Inflammation Cytokines
in JAK2V617F-mutated MPNs

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Before 2005

- **Hypersensitivity to cytokines** of MPN progenitors, used for diagnostic purposes:
  - Endogenous Erythroid Colony (EEC) growth
  - Low serum Epo

- **Chronic inflammation**: Elevated levels of certain cytokines (VEGF, b-FGF, IL-6…), known to be produced by MPN precursor cells (megakaryocytes) or/and cells of the bone marrow micro-environment
  - Progenitor cell survival & growth
  - Fibrosis of the bone marrow
  - Neo-angiogenesis
2005: Mutation JAK2V617F

Mutation 1849G>T (exon 14) in the gene JAK2, specific for MPNs


<table>
<thead>
<tr>
<th>JH7</th>
<th>JH6</th>
<th>JH5</th>
<th>JH4</th>
<th>JH3</th>
<th>JH2</th>
<th>JH1</th>
</tr>
</thead>
</table>
| FERM domain  
  4.1  
  Ezrin  
  Radixin  
  Meosin | V617F  
  pseudo-kinase domain | kinase domain |

Increased activation of the JAK2 / STAT5 pathway → Hypersensitivity to EPO
2013: Calreticulin (CALR) Mutations

Frameshift mutations caused by deletions or insertions in Exon 9:
**Type 1**: 52-bp deletion (p.L367fs*46)
**Type 2**: 5-bp TTGTC insertion (p.K385fs*47)


Mutant CALR requires both its mutant C-terminus and the TPO receptor (MPL) for transformation

*Elf S et al. Cancer Discov 2016;6:368-81
Araki M. Blood 2016;127:1307-16*
JAK2V617F, MPLW515L/K or CALR exon 9 mutations enhance myelopoiesis via increased activation of JAK2/STAT5 pathways

+ Disruption of the STAT3 and STAT1 pathways

MPNs: More than JAK2, CALR, MPL mutation?

- JAK2V617F is found in all subtypes of MPNs
- JAK2V617F+/+ clones are found in PV and PMF
- MPLW515L/K and CALR mutations are found in ET and in PMF
- Phenotype and evolution and JAK2, MPL or CALR mutant burden are not correlated

Unanswered questions:
- Why do JAK2V617F+ patients present with ET, rather than PV, or PMF?
- Why do CALR+ patients present with ET, or PMF?

Other pathogenic mechanisms likely at play in MPNs, presumably early and pre-disposing to JAK2, CALR, MPL mutation

Boissinot et al. Blood 2006
**Non-driver mutations**

*IDH1, IDH2, EZH2, SRSF2* (poor prognosis)  
*ASXL1, TET2, DNMT3A, CBL, RUNX1, SF3B1, TP53*

- Mutations in epigenetic regulators *TET2* and *DNMT3A* are involved in disease initiation and may precede the acquisition of *JAK2V617F*.

- Other mutations in epigenetic regulators such as *EZH2* and *ASXL1* also play a role in disease initiation and disease progression.

*Vainchenker W, Kralovics R. Blood 2017; 129:667-679*
Pre-disposition to JAK2, MPL or CALR mutation and MPN

Unknown event leading to chronic stimulation of myelopoiesis: may be genetic, germline or somatic, other
The haplotype 46/1 of chr. 9p is associated with a pre-disposition to mutations in the JAK2 gene on the same allele.

Olcaydu et al.; Jones et al.; Kilpivaara et al. Nature Genetics, 2009, March 15th

JAK2V617F can occur multiple times in the same patient (PV and ET)


JAK2V617F can occur as a consequence of a pre-disposition
Significant increase in frequency of the TNF238 GA genotype in MPNs compared to controls (OR=2.21, 95% CI=1.02-4.80, P<0.04)

Distribution of the genotypes and allelic frequencies of TNF-308 significantly different among the MPNs, JAK2V617F positive, PV and PMF, and controls

