

Article

# Anti-Glucosylsphingosine Autoimmunity, JAK2V617F-Dependent Interleukin-1 $\beta$ and JAK2V617F-Independent Cytokines in Myeloproliferative Neoplasms

Sophie Allain-Maillet, Adrien Bosseboeuf, Nicolas Mennesson, Mégane Bostoën, Laura Dufeu, Eun Ho Choi, Cédric Cleyrat, Olivier Mansier, Eric Lippert, Yannick Le Bris, Jean-Marc Gombert, François Girodon, Magali Pettazzoni, Edith Bigot-Corbel and Sylvie Hermouet

Supplementary Materials:

**Table S1.** Correlations between %JAK2V617F and blood counts in JAK2V617F-mutated MPN patients.

Blood Parameter	All MPNs			PV			ET			PMF		
	n	r	<i>p</i> value	n	r	<i>p</i> value	n	r	<i>P</i> value	n	r	<i>p</i> value
Leukocytes (x10 <sup>9</sup> /L)	53	0.4015	0.0029	25	0.7266	<0.0001	-	-	-	-	-	-
Neutrophils (x10 <sup>9</sup> /L)	51	0.4321	0.0015	24	0.7885	<0.0001	-	-	-	-	-	-
Monocytes (x10 <sup>9</sup> /L)	-	-	-	25	0.4018	0.0465	-	-	-	-	-	-
Hematocrit (L/L)	-	-	-	-	-	-	-	-	-	-	-	-
Hemoglobin (g/dL)	-	-	-	-	-	-	-	-	-	-	-	-
Platelets (x10 <sup>9</sup> /L)	-	-	-	-	-	-	-	-	-	-	-	-

- : non-significant.

**Table S2.** Characteristics of ET patients with JAK2V617F or CALR mutation.

	JAK2V617F –Mutated ET	CALR –Mutated ET
<b>Number</b>	21	15
<b>Sex, M/F (male%)</b>	8/13 (38.1%)	7/8 (46.7%)
<b>Age (Yr)</b>		
Median	68.0	61.1
Range	(43.0–95.0)	(41.2–85.8)
<b>%JAK2V617F</b>		
Median	13.0	0
Range	(4.0–61.0) <sup>a</sup>	(0–0)
<b>Blood Counts</b>		
Hematocrit (L/L)		
Median	46.2	42.9*
Range	(35.5–63.3)	(35.3–48.3)
Hemoglobin (g/dL)		

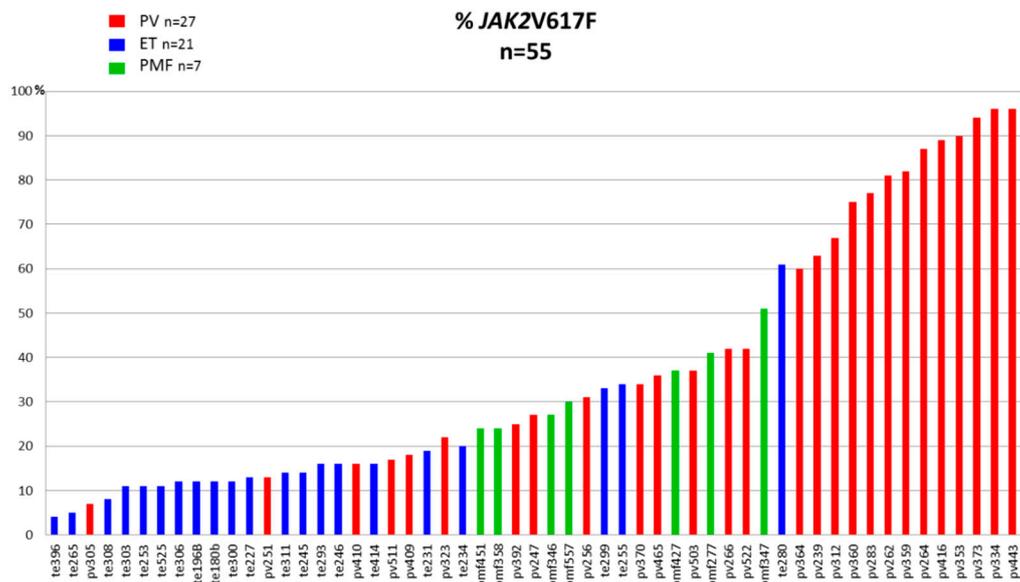
Median	14.8	14.0
Range	(10.4–20.0)	(11.6–11.6)
Leukocytes (x10 <sup>9</sup> /L)		
Median	9.1	7.9
Range	(4. –41.0)	(5.3–15.4)
Platelets (x10 <sup>9</sup> /L)		
Median	615	808**
Range	(191–1,851)	(360–2,300)

(a) Patient with myelofibrosis; \* $p = 0.0333$  and \*\* $p = 0.0510$  vs JAK2V617F-mutated ET, Mann-Whitney  $t$ -test.

