Histological and genetic characterization and follow-up of 130 patients with chronic triple-negative thrombocytosis

Sandrine Lemoine,[1-4] Clelia Mornet,[4,5] Isabelle Quintin-Roue,[6] Marie-Christine Rousselet,[7] Laurane Cottin,[1-4] Aurélie Georgeais,[5] Ludovic Dubouis,[8] Françoise Boyer,[3,4,9] Corentin Orvain,[2-4,9] Clara Caillon,[3,9] Maxime Renard,[1,3] Valoris Le Brun,[3,5] Lenaig Le Clech,[10] Jean-Christophe Ianotto,[3,4,11,12] Emmanuelle Génin,[13] Barbara Burroni,[4,14,15] Valérie Ugo,[1-4] Damien Luque Paz[1-4#] and Eric Lippert[3-5,13#]

[1]Laboratoire d'Hématologie, CHU Angers, Angers; [2]Université d'Angers, Nantes Université, CHU Angers, Inserm, CNRS, CRCI2NA, Angers; [3]Fédération Hospitalo-Universitaire "Grand Ouest Against Leukemia" (FHU GOAL), Angeres; [4]France Intergroupe des Syndromes Myéloprolifératifs (FIM), Paris; [5]Laboratoire d'Hématologie, CHRU Brest, Brest; [6]Département de Pathologie Cellulaire et Tissulaire, CHRU Brest, Brest; [7]Département de Pathologie Cellulaire et Tissulaire, CHU Angers, Angers; [8]Laboratoire de Biopathologie, CHRU Nancy, Nancy; [9]Service des Maladies du Sang, CHU Angers, Angers; [10]Service d'Hématologie Clinique, CHIC de Quimper, Quimper; [11]Service d'Hématologie Clinique, CHRU Brest, Brest; [12]Université Brest, Inserm, GETBO, Brest; [13]Université Brest, Inserm, EFS, UMR 1078, GGB, Brest; [14]Service de Pathologie, AP-HP, Hôpital Cochin, Paris and [15]Centre de Recherche des Cordeliers, Sorbonne Université, Inserm, Université de Paris, Paris, France.

[#]DLP and EL contributed equally as co-senior authors.

Correspondence:
E. LIPPERT - eric.lippert@chu-brest.fr
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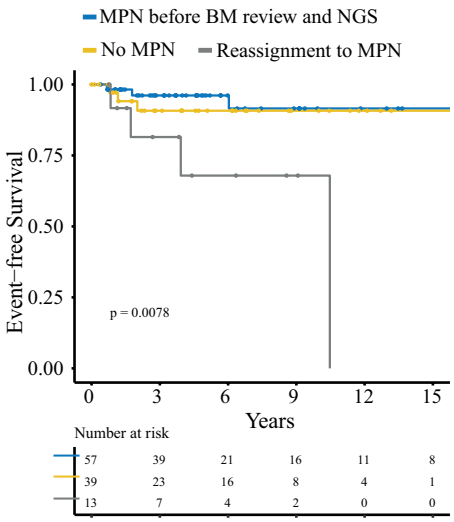
Supplemental Data

A



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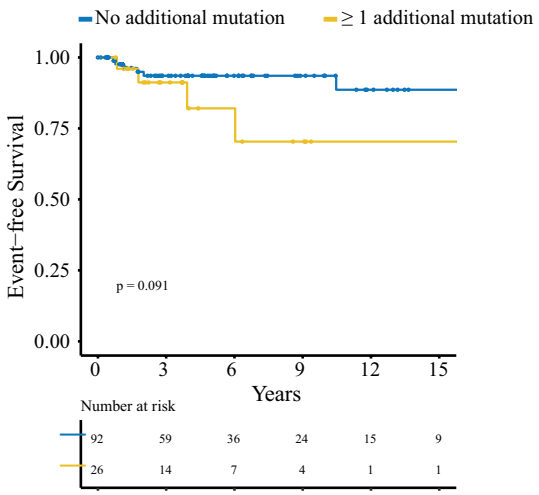

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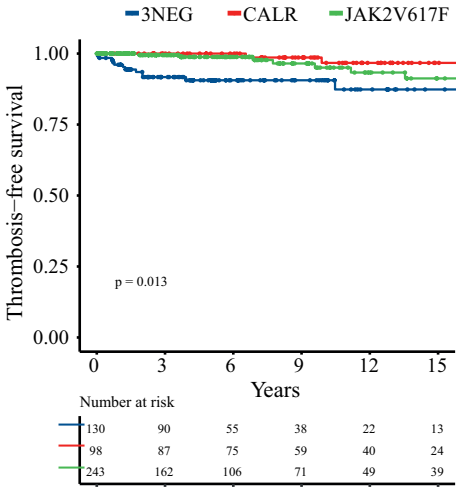

