	Total MF (61 cases)	JAK2$^{(V617F)}$ (38 cases)	CALR (11 cases)	MPL (6 cases)	TN (6 cases)
Median age, years (range)	70 (40-84)	65,5 (40-82)	72,5 (61-84)	65,5 (56-76)	77 (60-84)
Males, no. (%)	30 (49%)	19 (50%)	7 (64%)	1 (17%)	3 (50%)
Median Hemoglobin, g/dl; median (range)	11,5 (7,1-15,1)	12,4 (7,1-15,1)	10,0 (7,7-4,9)	9,1 (7,2-10,8)	9,7 (8,1-14,3)
Median Leukocytes, x 10^9/l; median (range)	9,4 (2,2-80,1)	9,4 (2,2-80,1)	7,5 (4,3-20,1)	15,9 (6,2-39,5)	10,8 (2,5-7)
Median Platelets, x 10^9/l; median (range)	229 (38-845)	229 (65-631)	217 (90-845)	280 (46-632)	121 (38-613)
Median Lymphocyte x 10^9/l; median (range)	1,7 (0,4-16,3)	1,4 (0,4-15,8)	1,85 (1,1-6)	2,4 (0,8 -16,3)	4,1 (0,4 -11,7)
Median Monocyte x 10^9/l; median (range)	0,6 (0,1-5,9)	0,6 (0,1-5,5)	0,6 (0,3-4,4)	1,3 (0,4-5,9)	0,7 (0,2-5,3)
BM fibrosis, no. of patients (%)					
Grade 1	30 (49%)	19 (50%)	5 (46%)	3 (50%)	3 (50%)
Grade 2	23 (38%)	14 (37%)	4 (36%)	2 (33%)	3 (50%)
Grade 3	8 (13%)	5 (13%)	2(18%)	1 (17%)	0
IPSS, Number of patients					
Low	4 (7%)	4 (10%)	0	0	0
Intermediate-1	20 (33%)	12 (32%)	5 (45,5%)	0	3 (50 %)
Intermediate-2	16 (26%)	11 (29%)	1 (9%)	3 (50%)	1 (17 %)
High	21 (34%)	11 (29%)	5 (45,5%)	3 (50%)	2 (33 %)
Previous treatment, no of patients (%)					
Hydroxyurea	30 (49%)	19 (50%)	3 (27%)	4 (67%)	4 (67%)
WHO Diagnosis					
PMF	39 (64%)	22 (58%)	7 (64%)	5 (83%)	5 (83%)
PPV-MF	14 (23%)	14 (37%)	0	0	0
PET-MF	8 (13%)	2 (5%)	4 (36%)	1 (17%)	1 (17%)

Supplementary Table 1. Clinical and laboratory features of MF patients according to mutational status *PMF: Primary Myelofibrosis; PPV-MF: Post Polycythemia Vera-Myelofibrosis; PET-MF: Post Essential Thrombocythemia-Myelofibrosis. The presence of 0, 1, 2 or 3 and >4 adverse factors defines low, intermediate-1, intermediate-2 and high-risk disease. IPSS: International Prognostic Scoring System. Comparisons of variables between groups of patients were carried out by Kruskal-Wallis test and by chi-square or Fisher exact test, as appropriate. No significant differences were observed among groups, except for Hb (JAK2$^{(V617F)}$ vs MPL, $p<0.05$) and PET-MF (JAK2$^{(V617F)}$ vs CALR, $p<0.05$).*

	Total ET (20 cases)	JAK2$^{(V617F)}$ (13 cases)	CALR (5 cases)	TN (2 cases)
Median age, years (range)	65,5 (61-79)	66 (61-72)	65 (63-79)	69 (62-76)
Males, no. (%)	8(40%)	4 (30,8%)	3 (60%)	1 (50%)
Median Hemoglobin, g/dl; median (range)	13,7 (10-16,2)	13,7 (10-15,2)	13,5 (10,9-16,2)	12,6 (10,5-14,8)
Median Leukocytes, x 10^9/l; median (range)	7 (4,7-15,45)	8,7 (7-15,45)	6,5 (5,7-7,3)	5,3 (4,7-5,8)
Median Platelets, x 10^9/l; median (range)	647 (542-1069)	655 (542-1069)	639 (631-939)	665 (617-712)
Previous treatment, no of patients (%)				
Hydroxyurea	8 (40%)	4 (31%)	4 (80%)	0

Supplementary Table 2. Clinical and laboratory features of ET patients according to mutational status *No significant differences were observed among groups. Comparisons of variables between groups of patients were carried out by Kruskal-Wallis test and by chi-square or Fisher exact test, as appropriate.*

