CHARACTERIZATION OF CD34+ HEMATOPOIETIC PROGENITOR CELLS IN *JAK2*V617F AND *CALR*-MUTATED MYELOPROLIFERATIVE NEOPLASMS

Running title: *JAK2*V617F and *CALR*-mutated stem cells in myeloproliferative neoplasms

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Abstract

Mutations in *JAK2* or *CALR* are observed in patients with myeloproliferative neoplasms (MPN). To get further insight in the dynamics of the mutant clone, we assessed the mutant allele burden in hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPCs) and granulocytes from 138 patients [51 polycythemia vera (PV), 58 essential thrombocythemia (ET) and 29 myelofibrosis (MF)]. *CALR*-mutated ET patients harbored a higher mutant load at progenitor level than *JAK2*V617F-positive ET (HSCs: 39.9% vs 7.5% p< 0.001, HPCs: 32.7% vs 7.7% p< 0.001). Moreover, HSCs of *CALR*-mutated ET patients showed a similar mutational load than patients with *CALR*-mutated MF (39.9% vs 48.2%, p=0.17). Regarding *JAK2*V617F MPN, PV and ET patients showed a low mutational burden at progenitor level whereas in the myelofibrotic phase the dominance of the mutated clone was a constant finding. In conclusion, the size of the mutated clone in chronic phase MPN is different according to genotype with *CALR*-mutated ET showing a pattern similar to that observed in MF.

Keywords: myeloproliferative neoplasms, *JAK2*V617F, *CALR*, allele burden, hematopoietic stem cells

Introduction

Polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) are myeloproliferative neoplasms (MPN) characterized by overproduction of one or more mature myeloid cell types. The origin of these neoplasms is in hematopoietic stem cells (HSCs) which display self-renewal capacity and ability to differentiate into more mature myeloid cells.

The *JAK2*V617F mutation is detectable in more than 95% of patients with PV and in 50-60% of those with ET or MF [1-4]. In the chronic phase of PV or ET, *JAK2*V617F is present in a low range at progenitor level with the percentage of CD34+ cells harboring *JAK2*V617F increasing at a low rate during the follow-up of the disease [5-9]. A constant finding in *JAK2*V617F-mutated PV and ET is the existence of a higher allele burden in granulocytes than in progenitors suggesting that mutated progenitors have a greater differentiation capacity than wild-type stem cells [5]. In contrast, in primary and secondary MF, *JAK2*V617F allele burden is very high in both granulocytes and progenitor cells indicating that the dominance of the *JAK2*V617F-positive clone at the CD34+ compartment is an important modifier of the disease phenotype in *JAK2*V617F-positive MPNs [10].

Recently, somatic mutations at exon 9 *CALR*, the gene encoding calreticulin, have been described in around 40-70% of *JAK2*V617F and *MPL* wild-type ET and MF patients [11, 12]. Many types of *CALR* mutations have been reported, but the most frequent alterations are the 52-bp deletion (mutation type 1) and the 5-bp insertion (mutation type 2). The mechanisms by

which *CALR* mutants causes MPN are still under investigation, but recent data show that they activate the thrombopoietin receptor and in turn JAK2/STAT pathway [13]. Interestingly, the mutant allele burden in granulocytes is higher in *CALR*-mutated ET than in *JAK2*V617F-mutated ET [14]. However, there is limited information regarding the size of the *CALR* mutant clone in the progenitor compartment.

The aim of this work was to study the mutant allele burden at the progenitor level in *CALR*-mutated MPN and compare it to the corresponding in *JAK2*V617F-positive progenitor cells.

Patients and methods

Patients

From a whole cohort of 395 *JAK2*V617F or *CALR*-mutated MPN, a total of 138 patients were included in the study. Overall, we studied 51 PV (all *JAK2*V617F positive), 58 ET (35 *JAK2*V617F and 23 *CALR*-mutated) and 29 MF (20 *JAK2*V617F and 9 *CALR*-mutated). Regarding *JAK2*V617F-mutated MF, 7 were primary MF, 11 post-PV MF and 2 post-ET MF. Additionally, 4 of *CALR*- mutated MF were primary MF and the others were post-ET MF. At the time of the study, 104 patients were in early chronic phase (< 5 years from diagnosis) and 34 patients in late chronic phase (more than 5 years from diagnosis). Patients were diagnosed according to World Health Organization criteria [15]. Patients with the pre-fibrotic form of MF were excluded. Informed consent for the scientific use of the patients' clinic hematological data and

biological samples was obtained, and the study was approved by the Hospital del Mar Ethics Committee.