Table 1

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>TNF-238</th>
<th>MPN overall N = 123</th>
<th>MPN JAK V617F positive N = 94</th>
<th>MPN JAK V617F negative N = 29</th>
<th>PV N = 33</th>
<th>ET N = 35</th>
<th>PMF N = 22</th>
<th>MPNa N = 33</th>
<th>Controls N = 123</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF-238</strong></td>
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</tr>
<tr>
<td>GG</td>
<td>101 (82%)</td>
<td>78 (82.9%)</td>
<td>27 (81.8%)</td>
<td>18 (81.8%)</td>
<td>27 (81.8%)</td>
<td>110 (89.4%)</td>
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<tr>
<td>GA</td>
<td>22 (17.8%)</td>
<td>16 (17.1%)</td>
<td>6 (18.2%)</td>
<td>6 (17.1%)</td>
<td>6 (18.2%)</td>
<td>11 (9%)</td>
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<tr>
<td>AA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (1.6%)</td>
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<tr>
<td><strong>TNF-308</strong></td>
<td></td>
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</tr>
<tr>
<td>GG</td>
<td>67 (54.5%)</td>
<td>48 (51.1%)</td>
<td>16 (48.5%)</td>
<td>20 (57.1%)</td>
<td>9 (40.9%)</td>
<td>85 (69.1%)</td>
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</tr>
<tr>
<td>GA</td>
<td>53 (43%)</td>
<td>44 (46.8%)</td>
<td>17 (51.5%)</td>
<td>13 (37.2%)</td>
<td>13 (59.1%)</td>
<td>36 (29.3%)</td>
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<td></td>
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</tr>
<tr>
<td>AA</td>
<td>3 (2.5%)</td>
<td>2 (2.1%)</td>
<td>0</td>
<td>2 (5.7%)</td>
<td>1 (3.0%)</td>
<td>2 (1.6%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>G</td>
<td>187 (76%)</td>
<td>140 (74.5%)</td>
<td>49 (74.2%)</td>
<td>53 (75.7%)</td>
<td>54 (81.8%)</td>
<td>206 (83.7%)</td>
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</tr>
<tr>
<td>A</td>
<td>59 (24%)</td>
<td>48 (25.5%)</td>
<td>17 (25.8%)</td>
<td>17 (24.3%)</td>
<td>13 (29.5%)</td>
<td>40 (16.3%)</td>
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</table>

Pathological process consisting of:

- **Cellular infiltration**
  granulocytes, monocytes, lymphocytes

- **Release of mediators**
  in blood vessels and tissues

Causes of inflammation can be physical, chemical, or biologic: infection, hypoxia, cancer.
Inflammation and Pathogenesis of MPNs?

Mediators of Inflammation in Myeloproliferative Neoplasms: State of the Art

Guest Editors: Sylvie Hermouet, Hans C. Hasselbalch, and Vladan P. Čokić

- MPNs as Inflammatory Diseases: The Evidence, Consequences, and Perspectives, Hans Carl Hasselbalch and Mads Emil Bjørn
  Volume 2015 (2015), Article ID 102476, 16 pages

- HSP90 and HSP70: Implication in Inflammation Processes and Therapeutic Approaches for Myeloproliferative Neoplasms, Margaux Sevin, François Giridon, Carmen Garrido, and Aurélie de Thonel
  Volume 2015 (2015), Article ID 970242, 8 pages

- The Hen or the Egg: Inflammatory Aspects of Murine MPN Models, Jonas S. Jutzi and Heike L. Pahl
  Volume 2015 (2015), Article ID 101987, 8 pages

- Cytokine Regulation of Microenvironmental Cells in Myeloproliferative Neoplasms, Gregor Hoermann, Georg Greiner, and Peter Valent
  Volume 2015 (2015), Article ID 869242, 17 pages

- The Role of Reactive Oxygen Species in Myelofibrosis and Related Neoplasms, Mads Emil Bjørn and Hans Carl Hasselbalch
  Volume 2015 (2015), Article ID 648090, 11 pages

- Pathogenesis of Myeloproliferative Neoplasms: Role and Mechanisms of Chronic Inflammation, Sylvie Hermouet, Edith Bigot-Corbel, and Betty Gardie
  Volume 2015 (2015), Article ID 145293, 16 pages

- Impact of Inflammation on Myeloproliferative Neoplasm Symptom Development, Holly L. Geyer, Amylou C. Dueck, Robyn M. Scherber, and Ruben A. Mesa
  Volume 2015 (2015), Article ID 284706, 9 pages

- Inflammation as a Driver of Clonal Evolution in Myeloproliferative Neoplasm, Angela G. Fleischman
  Volume 2015 (2015), Article ID 606819, 6 pages

- Circulating Cytokine Levels as Markers of Inflammation in Philadelphia Negative Myeloproliferative Neoplasms: Diagnostic and Prognostic Interest, Julie Mondet, Kais Hussein, and Pascal Mossuz
  Volume 2015 (2015), Article ID 670580, 10 pages

Special Issue "Mediators of Inflammation in Myeloproliferative Neoplasms: State of the Art" November 2015

More information on: http://www.hindawi.com/journals/mi/si/329170/
Inflammation present in almost all MPNs

Mondet J, Hussein K, Mossuz P. Circulating cytokine levels as markers of inflammation in Phinegative MPNs: Diagnostic and prognostic interest. Mediators of Inflammation Nov 13, 2015, Article 670580
Correlations between Cytokine Levels and Biological & Clinical Symptoms

**PV**
- Thrombosis: IL-12(p70), GM-CSF, C-Reactive Protein (CRP), Pentraxin 3
- Leukocytes: HGF
- Hematocrit: IL-11

**PMF**
- Quality of Sleep and Appetite: RANTES, ILR1-α
- Pruritis: Ferritin
- Weight Loss: Leptin
- Splenomegaly: HGF
- Leukocytosis: IL-8
- **Shorter Survival**: MIP-1, CRP
- **Poor Prognosis (inferior survival)**: IL-8, IL-12, IL-15, IL-2R