MVs type	Identified as	List of monoclonal antibodies
Megakaryocyte-MVs	CD61+/CD62P-	Anti-CD61 (Clone: SZ21; FITC-conjugated; Catalog number IM1758); Anti- CD62P (Clone: CLB-THROMB/6; PE-coniugated; Catalog number IM1759U). All antibodies from Beckman Coulter S.r.l.
Platelet-MVs	CD61+/CD62P+	Anti-CD61 (Clone: SZ21; FITC-conjugated; Catalog number IM1758); Anti- CD62P (Clone: CLB-THROMB/6; PE-coniugated; Catalog number IM1759U). All antibodies from Beckman Coulter S.r.l.
Monocyte-MVs	CD14+	Anti-CD14 (Clone: RM052; FITC-conjugated; Catalog number B36297) from Beckman Coulter S.r.l.;
Endothelial-MVs	CD144+/CD105+	Anti-CD144 (Clone: REA199; FITC-conjugated; Catalog number 130-100-742) from Miltenyi Biotec; Anti- CD105 (Clone; TEA3/17.1.1; PE-coniugated; Catalog number B92442) from Beckman Coulter S.r.l.

Supplementary Table 3. List of monoclonal antibodies according to MVs subtype

Cytokines	HD (cases 20)	Total MF (cases 61)	*P*-value
IL-1β (pg/mL)	0,2 (0-4,5)	1,5 (0,07-8,5)	<0.01
IL-6 (pg/mL)	5,4 (4,5-32,8)	24,8 (1,2-259)	<0.001
IFN-γ (pg/mL)	0,2 (0,02-0,8)	1,3 (0-6,4)	<0.0001
TNF-α (pg/mL)	0,4 (0-13,3)	6,8 (0,05-39,2)	<0.0001
TPO (pg/mL)	22,2 (11,2-88)	124,4 (10,4-539,7)	<0.01
sP-selectin (ng/mL)	**34,81 (26,9-43,1)**	**99,81 (47,6-205,9)**	**<0.0001**

Supplementary Table 4. Plasma levels of crucial pro-inflammatory cytokines, thrombopoietin and soluble P-selectin were increased in MF patients. *Interleukin (IL)1β, IL6, Interferon (IFN)-γ, Tumor Necrosis Factor (TNF)-α, Thrombopoietin (TPO),* ***soluble (s)P-selectin*** *plasma levels of HD (n=20), total MF patients (n=61) measured by ELISA. Results are expressed as median and range (Mann-Whitney test).*

Supplementary Material and Methods

Casistic

This is a pilot study where patients were enrolled from May 2015 to May 2018. Sixty-one MF and 20 ET patients were included into the study. Patients were at diagnosis (n=31 MF; n=12 ET) or at least 3 months after cytoreductive therapy (n=30 MF; n=8 ET) **(Supplementary Table 1, 2). Previous therapy did not alter the level of megakaryocyte- and platelet-MVs in MF/ET patients.** Twenty-seven MF patients (24 *JAK2*$^{(V617F)}$- and 3 *CALR*-mutated) at intermediate-1 (n=7), intermediate-2 (n=12) or high (n=8) International Prognostic Scoring System (IPSS) risk (O'Sullivan *et al*, 2018) were studied before and after 6 months of ruxolitinib therapy. Spleen response in ruxolitinib-treated patients was evaluated according to the 2013 International Working Group-MPN Research and Treatment criteria (Tefferi *et al*, 2013). Twenty age/sex-matched healthy donors (HD) were also included.

Blood collection and Platelet Poor Plasma (PPP) preparation

EDTA-anticoagulated PB was collected from patients and HD. The first 2 ml of blood were discarded. PPP was obtained (within 2 hours from blood collection) after two consecutive centrifugations at 2500 g for 15 minutes at room temperature. PPP was then aliquoted and stored at -80°C until testing. The study was approved by the local

Ethics Committee and was conducted accordingly to the Helsinki declaration (Informed consent was obtained from all subjects).

Flow cytometry MVs identification

Megakaryocyte-, platelet-, monocyte- and endothelial-MVs were analyzed in Platelet Poor Plasma (PPP; after thawing at 37°C) by flow cytometry (Cytoflex, Beckman Coulter, Milan Italy) ***(Supplementary Fig 1 and Supplementary Table 3***). The Violet Side Scatter laser (VSSC) is used as a trigger signal to discriminate the noise. To detect MVs the instrument was calibrated with MegaMix Beads (Stagò, Marseille, France). MVs identification was based on size (500-900 nm) and on the ability to bind lineage-specific monoclonal antibodies (**Supplementary Fig 1 and Supplementary Table 3**). Matched isotype controls were used to select the cut-off. Results are expressed as percentage of total MVs.