Isolation of granulocytes and CD34+ cells

Granulocytes were isolated from peripheral blood or blood marrow samples by density gradient, whereas CD34+ cells were purified by immunomagnetic positive selection (Miltenyi Biotech, Bergisch Gladbach, Germany) from the mononuclear cell layer obtained after Ficoll centrifugation. Stem cells CD34+CD38- (HSCs) and progenitor cells CD34+CD38+ (HPCs) were further separated by fluorescence-activated cell sorting using a Becton Dickinson sorter (BD Biosciences, San Jose, CA, USA).

*JAK2*V617F and *CALR* mutation allele burden quantification

*JAK2*V617F allele burden was assessed in DNA from HSCs, HPCs and granulocytes by quantitative allele specific polymerase chain reaction [16].

The mutational analysis of exon 9 of the *CALR* gene was performed in the three populations by PCR, using a 6-carboxyfluorescein labelled reverse primer, followed by fragment analysis in a Genetic Analyzer 3500DX (Applied Biosystems).

Statistical methods

The mutant allele load in the different cell populations (HSCs, HPCs and granulocytes) was compared according to disease type (*JAK2*V617F PV, *JAK2*V617F ET, *CALR*-mutated ET, *JAK2*V617F MF and *CALR*-mutated MF).

Differences between groups were analyzed with t-Student test or one-way ANOVA followed by post-hoc analysis using the Student-Newman-Keuls test for continuous variables and the Chi-square test for categorical variables. Significance was considered for *p* values <0.05. The statistical analysis was performed using the SPSS 21.0 package (SPSS,Chicago, IL).

Results

Patients' characteristics

The main clinical and hematological data at the time of the study of the 138 MPN patients are summarized in Table 1. At the moment of CD34+ cell purification, patients with MF and those with *CALR*-mutated ET were more frequently on cytoreductive therapy than patients with PV or *JAK2*V617F-mutated ET. Type 1, type 2 and other types of *CALR* mutations were detected in 16 (ET n=8, MF n=8), 11 (ET n=11) and 5 (ET n=4, MF n=1) cases, respectively.

Mutant allele burden in HCSs, HPCs and granulocytes according to the disease

The quantification of *JAK2*V617F or *CALR* mutation allele burden in each population according to the disease is shown in Figure 1.

The mutant allele burden was significantly lower in *JAK2*V617F ET than in *JAK2*V617F PV in the three cell populations studied: HSCs (7.5% vs 16.7%, p =0.002) HPCs (7.7% vs 26.9%, p < 0.001) and granulocytes (21% vs 62.9%, p < 0.001) (Figure 2a).

As can be seen in Figure 2b, *CALR*-mutated ET patients harbored a higher mutant load at progenitor level than *JAK2*V617F-positive ET (HSCs: 39.9% vs 7.5% p< 0.001, HPCs: 32.7% vs 7.7% p< 0.001). These differences

were also observed in granulocytes (35.1% vs 21% p< 0.001). In addition**,** the comparison of *CALR*-mutated ET with *CALR*-mutated MF showed no statistically significant differences in the mutant allele burden of stem cells (39.8% vs 48.2%, p=0.17) and progenitors (32.7% vs 47.7%, p=0.09) (Figure 2c). Similar results were observed when patients with early or late chronic phase were separately considered.

Regarding *CALR*-mutated ET patients, no significant differences according to the type of the mutation were found neither at progenitor level nor in granulocytes (type 1 in comparison with type 2: HSCs 43.2% vs 36.7% p=0.54, HPCs 41.2% vs 25.9% p=0.16, granulocytes 35.6% vs 37.1% p =0.8) (Figure 2d).

Patients with MF showed the highest mutant load at the progenitor level in comparison with the remaining groups. Taking into consideration the mutational status of MF, *JAK2*V617F-MF patients showed a significantly higher mutant load in granulocytes than those with mutation in *CALR* gene (66.4% vs 51.4%, p =0.04), whereas no significant differences were observed at the progenitor level (Figure 2e).