=> Prognostic biomarkers

*Tefferi A et al. J. Clin Oncol. 2011;29:1356*
Molecular Pathways of Inflammation & Cytokine Production

- JAK2/STAT5?
- NF-κB
- JAK1/STAT1/STAT3
- HIF-1α
Quantification of 40 inflammation cytokines + 2 receptors in serum from 72 MPN patients at the time of diagnosis (51 with JAK2V617F mutation, 21 with CALR or MPL mutation)

Methods: Multiplex Luminex technology, BioRad kits

All 72 MPN patients over-produce >15 inflammation cytokines

- **13 cytokines are over-expressed by all patients**
  - Anti-inflammatory: IL-4, IL-10
  - Pro-inflammatory: TNF-α, IL-1β, IL-12p70, IL-8, IL-17
    - G-CSF, Eotaxin, MIP-1α, MIP-1β
    - RANTES, SDF-1α

- **2 are over-expressed by ~50% patients: LIF and MIG**

- **1 receptor and 4 cytokines are occasionally elevated:** GM-CSF, IL-33, MCP-1, IL-2Rα, IL-15
  (poor prognosis markers in PMF)
Cytokines in excess for all MPN patients

- IL-1β
- IL-12p70
- SDF-1
- LIF
- IL-33
- GM-CSF

**ET**
- Essential thrombocythemia
- Polycythemia vera
- Primary myelofibrosis

**PV**

**PMF**

- Always
- 50%
- Rarely
51 JAK2V617F+ Patients

28 PV, 18 ET, 7 PMF

%JAK2V617F: 5% -> 96%

% JAK2 V617F
n=51

PV n=26
ET n=18
PMF n=7
Cytokine levels and %JAK2V617F NOT, or weakly, correlated

51 JAK2V617F+ patients (all phenotypes)
No correlation between %JAK2V617F and 39/40 inflammation cytokines
Multiparametric studies: on-going

Weak positive correlations between %JAK2V617F and IL-1Rα and IP-10
Spearman correlation test

IL-1Rα
p = 8.317e-005
r = 0.5227

IP-10
p = 0.002034
r = 0.4261
26 JAK2V617F+ PV patients: No correlation between %JAK2V617F and 37/40 cytokines

Weak correlations between %JAK2V617F and IL-1Rα and IL-1β

Good inverse correlation between %JAK2V617F and leptin (negative feed-back)
18 JAK2V617F+ ET patients

No correlation between %JAK2V617F and 40/40 inflammation cytokines
26 JAK2V617F+ PV patients vs 18 JAK2V617F+ ET patients
18 JAK2V617F+ ET patients vs 15 CALR+ ET patients

- **JAK2** V617F+ ET patients vs CALR+ ET patients
- CALR mutation + TET2 mutation

**CHARTS**

- **TNF-a**
  - p* = 0.022

- **IFN-g**
  - p* = 0.0143

- **IFN-a2**
  - p* = 0.0232

**Note:** The charts show the levels of TNF-a, IFN-g, and IFN-a2 for JAK2V617F+ ET patients and CALR+ ET patients, with statistical significance indicated by p* values.
**JAK2V617F+ ET vs CALR+ ET**

18 *JAK2V617F*+ ET patients vs 15 *CALR*+ ET patients

- **Green Circles**: *CALR* mutation + *TET2* mutation

**Acute inflammation**

- **TNF-a**
  - *p* = 0.022

- **IFN-g**
  - *p* = 0.0143

**Immuno-stimulant**

- **IFN-a2**
  - *p* = 0.0232
JAK2V617F+ ET vs CALR+ ET

18 JAK2V617F+ ET patients vs 15 CALR+ ET patients

- **CALR mutation + TET2 mutation**

**Acute inflammation**
- Inhibits viral replication

**Immuno-stimulant**
- Anti-viral, anti-microbial
CALR+ ET vs JAK2V617F+ ET

18 JAK2V617F+ ET

15 CALR+ ET

IL-4

\[ p^{**}=0.0010 \]

IL-9

\[ p^* = 0.0147 \]
CALR+ ET vs JAK2V617F+ ET

18 JAK2V617F+ ET
15 CALR+ ET

**CALR** mutation
+ TET2 mutation

**IL-4**

\[ p^{***} = 0.0010 \]

**IL-9**

\[ p^* = 0.0147 \]

*Increases IL-4 production*
• Inflammation is important in MPNs

• Determination of a patient’s inflammation status is simple (multiplex cytokine assay)

• There are predictive inflammation markers in PMF: IL-2R, IL-8, IL-12, IL-15, MIP-1

• Inflammation is specific: Cytokine profiles differ in MPNs and in MGUS/MM (similar in MGUS and MM)

• IL-1Rα and IL-1β levels and %JAK2V617F are -weakly- correlated in JAK2V617F-mutated PV

• IL-1Rα is the main difference between JAK2V617F+ PV and JAK2V617F+ ET

• Inflammation differs in JAK2V617F+ ET and in CALR-mutated ET
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