CD34+ and MK cells, independently of JAK2V617F



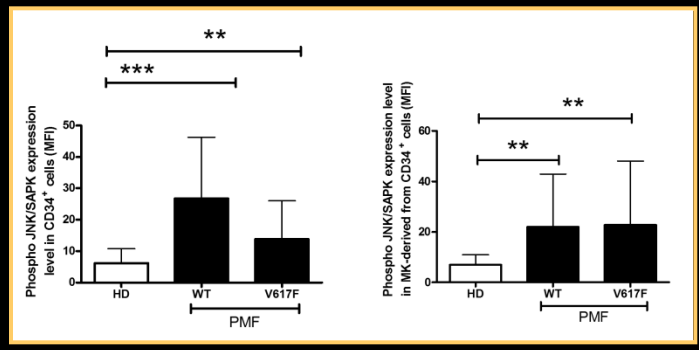
Meshinchi S et al; Clin Cancer Res 2009;15:4263-4269



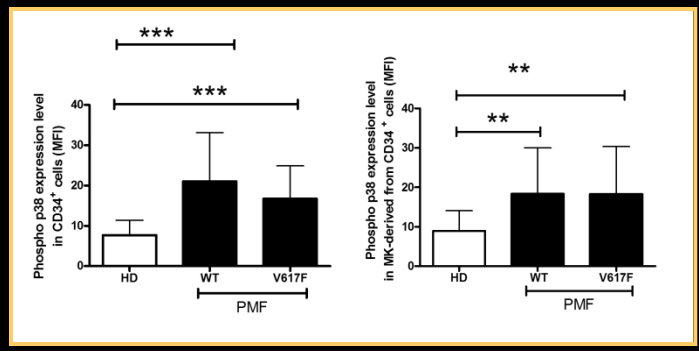
CD34+ MK



Phospho ERK/p42-p44



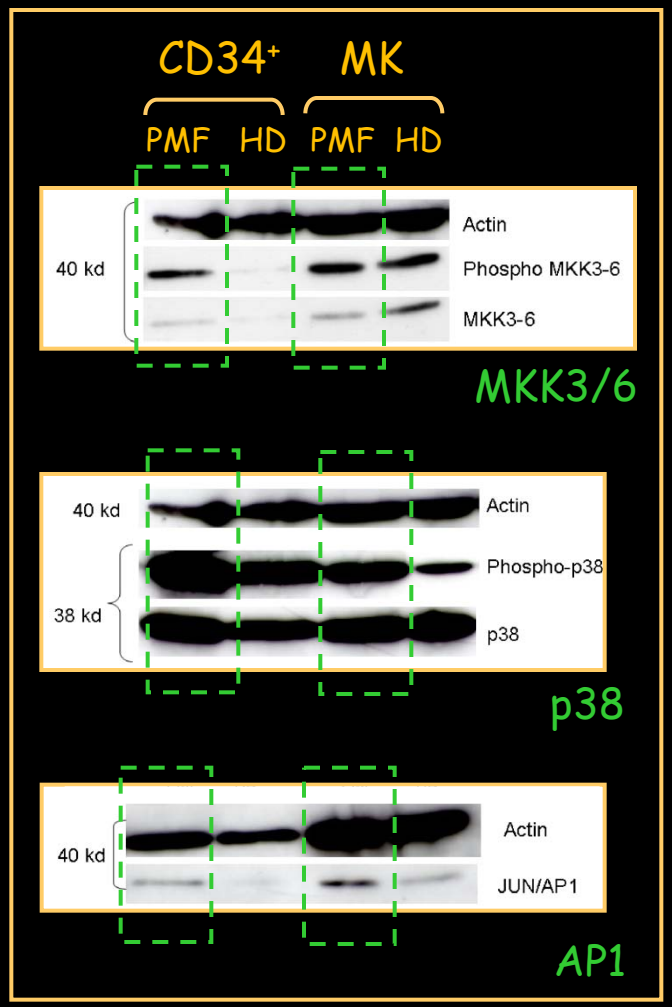
Phospho JNK