D

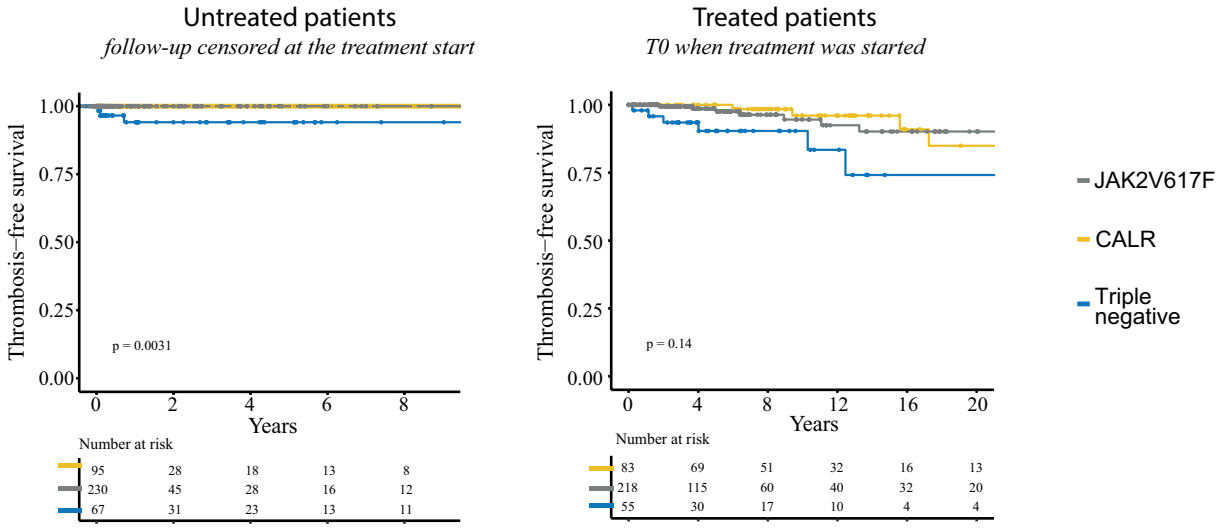

Figure S1: Outcomes of patients with Triple Negative Thrombocytosis.
(A) Kaplan-Meier curve representing the event free survival according to the reassignment after BMB review and NGS; (B) Kaplan-Meier curve representing the event free survival according to the presence of pathogenic or likely pathogenic mutation (constitutional thrombocythemia were excluded) ; (C) Kaplan-Meier curve representing thrombosis free survival according to the driver mutation; (D) Kaplan-Meier curves representing the sensitivity analysis of thrombosis-free survival of triple negative ET compared to *JAK2*V617F- and *CALR*-mutated controls, in untreated and treated patients considering only triple negative patients with either a histology of MPN or a clonal marker detected by NGS (related to Figure 1C).

Panel of 24 genes used in target next generation sequencing (NGS) :							
ASXL1	*BCOR*	*CBL*	*CSF3R*	*DNMT3A*	*ETNK1*	*ETV6*	*EZH2*
IDH1	*IDH2*	*JAK2*	*KRAS*	*MPL*	*NRAS*	*RUNX1*	*SETBP1*
SF3B1	*SH2B3*	*SRSF2*	*STAG2*	*TET2*	*TP53*	*U2AF1*	*ZRSR2*

Variant classification :		
Type	**Signification**	**Criteria**
A	**Pathogenic**	Deleterious variant described in myeloid neoplasms in COSMIC and validated somatic. Undescribed variant causing a truncated protein.
B	**Likely pathogenic**	Variant described in myeloid neoplasms in COSMIC but not validated somatic, absent from the "SNP" bases or with MAF <0.01 (1%). Variant not described in myeloid neoplasms in COSMIC, with MAF <0.01 (1%) and described as deleterious by Polyphen and / or SIFT.
VUS	**Of undetermined significance**	Variant not described in myeloid neoplasms in COSMIC, with MAF <0.01 (1%) without impact predicted by Polyphen and SIFT.
D	**Germline**	Constitutional status, controlled on nail DNA and/or described constitutional in literature. Or likely constitutional (JAK2 and MPL variants with VAF ≈ 50%, without germline control available).

Table S1: Panel of 24 genes used in targeted next generation sequencing (NGS) and variant classification according to the presumed impact on protein function.