The mutant allele burden in each population was compared between patients who were under cytoreductive treatment at the moment of CD34+ selection and those who were free of therapy. No significant differences were observed nor in PV, ET (*JAK2*V617F and *CALR*-mutated) neither *CALR*-mutated MF. However, in *JAK2*V617F MF, the mutant load was significantly higher in the three cell populations in patients who were receiving cytoreductive therapy in contrast to those without treatment (data not shown).

Dynamics of mutant allele burden during the evolution of the disease

We compared the mutant allele burden in each population according to the evolutive phase of the disease (early chronic phase or late chronic phase) (Table 2). As can be seen patients with shorter disease duration harbored a significantly lower allele burden in the three populations than patients with more follow-up. The increase in the mutant allele burden at progenitor level was higher in *CALR*-mutated than in *JAK2*V617F-mutated MPN (Table 2). We analyzed *CALR*-mutated ET patients according to type of the mutation, and in both cases the mutant allele burden increased in all populations during the follow-up (data not shown).

Discussion

Around 90% of MPN patients carry at least one somatic mutation, being *JAK2*V617F the most frequent (69%), followed by mutations in exon 9 of *CALR* gene (15%) [17]. In this study we assessed the mutant allele burden in HSCs, HPCs and granulocytes of 138 MPN patients. The main findings were a significantly higher mutant allele burden at progenitor level in *CALR*-mutated ET than in *JAK2*V617F ET being the *CALR* mutant allele load in ET similar to the one observed in patients with MF.

It has been proposed that *JAK2*V617F-mutated ET and PV represent a biological spectrum of the same disease modulated by several acquired and constitutional factors [18]. *JAK2*V617F-homozygous erythroid progenitors have

been reported to occur in most patients with PV but rarely in those with ET [19]. Moreover, Godfrey *et al*, using microsatellite PCR to map loss-of-heterozygosity in colony assays, have observed that PV is associated with expansion of a dominant homozygous clone [20]. Furthermore, other factors have been involved in the acquisition of a particular phenotype such as the polymorphism HBS1L-MYB (rs9376092) [21], a defect in STAT1 phosphorylation [22] and the order of acquisition of additional mutations [23]. In our study we observed that the mutant load at the progenitor level in *JAK2*V617F mutated patients increases in accordance to the phenotype, being higher in *JAK2*V617F PV than in *JAK2*V617F ET. This finding is in accordance with more frequent homozygous cells observed in colonies derived from PV patients [20]. When *JAK2*V617F MF patients were analyzed, the mutant load was higher than 50% in the majority of patients indicating that *JAK2*V617F clonal dominance is a constant finding in these patients, as previously reported [7, 10]. All this data support the idea that *JAK2*V617F ET and *JAK2*V617F PV represent a continuum of the same disease and MF transformation is a late event during the follow-up.

Recently, distinct phenotype and clinical characteristics have been described between *JAK2*V617F and *CALR*-mutated ET. Thus, mutation in *CALR* gene is associated with younger age, lower level of hemoglobin and leukocytes, and higher platelet count, and interestingly, a lower risk of thrombosis in comparison with *JAK2*-mutated patients [14, 24]. In addition to this different clinical profile, several studies argue that *JAK2*V617F and *CALR* mutations define two distinct diseases at the molecular level. In this sense, X chromosome inactivation patterns (XCIP) analyses showed clonality in 88% of

CALR-mutated ET patients in comparison with 26% of *JAK2*V617F ET patients [25]. Moreover, some studies suggest that mutation acquisition is a late event in *JAK2*V617F-positive MPN whereas C*ALR* mutations are typically initiating events [11, 12, 17].