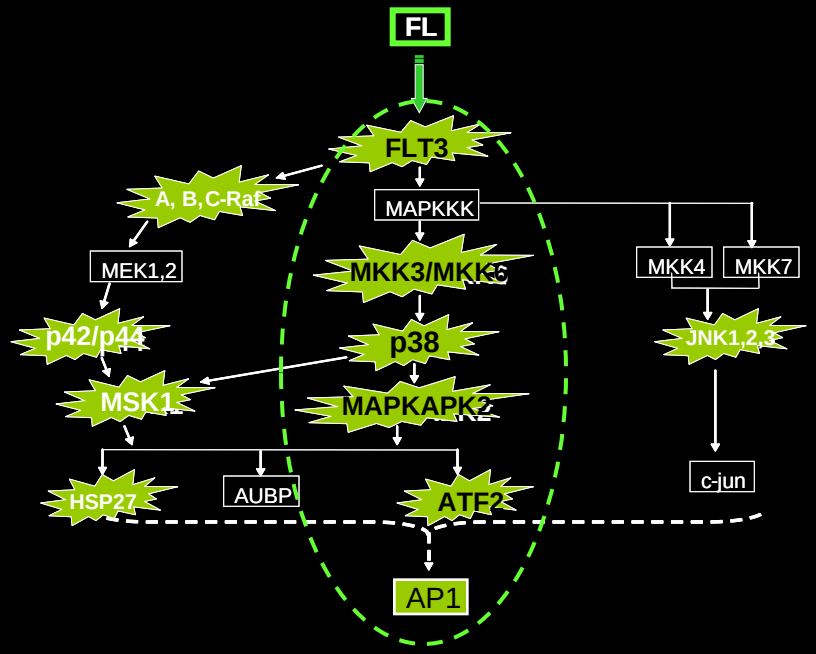


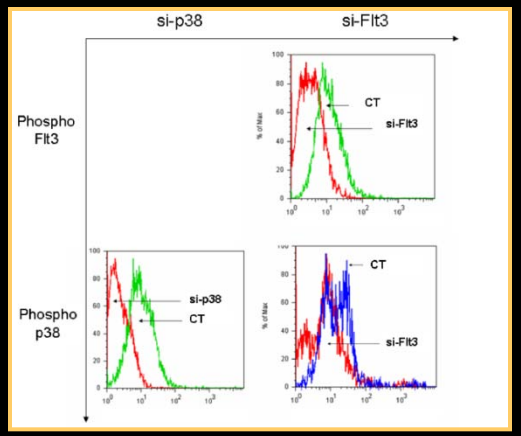
Phospho p38

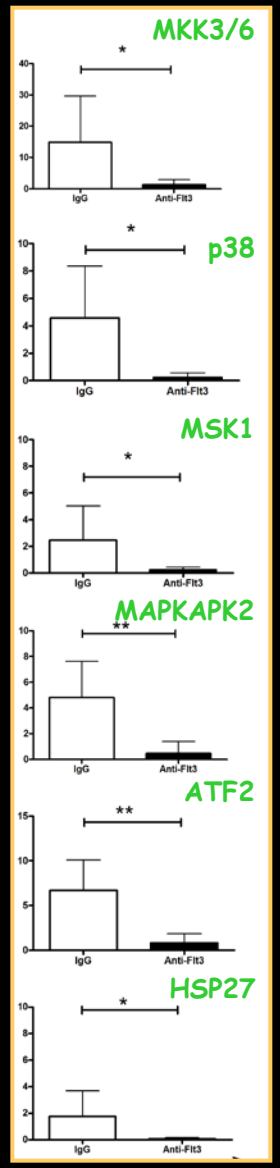
(Desterke et al; Cancer Research, April 2011)

Phosphorylation of p38 MAPK and its up/down effectors is increased in PMF CD34 and MKs and is Flt3 dependent