ELISA assay

Plasma crucial pro-inflammatory cytokines (*Interleukin (IL)1β, IL6, Interferon (IFN)-γ, Tumor Necrosis Factor (TNF)-α), Thrombopoietin (TPO) and* ***soluble (s)P-selectin*** of MF patients and HD were analyzed by ELISA (R&D Systems, Milan, Italy).

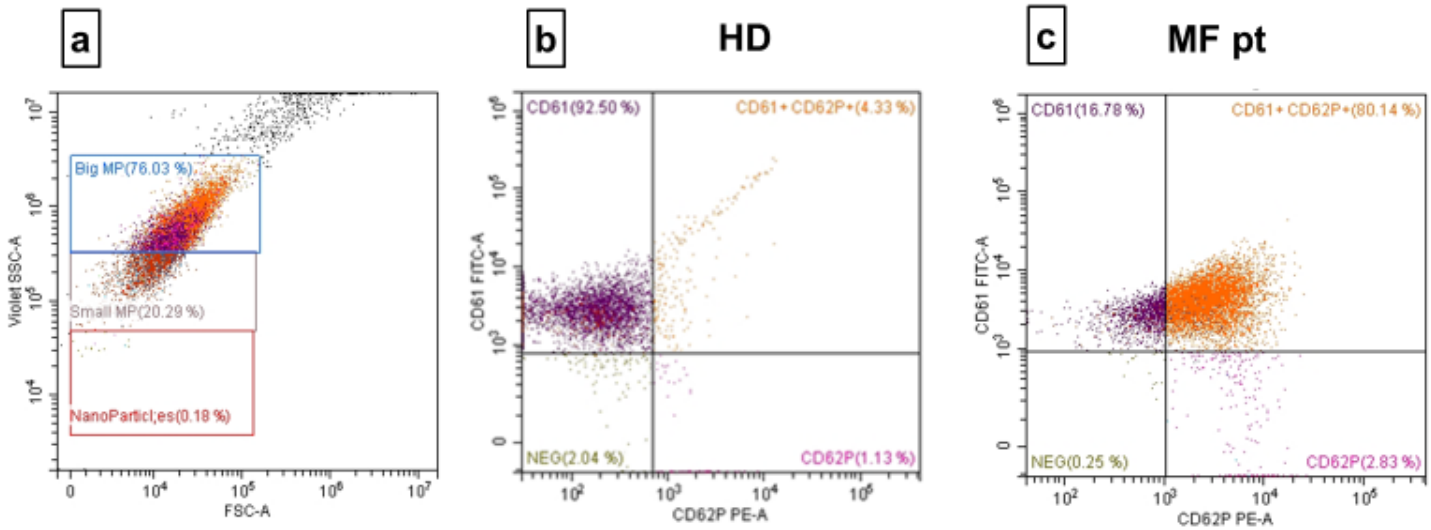
Genotype

Molecular genotyping was performed as previously described (Romano *et al*, 2017).