Moreover, it is important to note that *CALR*-mutated ET patients have a higher mutant load at the progenitor level than *JAK2*V617F-positive ET and *JAK2*V617F PV patients, conferring a higher potential to proliferate during the chronic phase of MPN. Interestingly, when we compared *CALR*-mutated ET and *CALR*-mutated MF, no significant differences in the mutant allele burden at the progenitor level were observed. The existence of an expanded mutated clone at progenitor level in *CALR*-positive ET might indicate that MF and ET are a continuum of the same disease. In this sense, it has been reported a higher frequency of MF transformation in patients with *CALR*-mutated ET than in those with *JAK2*V617F [12, 26]. In addition, *CALR*-mutated ET showed at diagnosis some MF-like clinical features such as lower hemoglobin [14, 24, 26-28], or higher LDH serum level [28] than *JAK2*V617F ET. Finally, bone marrow histology is different according to genotype with *CALR*-positive ET being more frequently associated with megakaryocyte abnormalities and prefibrotic PMF [29]. In this sense, it would be interesting to study the allele burden at the stem cell level in patients with pre-fibrotic form of MF according to genotype. It has been reported that ET patients with type 1 *CALR* mutation showed a higher risk of MF transformation compared with type 2 or other mutations [30]. We could not demonstrate differences in the mutant allele burden of progenitor cells according to the type of *CALR* mutation in ET. However, this finding must be interpreted cautiously due to the limited number of cases.

Regarding MF, we could observe the presence of clonal dominance in the majority of *JAK2*V617F-positive cases as previously described [7, 10], and the same was observed in *CALR*-mutated MF. However, while *JAK2*V617F allele burden showed a slight rise between HSCs and granulocytes, the mutant allele burden in those patients harboring *CALR*-was similar in the three populations, suggesting higher differentiation potential of *JAK2*V617F.

We observed that *JAK2*V617F allele burden increased steadily during the hematopoiesis process in PV and, in a lower range, also in ET. However, when we analyzed *CALR*-mutated ET, the mutant load remained stable in HSCs, HPCs and granulocytes. These results support the fact that *JAK2*V617F confers a higher differentiation potential than *CALR* mutation. In this regard, it has been reported a higher frequency of monoclonal hematopoiesis and suppression of wild-type myeloid cells at the hematopoietic stage in the *CALR*-mutated ET but not in *JAK2*V617F ET [25].

Finally, our work provides data concerning the changes of the mutant allele burden during the evolution of the disease. Thus, we have shown that the mutant allele burden increases significantly in the three populations both in *JAK2*V617F and *CALR*-mutated MPN. Interestingly, in *CALR*-mutated MPN, the clonal dominance remained stable during the evolution of the disease, suggesting a continuous proliferation of the mutated clones.

One of the limitations of this study is the scarce number of patients analyzed. Another important limitation is that we used only CD38 and CD34 staining to define HSCs and HPCs in contrast to other researchers that have added CD90 and CD45RA to improve the discrimination between HSCs and HPCs [31]. However, taking into consideration that CD38 positivity showed a

continuous gradient, the double CD38intermediate+/CD34+ population was excluded in order to be sure that HSCs and HPCs were CD38- and CD38+ positive, respectively. Finally, we assumed the mutated clone is embedded in an environment of non-mutated cells that maintain a normal proliferative and differentiate capacity. However, the cross-talks of malignant cells with the microenvironment and residual normal haematopoiesis may be different according to genotype.

In conclusion, the size of the mutated clone at the stem cell level is different according to the genotype of MPN. *CALR*-mutated MPN are characterized by an expansion of mutated progenitors with suppression of wild type mature cells whereas *JAK2*V617F-mutated PV and ET have a low proportion of mutated progenitors with an enhanced ability of differentiation.

Authorship

A.Ang. designed the study, collected the data, performed the molecular studies, performed the statistical analysis, analyzed and interpreted the results and wrote the paper. A.A.L. designed the study, collected the data, performed the statistical analysis, analyzed and interpreted the results and wrote the paper. B.B. and C.B. designed the study, interpreted the results, wrote the paper and approved the final version. R.L., L.C., S.P. and C.F.R. performed the molecular studies and approved the final version.

No relevant conflict of interest to declare regarding this article.

References

[1] Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton 2, *et al*.; Cancer Genome Project. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054-1061.

[2] James C, Ugo V, Le Couédic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005; 434: 1144-1148.

[3] Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, *et al*. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005; 352: 1779-1790.

[4] Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, *et al*. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005; 7: 387-397

[5] Jamieson CH, Gotlib J, Durocher JA, Chao MP, Maiappan MR, Lay M, *et al*. The JAK2 V617F mutation occurs in hematopoietic stem cells in polycythemia vera and predisoposes toward erythroid differentiation. Proc Natl Acad Sci U S A 2006; 103: 6224-9.