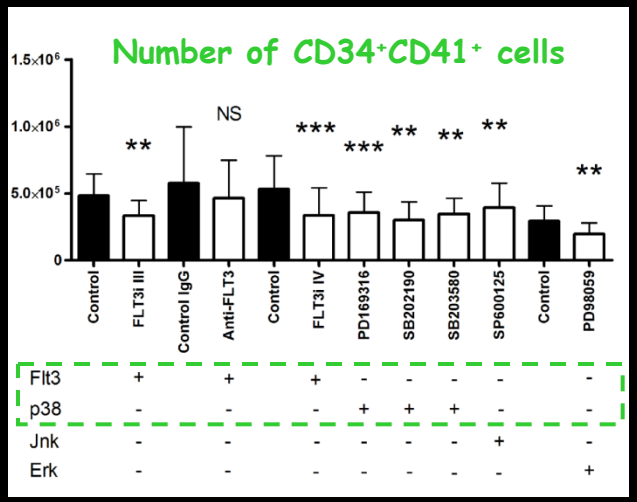


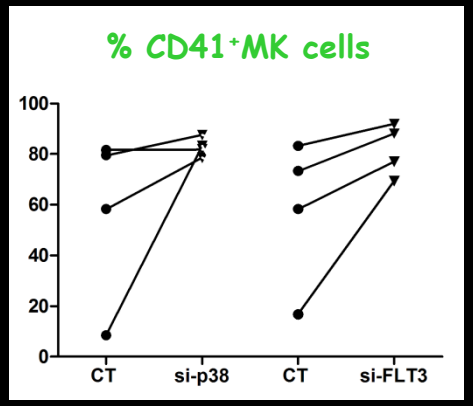


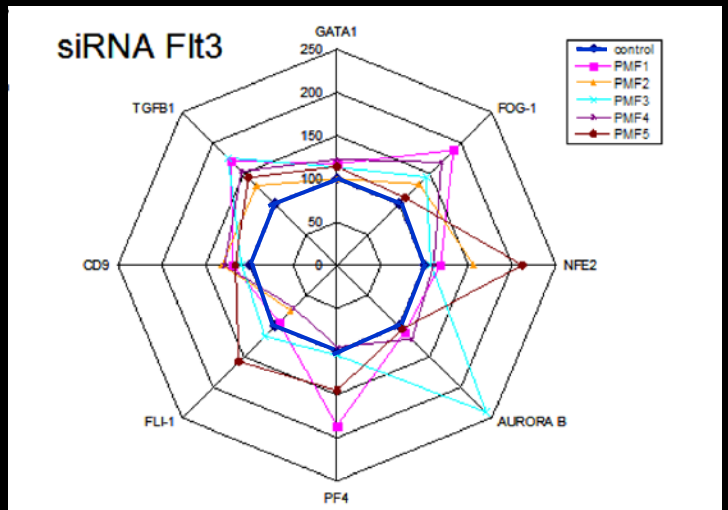


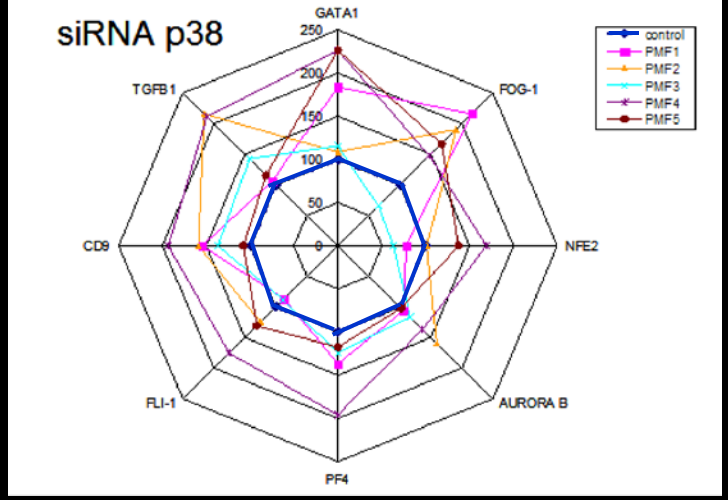
(Desterke et al; Cancer Research, April 2011)

FL/Flt3 couple is involved in PMF dysmegakaryopoieisis through p38 axis



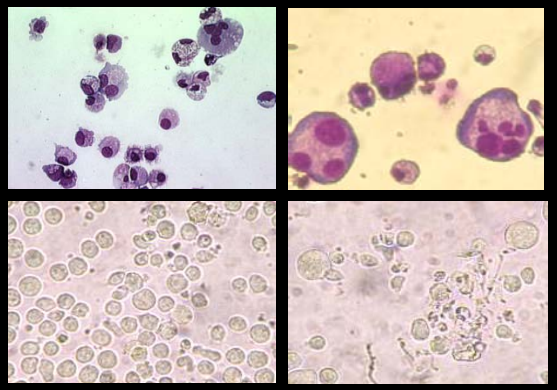






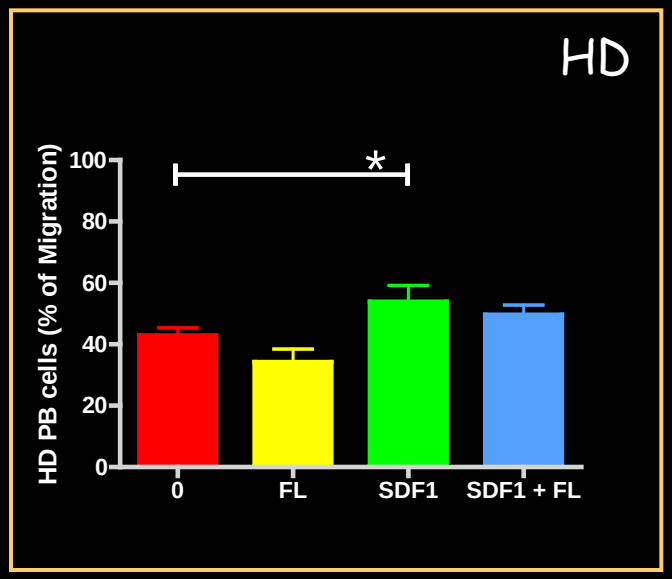
Silencing of Flt3 or p38 in PMF MK:

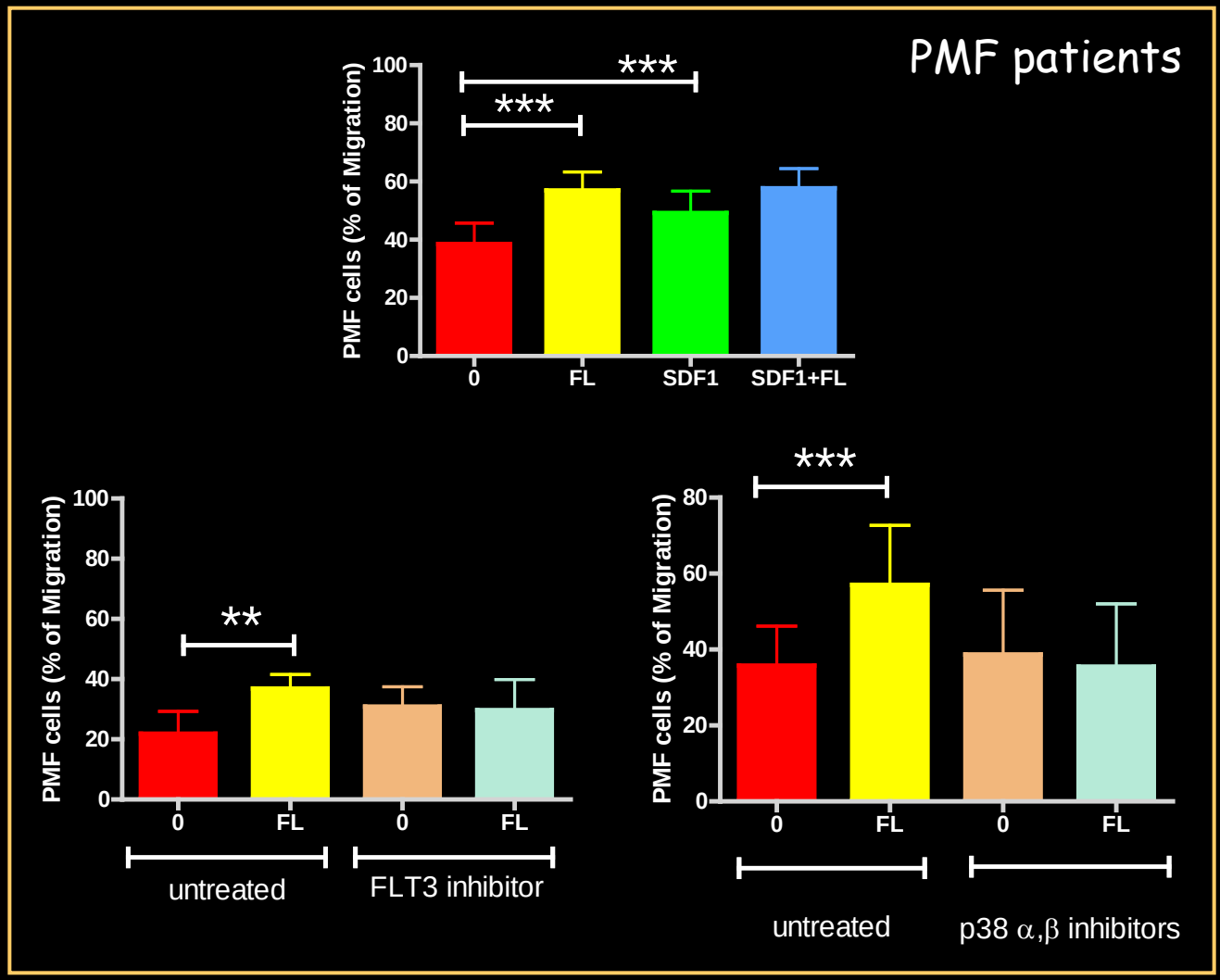
- ✓ Decreases proliferation
- ✓ Restores differentiation
- ✓ Increases ploidization and pro-platelet formation
- ✓ Increases expression of genes involved in megakaryopoiesis (TFs, GFs…)



(Desterke et al; Cancer Research, April 2011)