The analysis of mutations was performed with targeted next generation sequencing (NGS), in each center, using a custom targeted panel of all coding exons of 24 genes. DNA libraries, built with the Sureselect® target enrichment protocol (Agilent Technologies, Santa Clara, CA, USA), were paired-end sequenced with a NextSeq 500® (Angers) or MiniSeq (Brest) Instrument (Illumina, San Diego, CA, USA). Variants were called using an in-house pipeline including trimmed reads alignment to the GRCh37 human reference genome, tumor variants detection by several variants callers (GATK HaplotypeCaller, VarScan) followed by annotation with public databases (gnomAD, COSMIC, dbSNP, ClinVar) and in silico predictors in the case of unknown variants (SIFT, PolyPhen-2), using updated versions whenever available. Mutations were considered significant if they reached a good quality score, a minimum variant allele frequency of at least 2% and a minimum of 20 reads supporting the variant for hotspots or 50 reads for non-hotspot variants. After removing polymorphisms (MAF>0.1%), intronic and synonymous variants, mutations were classified as either (i) pathogenic (non-sense/frameshifts or, previously described in myeloid malignancies) (ii) likely pathogenic (VAF <40% or >60%), (iii) germline (controlled on nail DNA and/or described germline in the literature or (iv) likely germline variants with VAF≈50% without germline control available.

Pt #	Age at diag.	Sex	Hgb g/L	Platelets (×10^9/L)	Leukocytes (×10^9/L)	Neutro (×10^9/L)	LDH ratio	CD 34+ (cells/x10-^{6}L)	SMG	History of thrombosis	Cyto-reduction	Initial Diag.	Diag. with BMB review	Mutations (VAF)	Diag. with NGS
A_001	60	M	148	1700	9.3	8.0	1.8	5.5	N	N	Y	ET	ET	***MPL* S505N (25%) / *DMNT3A* R882H (31%)**	ET
A_002	68	F	132	713	6.8	4.5	0.7	3.5	N	N	Y	ET	MPN	***TET2* L957Tfs*13 (8%) / *TET2* Q860X (3%)**	MPN
A_003	38	M	136	685	5.7	3.7	1.1	4.0	Y	Y	Y	ET	ET	/	ET
A_004	32	M	89	1990	12.0	8.3	1.5	NR	N	N	Y	No MPN	No MPN	*ASXL1* D1180E (47%) / *SH2B3* R265Q (48%)	No MPN
A_006	29	F	140	969	6.7	3.8	0.7	2.9	N	N	N	ET	not reviewed	/	ET
A_007	84	F	153	765	7.6	4.6	1.3	2.0	N	N	Y	ET	ET	***CBL* C404Y (4%)**	ET
A_008	67	F	125	996	10.3	7.3	1.1	1.9	N	N	Y	ET	pMF	/	pMF
A_009	37	F	150	714	14.8	9.9	0.8	4.0	N	N	N	No MPN	not reviewed	*SH2B3* R566Q (50%) / *ASXL1* A889P(49%)	No MPN
A_010	63	M	157	822	6.9	4.4	0.6	2.7	N	N	Y	pMF	pMF	/	pMF
A_011	65	F	127	698	4.2	2.3	0.7	1.9	N	N	Y	ET	not reviewed	***MPL* W515A (12%) / *TET2* N1387S (4%) / *TET2* H1904D (2%)**	ET
A_012	56	M	150	664	7.2	4.6	1.0	3.3	N	N	N	ET	ET	***DNMT3A* F354Sfs*52 (5%)**	ET
A_013	59	F	117	812	10.2	6.3	2.5	NR	N	Y	Y	ET	ET	/	ET
A_015	48	F	135	699	6.2	2.5	NR	NR	N	Y	Y	ET	not reviewed	***ASXL1* G646Wfs*12 (2%) / *SH2B3* S433L (94%) / *TP53* G245D (5%) / *TP53* R248W (2%)**	ET
A_016	77	F	115	556	5.4	3.4	0.9	0.8	N	N	N	pMF	No MPN	***DNMT3A* Y735C (2%) / *BCOR* R63K (53%)**	MPN
A_017	51	F	138	512	8.1	4.7	0.8	2.1	N	N	N	ET	No MPN	/	No MPN
A_018	70	F	120	531	8.0	5.2	0.7	6.8	N	N	N	No MPN	unclear	/	unclear
A_019	67	M	135	679	6.7	3.9	1.4	8,0	N	N	Y	ET	ET	***SH2B3* S417X (4%)**	ET
A_020	69	M	145	482	6.0	3.7	0.8	4.1	N	Y	Y	MPN	ET	/	ET
A_021	73	F	134	504	8.5	4.8	0.8	3.6	N	N	N	No MPN	MPN	***DNMT3A* R635Q (11%)**	MPN
A_023	78	M	139	684	8.5	6.3	0.7	2.8	Y	N	Y	ET	No MPN	/	No MPN
A_024	78	F	142	779	9.07	5.62	1.2	2.3	N	N	Y	ET	MPN	***MPL* W515S (10%) / *JAK2* V617I (12%) / *TET2* A1301fs (7%) / *SF3B1* G742D (7%)**	MPN
A_025	51	F	115	1010	6.0	3.8	NR	1.8	N	N	N	ET	ET	/	ET
A_026	36	F	NR	1800	NR	NR	NR	NR	N	N	Y	ET	not reviewed	/	ET
A_029	42	M	140	659	6.0	4.4	0.8	3.0	Y	N	N	ET	ET	/	ET
A_030	49	M	151	680	5.0	4.5	1.0	2.3	N	N	Y	ET	ET	/	ET
A_031	35	F	135	527	6.7	4.8	0.9	2.3	N	N	N	MPN	ET	/	ET
A_032	62	F	123	827	5.5	2.9	NR	NR	N	N	Y	ET	not	*ASXL1* G680C (48%) / *ZRSR2* S445_R448del (43%)	ET