[6] Dupont S, Massé A, James C, Teyssandier I, Lécluse Y, Larbret F, *et al*. The JAK2 617V>F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. Blood 2007; 110:1013-21.

[7] Moliterno AR, Williams DM, Rogers O, Isaacs MA, Spivak JL. Phenotypic variability within the JAK2 V617F-positive MPD: roles of progenitor cell and neutrophil allele burdens. Exp Hematol. 2008;36:1480-6.

[8] Anand S. Stedham F, Beer P, Gudgin E, Ortmann CA, Bench A, *et al*. Effects of the JAK2 mutation on the hematopoietic stem and progenitor compartment in human myeloproliferative neoplasms. Blood 2011; 118:177-81.

[9] Angona A, Alvarez-Larran A, Bellosillo B, Martínez-Avilés L, Camacho L, Fernández-Rodríguez C, *et al*. Hematopoietic clonal dominance, stem cell mutations, and evoutionary pattern of JAK2V617F allele burden in polycythemia vera. Eur J Haematol 2015; 95: 251-257.

[10] Stein BL, Williams DM, Rogers O, Isaacs MA, Spivak JL, Moliterno AR. Disease burden at the progenitor level is a feature of primary myelofibrosis: a multivariable analysis of 164 JAK2 V617F-positive myeloproliferative neoplasm patients. Exp Hematol. 2011; 39:95-101.

[11] Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD *et al*. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med 2013; 369: 2379-90

[12] Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, *et al*. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med 2013; 369: 2391-405

[13] Chachoua I, Pecquet C, El-Khoury M, Nivarthi H, Albu RI, Marty C, *et al*. Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. Blood 2015 [Epub ahead of print]

[14] Rumi E, Pietra D, Ferretti V, Klampfl T, Harutyunyan AS, Milosevic JD, *et al*. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood 2014; 123:1544-51.

[15] Tefferi A, Thiele J, Orazi A, Kvasnicka HM, Barbui T, Hanson CA, *et al*. Proposals and rationale for revision of the world health organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary

myelofibrosis: Recommendations from an ad hoc international expert panel. Blood 2007; 110: 1092-7.

[16] Larsen TS, Christensen JH, Hasselbalch HC, Pallisgaard N. The JAK2 V617F mutation involves B- and T-lymphocyte lineages in a subgroup of patients with Philadelphia-chromosome negative chronic myeloproliferative disorders. Br J Haematol 2007; 136: 745-51.

[17] Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen Hui, Nissen I, *et al*. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood 2014; 123: 2220-8.

[18] Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, *et al*. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet 2005; 366: 1945-53.

[19] Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. Blood 2006, 108:2435-7.

[20] Godfrey AL, Chen E, Pagano F, Ortmann CA, Silber Y, Bellosillo B, *et al*. JAK2V617F homozygosity arises commonly and recurrently in PV and ET, but PV is characterized by expansion of a dominant homozygous subclone. Blood 2012, 120:2704-7.

[21] Tapper W, Jones AV, Kralovics R, Harutyunyan AS, Godfrey, AL, Guglielmelli P, *et al.* Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. *Natu Commun* 2015; 6:6691.

[22] Chen E, Beer PA, Godfrey AL, Ortmann CA, Li J, Costa-Pereira AP, e*t al*. Distinct clinical phenotypes associated with JAK2V617F reflect differential STAT1 signaling. Cancer Cell 2010; 18:524-35

[23] Ortmann C, Kent D, Nangalia J, Silber Y, Wedge DC, Grinfeld J, *et al*. Effect of mutation order on myeloproliferative neoplasms. N Engl J Med 2015, 372, 601-612.

[24] Rotunno G, Mannarelli C, Guglielmelli P, Pacilli A, Pancrazzi A, Pieri L, *et al*. Impact of Calreticulin Mutations on Clinical and Hematological Phenotype and Outcome in Essential Thrombocythemia. Blood 2014; 123:552-5.

[25] Allen C, Lambert JR, Linch DC, Gale RE. X chromosome inactivation analysis reveals a difference in the biology of ET patients with JAK2 and CALR mutations. Blood 2014*,* 124:2091-3.

[26] Al Assaf C, Van Obbergh F, Billiet J, Lierman E, Devos T, Graux C, *et al.* Analysis of phenotype and outcome in essential thrombocythemia with CALR or JAK2 mutations. Haematologica *2015;* 100:893-7.