FL/Flt3 couple is involved in PMF MK precursor migration through p38 axis

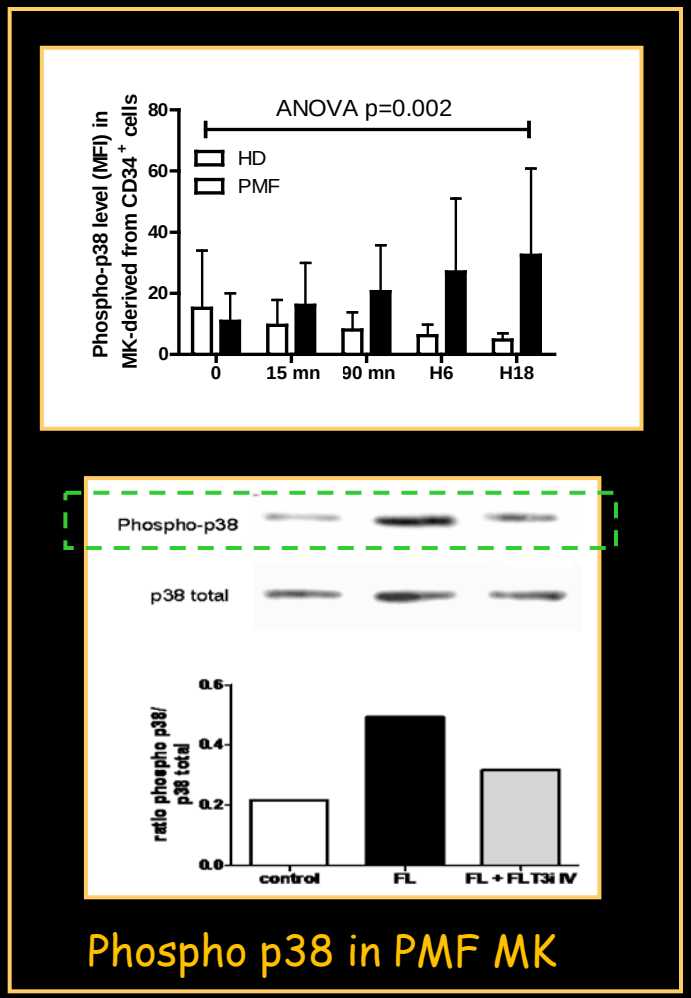




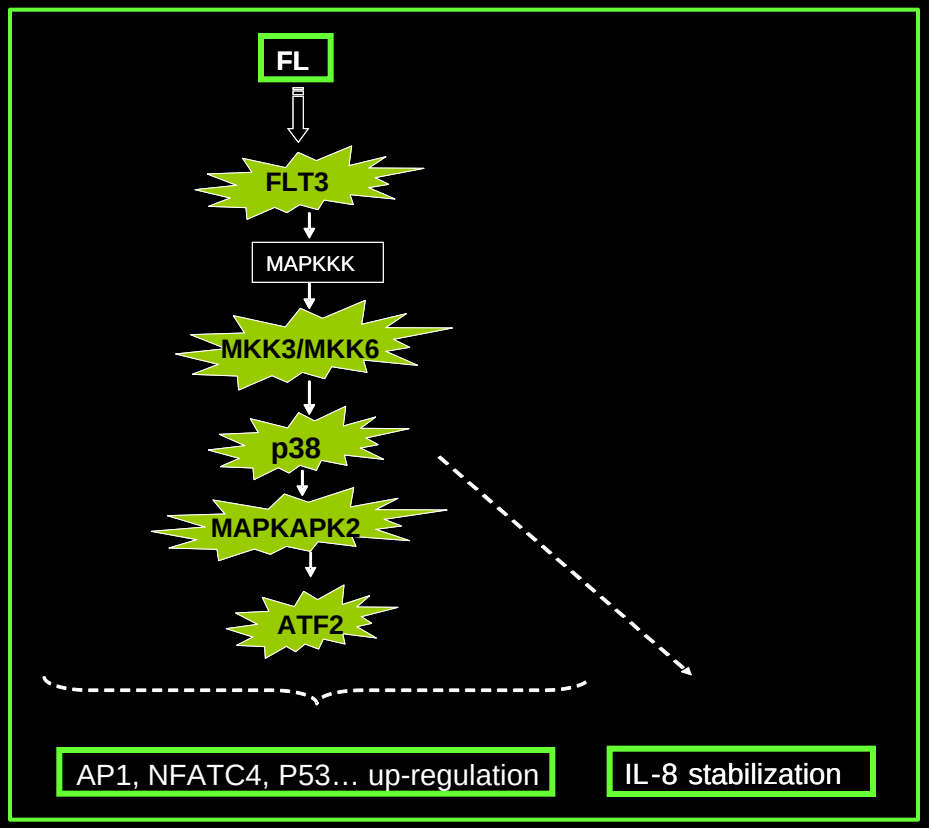
10 ng/ml FL
100 ng/ml SDF-1/CXCL12

(Desterke et al; Cancer Research, April 2011)

Activation of Flt3 in PMF MK cells by FL induces p38 phosphorylation and increases p38 target gene expression



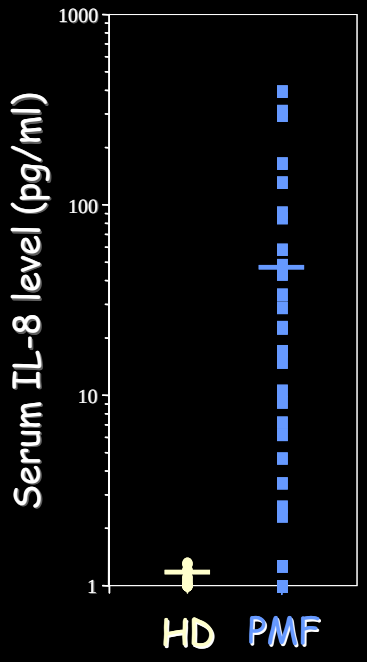
Phospho p38 in PMF MK

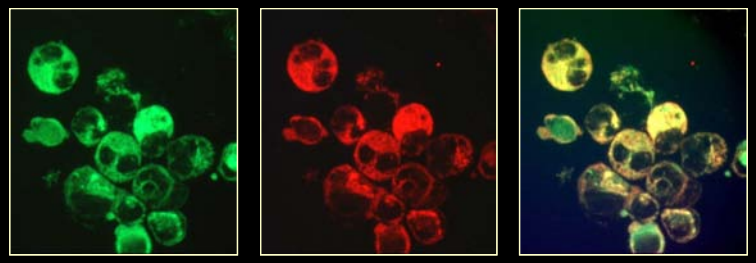


- Activation of ATF2, a key cross talk molecule for transcriptional activity of p38
- Up-regulation of p38-associated downstream TF transcripts (AP1, P53, ATF2, NF-κB and NFATC4, ...)
- Up-regulation and stabilisation of IL8 transcripts