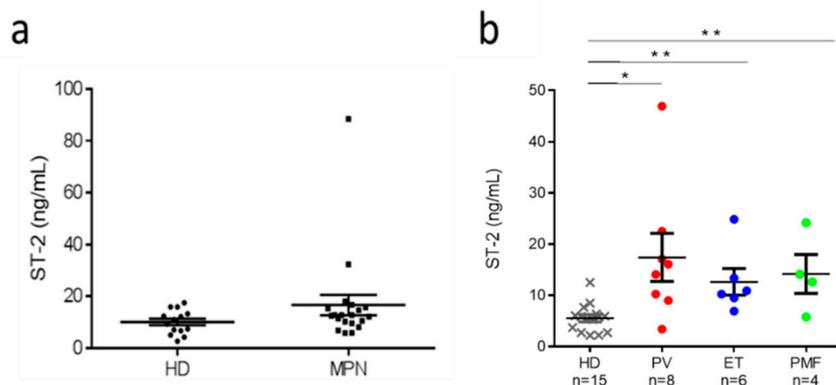
**Table S3.** Characteristics of MPN patients with GlcSph-reactive IgGs.

	All Patients	PV	ET	PMF
<b>Patients with GlcSph-reactive IgGs</b>				
<b>Number</b>	15	3	8	4
<b>Sex, M/F (male%)</b>	6/9 (40.0%)	2/1 (66.7%)	2/6 (25.0%)	2/2 (50.0%)
<b>Age (Yr)</b>				
Median	67.0	67.0	67.4	64.0
(Range)	(33–95)	(33–76)	(41–95)	(50–73)
<b>%JAK2V617F</b>				
Median	16.0	25.0	0	27.0
(Range)	(0–96)	(17.0–96)	(0–34)	(0–37)
<b>Blood Counts</b>				
<b>Hematocrit (L/L)</b>				
Median	42.6	48.7	42.9	37.4
(Range)	(29.3–63.3)	(43.1–52.2)	(35.3–63.3)	(29.3–41.0)
<b>Hemoglobin (g/dL)</b>				
Median	14.0	14.1	14.0	11.6
(Range)	(9.6–20.0)	(13.8–16.7)	(11.6–20.0)	(9.6–13.0)
<b>Leukocytes (x10<sup>9</sup>/L)</b>				
Median	7.3	7.8	6.9	6.5
(Range)	(4.5–37.8)	(7.3–37.8)	(4.5–15.4)	(5.0–12.5)
<b>Platelets (x10<sup>9</sup>/L)</b>				
Median	483	349	765	116
(Range)	(47–2,300)	(239–493)	(191–2,300)	(47–483)
<b>Patients without GlcSph-reactive IgGs</b>				
<b>Number</b>	60	24	31	5
<b>Sex, M/F (male%)</b>	32/28 (53.3%)	14/10 (58.3%)	13/18 (41.9%)	5/0 (100%)
<b>Age (Yr)</b>				
Median	67.0	69.0	62.0	80.0
(Range)	(37–93)	(37–93)	(37–93)	(69–86)
<b>%JAK2V617F</b>				
Median	15.5	17.6	14.4	10.5
(Range)	(7.9–22.4)	(15.5–22.4)	(10.4–16.2)	(7.9–12.1)
<b>Blood Counts</b>				

Hematocrit (L/L)				
Median	47.2	55.2	44.4	33.7
(Range)	(25.1–70.0)	(48.2–70.0)	(35.5–51.8)	(25.1–38.8)
Hemoglobin (g/dL)				
Median	15.5	17.6	14.4	10.5
(Range)	(7.9–22.4)	(15.5–22.4)	(10.4–16.2)	(7.9–12.1)
Leukocytes ( $\times 10^9/L$ )				
Median	9.9	9.8	8.7	17.3
(Range)	(2.8–41.0)	(2.8–24.6)	(5.3–41.0)	(10.2–27.5)
Platelets ( $\times 10^9/L$ )				
Median	540	383	634	239
(Range)	(76–2,160)	(89–851)	(360–2,160)	(76–586)