[27] Gangat N, Wassie EA, Lasho TL, Finke C, Ketterling RP, Hanson CA, *et al*. Mutations and thrombosis in essential thrombocythemia: prognostic interaction with age and thrombosis history. Eur J Haematol 2015; 94:31-6.

[28] Palandri F, Latagliata R, Polverelli N, Tieghi A, Crugnola M, Martino B, *et al*. Mutations and long-term outcome of 217 young patients with essential thrombocythemia or early primary myelofibrosis. Leukemia *2015;* 29: 1344-9.

[29] Alvarez-Larrán A, Ancochea A, García M, Climent F, García-Pallarols F, Angona A, *et al*. WHO-histologic criteria for myeloproliferative neoplasms: reproducibility, diagnostic accuracy and correlation with gene mutations and clinical outcomes. Br J Haematol 2014; 166 (6): 911-9.

[30] Pietra D, Rumi E, Ferretti VV, Di Buduo CA, Milanesi C, Cavalloni C, *et al*. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia 2016; 30:431-8.

[31] Majet R, Park C, Weissman I. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell 2007; 1: 635-645.

Table 1. Main clinical and hematological characteristics at the time of CD34+ cells purification in 138 MPN patients included in the study						
	PV N = 51	**TE *JAK2*V617F** N = 35	**TE *CALR*- mut** N = 23	**MF *JAK2*V617F** N = 20	**MF *CALR*-mut** N = 9	***p***
Time from diagnosis, months[1]	1 (0-271)	0 (0-179)	51 (0-269)	7 (0-131)	8 (0-223)	< 0.001
Early/late chronic phase, n	36/15	34/1	12/11	16/4	6/3	0.002
Age, years[1]	68 (41-95)	65 (31-85)	66 (32-93)	72 (51-85)	72 (35-90)	0.56
Male/female, n	35/16	13/22	9/14	8/12	4/5	0.02
Hemoglobin, g/L[1]	161 (106-222)	147 (124-169)	125 (95-153)	109 (91-146)	110 (66-140)	< 0.001
Leukocytes, $x10^9/L^1$	11.7 (5-21.1)	8.6 (5.8-16.4)	7.4 (3.4-10.9)	9.9 (3.4-48.4)	9.3 (2.8-51.9)	0.001
Platelets, $x10^9/L^1$	558 (160-1138)	617 (450-876)	623 (294-1149)	192 (44-770)	443 (43-645)	< 0.001
Cytoreductive treatment, n (%)	16 (31.4)	2 (5.7)	10 (43.5)	13 (65)	6 (66.7)	< 0.001
Hydroxyurea	13	1	7[#]	10[##]	2	
Ruxolitinib	1	-	-	1	-	
Others	2	1	3	2	4	

Table 2. Mutant allele burden in HSCs, HPCs and granulocytes according to the evolutive phase of the disease			
*JAK2*V617F MPN			
	Early chronic phase n=86	**Late chronic phase** n=20	*p*
HSCs	18.4 (23.6)	32.9 (25.9)	0.017
HPCs	22.7 (24.2)	39.7 (27.04)	0.007
Granulocytes	46.2 (29.7)	65.1 (26.7)	0.011
CALR-mutated MPN			
	Early chronic phase n=18	**Late chronic phase** n=14	*p*
HSCs	35.4 (17.8)	55.4 (10.9)	0.004
HPCs	28.3 (20.4)	54.9 (9.5)	0.001
Granulocytes	33.7 (14.2)	46.02 (12.8)	0.02