(Desterke et al; Cancer Research, April 2011)

Il-8 is over expressed in patients and participates in PMF dysmegacaryopoiesis



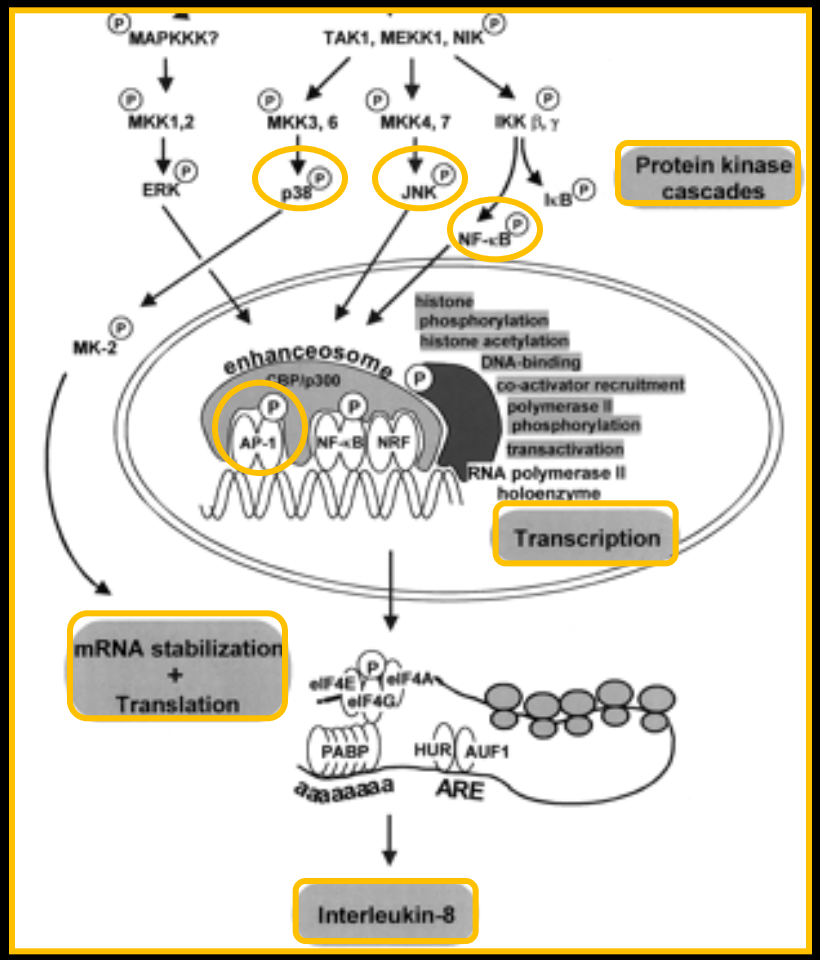


CD41 IL-8 CD41/IL-8

- IL-8 level is highly increased in PMF patients and is mainly produced by megacaryocytes, HSC/PH and stromal cells
- Plasma level of IL-8 is not correlated with JAK2 mutation and is a prognostic marker in PMF (Tefferi et al., 2011)
- IL-8/receptors are involved in PMF dysmegacaryopoieis (Emadi et al., 2005)
- Il-8 chemokine is one of the major mediators of the inflammatory process and is produced in response to pro-inflammatory molecules (TNF, IL-1..) and cellular stress (Hoffmann et al., 2002)

(Emadi et al, Blood 2005)

P38 MAPK activation is involved in IL-8 over expression in PMF patients



(Hoffmann et al, 2002)