**Figure S1.** Representation of the % of *JAK2V617F*-mutated alleles of the MPN patients examined in this study.

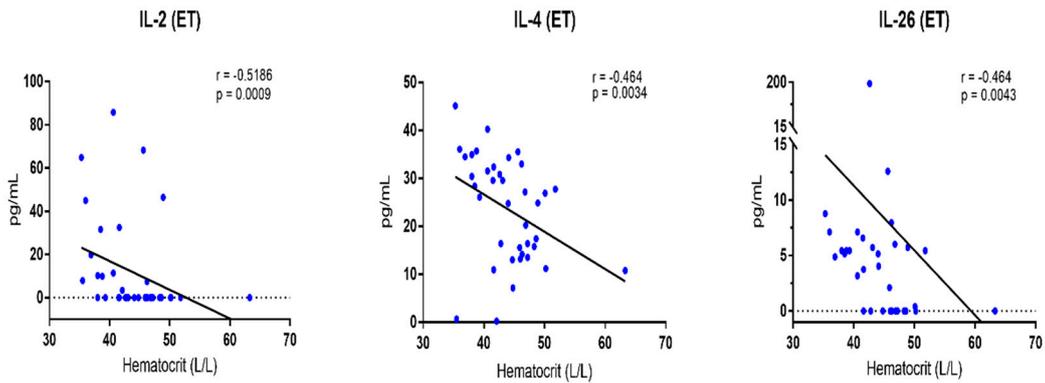


**Figure 2.** Levels of ST-2 (soluble IL-33 receptor) in MPN patients. ST-2 was measured in parallel in serum (a) and in plasma (b) of MPN patients and healthy donors (HD), as previously described (Levescot et al. 2014; Mager et al. 2018). Data are presented as means  $\pm$  SEM. (\*)  $p < 0.05$ , (\*\*)  $p < 0.01$ , Mann-Whitney  $t$ -test. Note the change in scale between (a) and (b).

## References

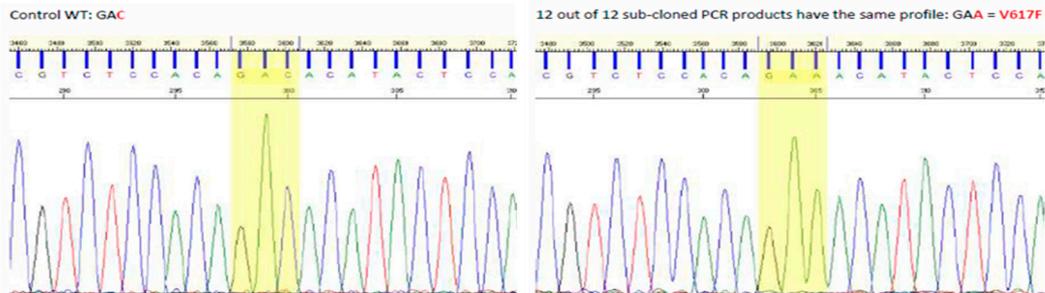
Levescot, A.; Flamant, S.; Basbous, S.; Jacomet, F.; Féraud, O.; Bourgeois, E.A.; Bonnet, M.-L.; Giraud, C.; Roy, L.; Barra, A.; Chomel, J.-C.; Turhan, A.; Guilhot, F.; Girard, J.-P.; Gombert, J.-M.; Herbelin, A. BCR-ABL-induced Deregulation of the IL-33/ST2 Pathway in CD34+ Progenitors From Chronic Myeloid Leukemia Patients. *Cancer Res* **2014**, *74*, 2669–2676.

Mager, B.L.F.; Riether, C.; Schurch, C.M.; et al. IL-33 signalling contributes to the pathogenesis of myeloproliferative neoplasms. *J Clin Invest* **2015**, *125*, 2579–2591.