Fig 1

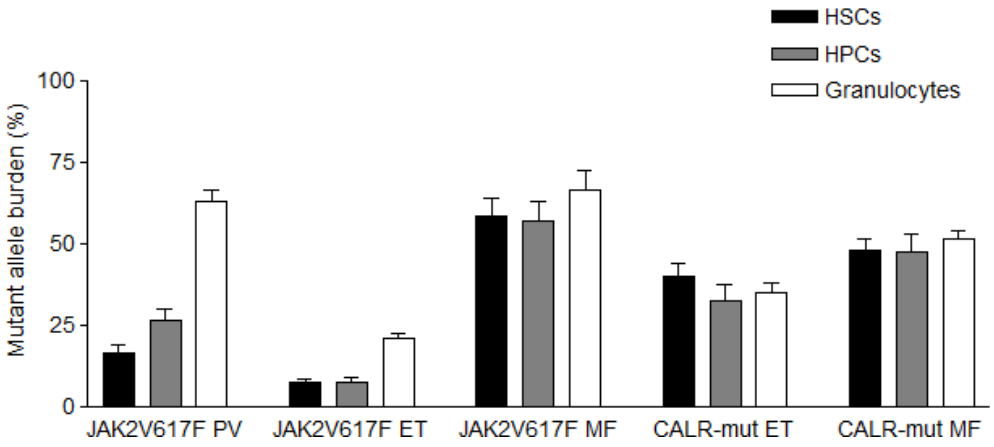

Fig 2a

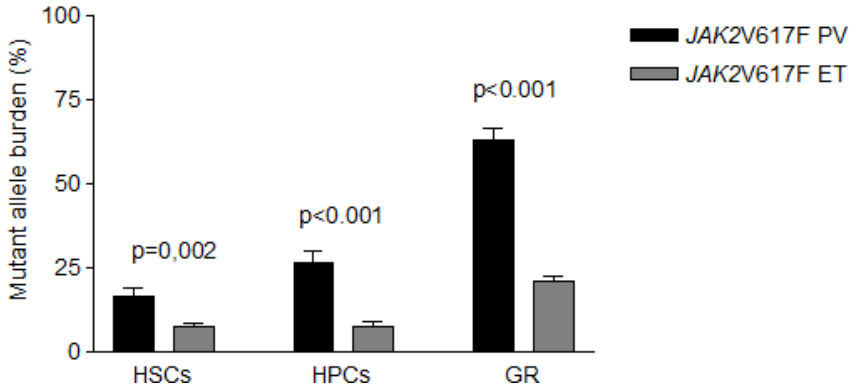

Fig 2b

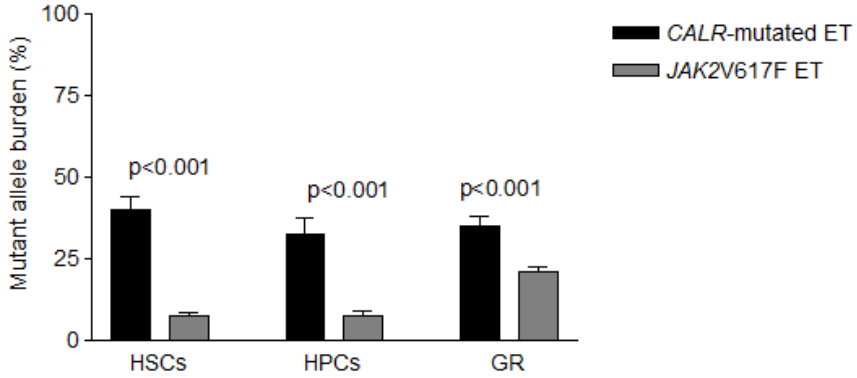

Fig 2c

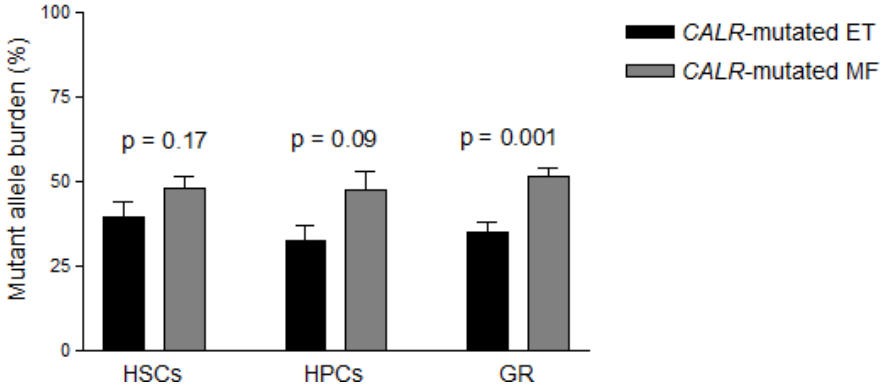

Fig 2d

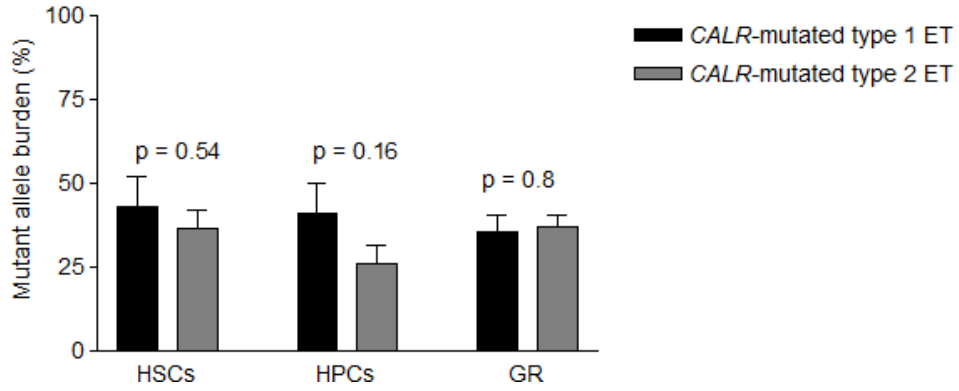

Fig 2e

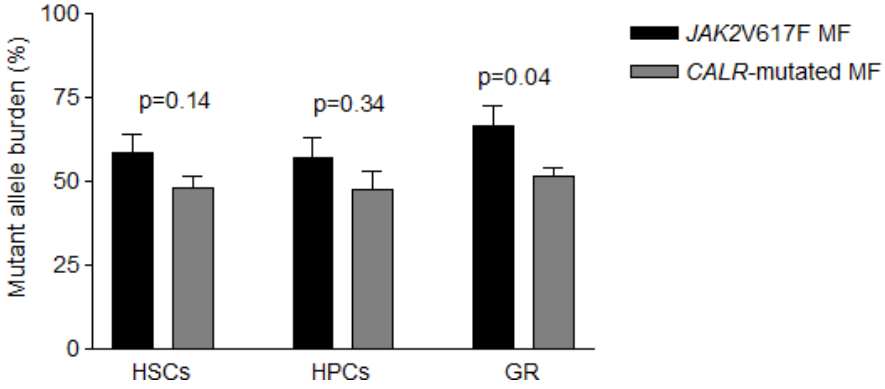

Table legends

Table 1. Main clinical and hematological characteristics at the time of CD34+ cells purification in 138 MPN patients included in the study

MPN: myeloproliferative neoplasms; PV: polycythemia vera; TE: essential thrombocythemia. [1] Median (range). ns: no significance. Cytoreductive treatment included hydroxiurea, busulphan, P32, anagrelide and ruxolitinib. p value compared to one way ANOVA or Chi-square test for continuous or quality variables, respectively. [#]In two patients, Hydroxyurea + Anagrelide; [##]In one patient, Hydroxyurea + Anagrelide

Table 2. Mutant allele burden in HSCs, HPCs and granulocytes according to the evolutive phase of the disease