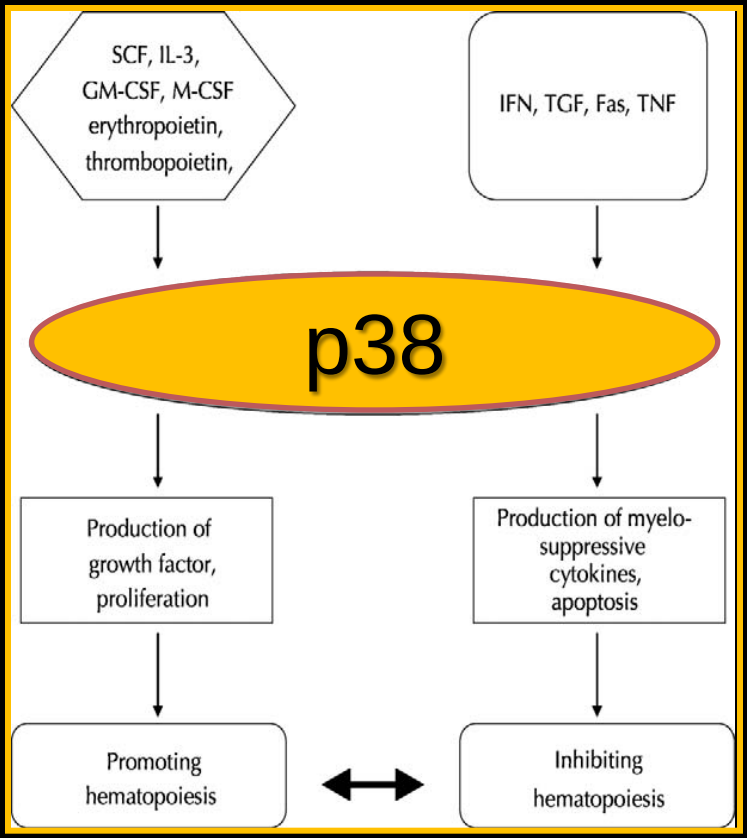
- Maximal IL-8 expression requires the coordinate activation of NF-κB, JNK and p38 pathways as well as a functional AP-1 transcription factor site

- In PMF patients, these different pathways are activated and AP1 expression is increased (Rameshwar et al. 2000; Komura et al. 2005; Desterke et al. 2011)

  Inhibition of Flt3 or silencing of p38 down-regulates IL-8 and AP-1 expression in PMF Mk cells (Desterke et al. 2011)

  Therefore, activation of NF-κB, JNK, AP-1 and especially p38, likely participates in IL-8 over-expression in patients

p38, a dual pro-inflammatory kinase in balancing hematopoieis

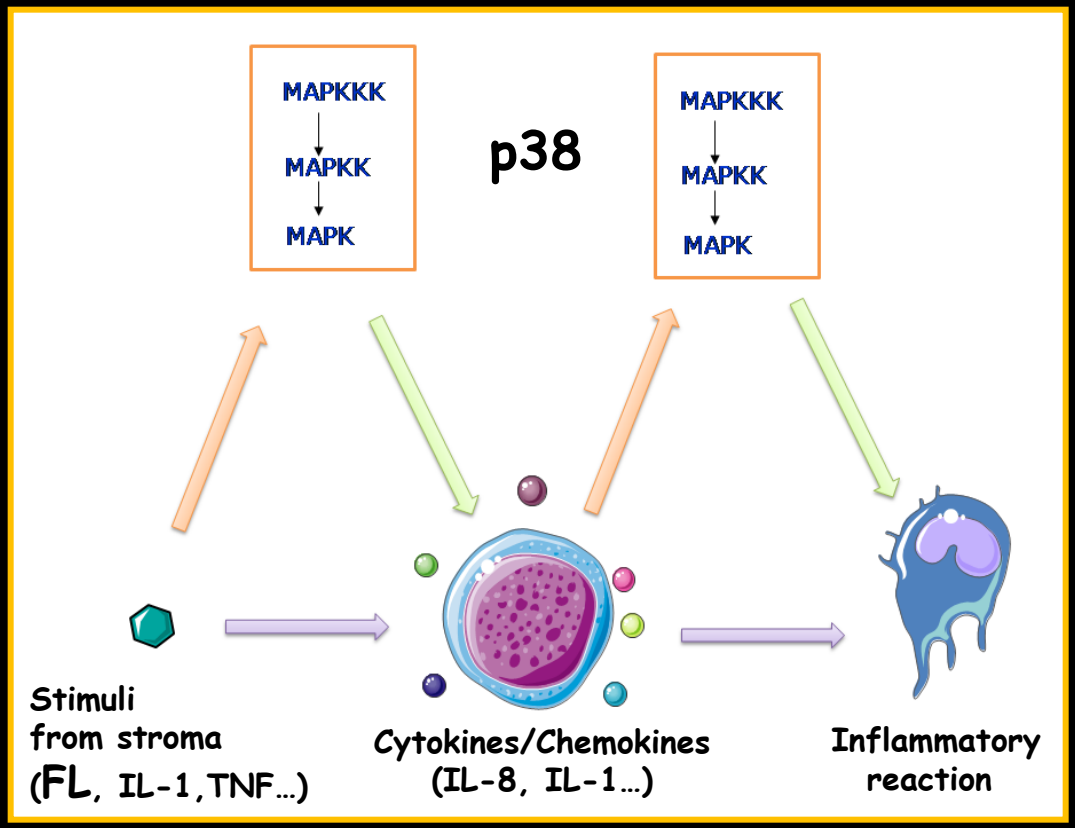


(Feng et al, 2009)