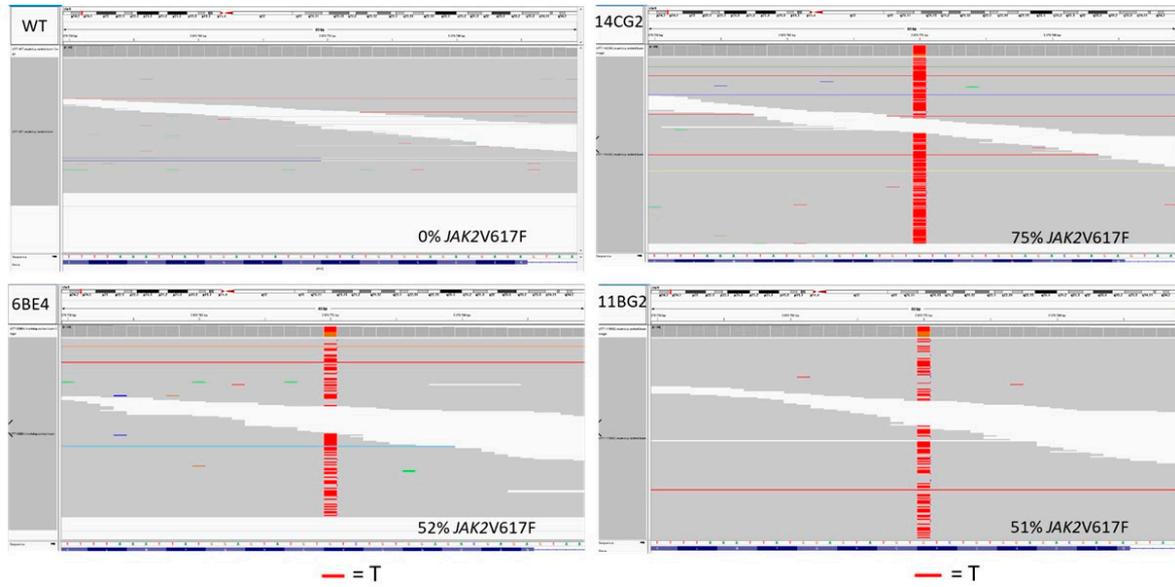


**Figure S3.** Inverse correlations between hematocrit and IL-2, IL-4 and IL-26 in ET patients. Analysis of the cytokine levels in serum and blood parameters in ET patients, using Spearman's  $t$ -test, revealed negative correlations between hematocrit and the levels of IL-2, IL-4, and IL-26.

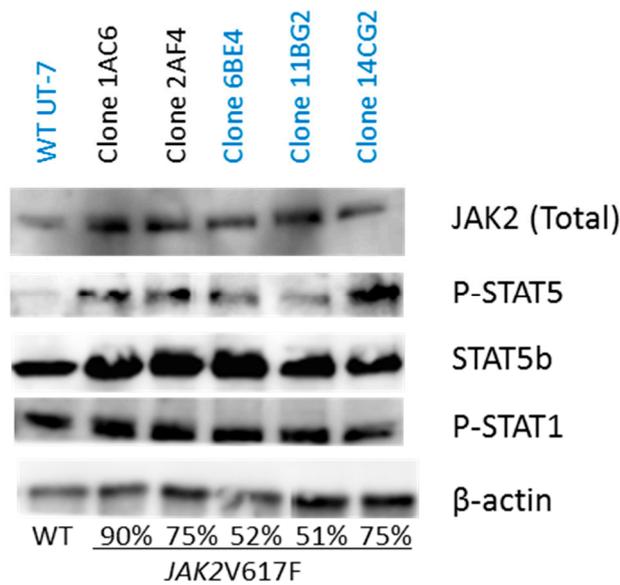
a



**b**



**c**



**Figure S4.** Description of UT-7 clones expressing 50% or 75% *JAK2V617F*-mutated alleles. (a) Sanger sequencing: Reverse sequencing of wild-type (WT) *JAK2* UT-7 cells (left panel) showed the expected GAC sequence of WT *JAK2* (sense sequence: GTC) whereas *JAK2V617F*-mutated UT-7 cells showed the GAA sequence corresponding to *JAK2V617F* (sense sequence: **TTC**) (right panel). (b) Whole genome sequencing: Results of *JAK2* sequencing obtained using the Integrative Genomics Viewer (IGV) (<http://software.broadinstitute.org/software/igv/>). Alleles with a sequence identical to the *JAK2* reference sequence (GTC) appear in grey (WT UT-7 cells), whereas red sequence reads correspond to a **T** mutation (TTC). The proportion of red reads is similar for UT-7 clones 6BE4 and 11BG2, and higher for clone 14CG2. The % of *JAK2V617F*-mutated alleles, quantified using allele specific qPCRs, were 52% for the 6BE4 UT-7 clone, 51% for the 11BG2 UT-7 clone, and 75% for the 14CG2 UT-7 clone. (c) *JAK2*/STAT5 pathway in UT-7 clones expressing 50% or 75% *JAK2V617F*-mutated alleles: WT *JAK2* UT-7 cells and the 3 UT-7 clones used in this study (shown in blue, expressing 51%, 52% or 75% *JAK2V617F*-mutated alleles) were grown in the presence of GM-CSF, then washed three times, numerated, seeded at  $10^5$  cells/ml in MEM with 10% FCS and a reduced dose of GM-CSF (1 ng/ml), and incubated at 37°C for 18 hrs. Cells were then collected, washed, and cell pellets were frozen at -80°C until use. Cell lysates were prepared and submitted to SDS-PAGE and transfer to PDVF





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