HSCs: hematopoietic stem cells; HPCs: hematopoietic progenitor cells. Results are expressed as mean (SD). Differences between groups are analyzed with t-Student test. Overall 31/106 *JAK2*V617F MPN and 16/32 *CALR*-mutated MPN (p=0.03) were under cytoreductive treatment (hydroxiurea, busulphan, P32, anagrelide and ruxolitinib)

Footnotes

Figure 1. Mutant allele burden in haematopoietic stem cells (HSCs), haematopoietic progenitor cells (HPCs) and granulocytes according to the disease.

PV: polycythaemia vera; ET: essential thrombocythemia; MF: myelofibrosis.

Figure 2. Comparison of the mutant allele burden in hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPCs) and granulocytes according to the disease. Differences between groups are analyzed with t-Student test. Significance was considered for *p* values <0.05.

a) *JAK2*V617F PV in comparison with *JAK2*V617F ET
b) *CALR*-mutated ET in comparison with *JAK2*V617F ET
c) *CALR*-mutated ET in comparison with *CALR*-mutated MF
d) *CALR*-mutated ET type 1 in comparison with *CALR*-mutated type 2
e) *JAK2*V617F MF in comparison with *CALR*-mutated MF

PV: polycythemia vera; ET: essential thrombocythemia; MF: myelofibrosis