- p38 cascade is activated by pro-inflammatory stimuli and cellular stress
- Its effects are isoform, cell, stimulus and signal intensity specific:
  - Common signaling mediator for hematopoietic GF promoting hematopoiesis and for myelosuppresive cytokines inhibiting hematopoiesis
  - Strong activation engages to apoptosis and senescence whereas lower activation is associated with survival

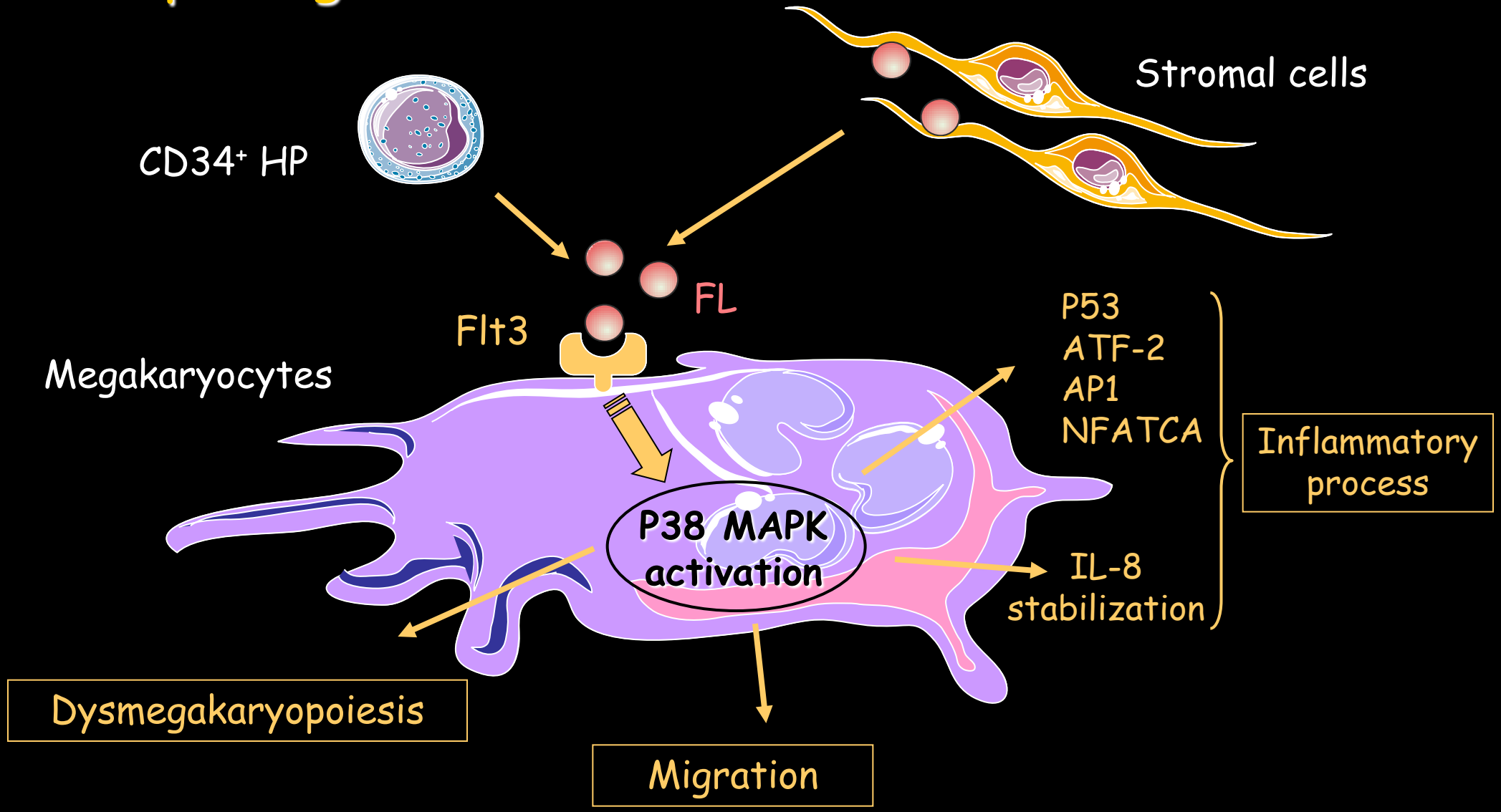
In PMF, strong activation of p38 likely participates in hematopoiesis/megacaryopoiesis deregulation and in inflammation by stabilizing transcripts of pro-inflammatory GFs

In PMF, alteration of stroma likely contributes to persistent production of proinflammatory cytokines capable to activate p38 and to maintain inflammation



Targeting the common p38 pathway by selective pharmacologic inhibitors could be a promising approach and could be preferable than targeting individual cytokines

Potential role of FL/Flt3 and p38 MAPK axis deregulation in PMF pathogenesis



(Desterke et al; Cancer Research, April 2011)

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