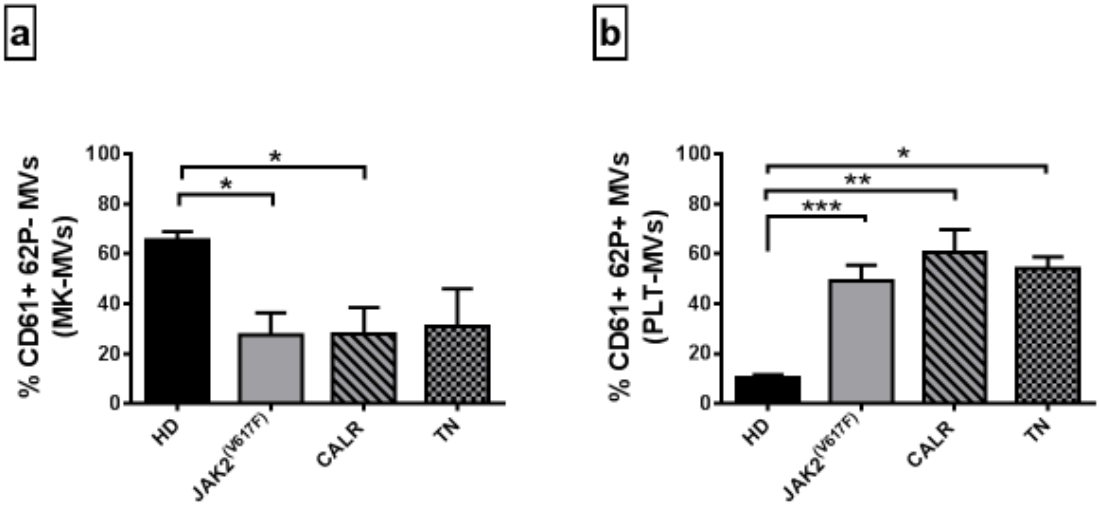
Statistical analysis

Statistical analysis was performed with GraphPad Prism 6 using Kruskal-Wallis test, chi-square or Fisher exact test, as appropriate and the Spearman's correlation test. ROC analysis was performed with STATA Software 15. P values <0.05 were considered significant.

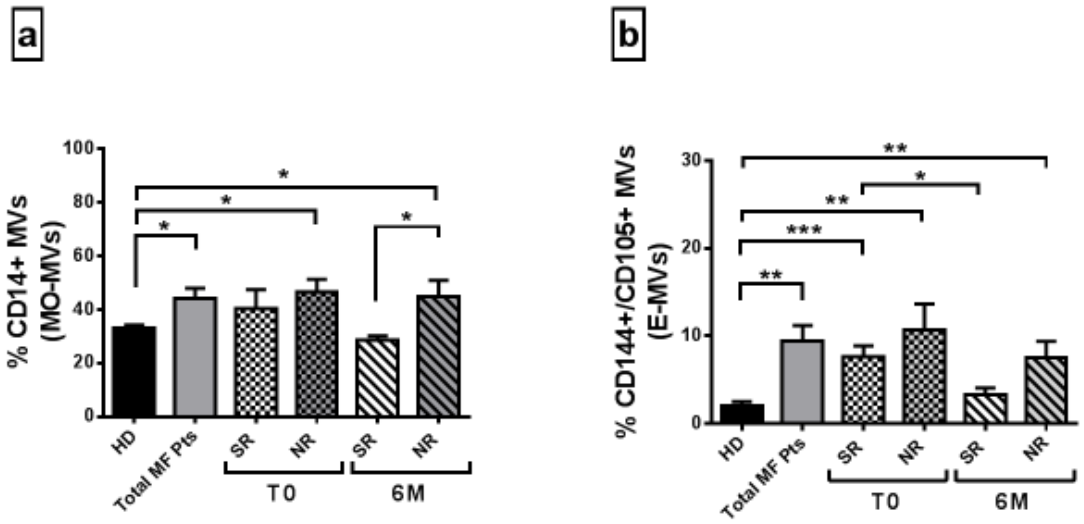
Supplementary Fig 1



Supplementary Fig 2



Supplementary Fig 3



Legend to Supplementary Figures:

Supplementary Fig 1. Gating strategy of circulating megakaryocyte- and platelet-MVs of 1 HD and 1 MF patient. (a) Fluorescence gated polystyrene beads of different sizes were used to determine the gates identifying big (500-900 nm), small (200-300 nm) and nano (100-160 nm) MVs. Gating strategy to identify big MVs (500-900 nm) is shown. **(b)** and **(c)** show representative dot-plots of megakaryocyte- and platelet-MVs in plasma samples from 1 HD and 1 MF patient. Using the defined gate for big MVs, all events positive for surface markers staining (CD61+CD62P- megakaryocyte-MVs and CD61+CD62P+ platelet-MVs) were recorded.

Supplementary Fig 2. **Circulating megakaryocyte- and platelet-MVs frequency in ET according to mutation status.** Megakaryocyte-MVs (MK-MVs; CD61+CD62P-) **(a)** and platelet-MVs (PLT-MVs; CD61+CD62P+) **(b)** frequency of ET patients (n=20) according to mutation status and HD (n=20) is shown. Results are reported as mean ± SEM. (Kruskal-Wallis test; *p<0.05; **p<0.01; ***p<0.001)

Supplementary Fig 3. **Circulating monocyte- and endothelial-MVs frequency of MF patients according to ruxolitinib therapy response.** Monocyte-MVs (MO-MVs; CD14+) and endothelial-MVs (E-MVs; CD144+/CD105+) frequency of HD (n=20), spleen responders (SR; n=12) and non-responder (NR; n=15) MF patients before (T0) and after 6 months ruxolitinib therapy (6M) are shown in **(a)** and **(b)**, respectively. Results are reported as mean ± SEM. (Kruskal-Wallis test*;* *p<0.05; **p<0.01; ***p<0.001)