													reviewed		
A_033	59	F	131	649	8.8	6.7	1.2	5.12	N	N	Y	ET	ET	***MPL* S204P (2%)**	ET
A_034	24	F	134	598	11.9	8.8	NR	1.7	N	N	N	ET	not reviewed	/	ET
A_035	35	F	117	940	9,0	5.3	0.7	5.4	N	N	N	ET	ET	/	ET
A_037	69	F	113	828	7.7	6.4	1.1	2.3	N	N	Y	ET	unclear	***DMNT3A* T835M (2%)**	MPN
A_038	50	M	134	500	9.5	4.4	0.9	10.7	N	Y	Y	ET	No MPN	/	No MPN
A_039	63	F	135	1152	9.6	7.8	1.0	1.1	N	N	Y	ET	ET	/	ET
A_040	40	M	151	1069	10.7	7.4	0.9	NR	N	N	Y	ET	not reviewed	/	ET
A_041	56	F	138	1122	4.9	2.6	1.2	NR	Y	N	Y	ET	ET	***MPL* S264F (6%)**	ET
A_042	53	F	156	579	9.0	6.3	0.7	NR	Y	N	N	No MPN	No MPN	/	No MPN
A_044	84	F	124	1200	10.4	7.4	NR	11.3	N	N	Y	ET	ET	***MPL* W515A (33%)**	ET
A_045	74	F	146	682	6.1	3.84	1.1	1.5	N	Y	Y	ET	ET	*TET2* V1718L (51%)	ET
A_046	58	F	134	523	5.39	3.4	0.9	4.1	N	N	N	MPN	No MPN	*SH2B3* E449K (48%)	No MPN
A_047	46	F	131	1064	10.2	6.9	1.0	13.9	N	N	N	ET	ET	*SH2B3* P19L (54%)	ET
A_048	76	F	123	922	9.2	5.1	1.0	3.6	N	N	Y	ET	ET	***MPL* L599P (11%)/ *CBL* Y371C (3%) / *TET2* E351X (13%) / *TET2* Q1624X (33%) / *EZH2* splicing (7%) / *SH2B3* splicing (39%)**	ET
A_050	29	F	127	592	9.7	5.8	0.6	NR	N	N	N	No MPN	No MPN	/	No MPN
A_051	53	F	134	563	6.04	3.74	1.2	1.8	Y	Y	N	MPN	No MPN	/	No MPN
A_052	29	F	132	600	8.2	4.7	0.8	1.93	N	N	N	ET	ET	*SETBP1* R1162Q (48%)	ET
A_053	64	F	150	647	4.9	4.1	1.1	1.7	N	N	Y	pMF	pMF	***MPL* S204P (87%) / *TET2* H1036Qfs*5 (41%)**	pMF
A_055	71	M	150	615	7.5	4.1	0.8	2.07	N	N	Y	ET	No MPN	/	No MPN
A_056	79	F	122	556	6.8	5.7	0.7	3.3	N	N	N	No MPN	unclear	/	unclear
A_057	25	F	134	572	5.7	3.3	0.6	NR	N	N	N	No MPN	No MPN	/	No MPN
A_058	52	F	150	469	8.5	5.8	1.0	3.7	N	N	N	No MPN	No MPN	/	No MPN
A_060	23	M	149	668	7.2	4.4	0.8	3.2	N	N	N	ET	ET	/	ET
A_061	41	F	149	650	13.2	6.4	0.8	6.22	N	N	Y	No MPN	No MPN	***ASXL1* Q695X (44%)**	MPN
A_062	31	F	131	1115	6.5	3.9	1.0	2.42	N	N	N	ET	ET	/	ET
A_063	37	M	149	599	5.6	3.6	0.7	2.1	N	Y	Y	ET	ET	/	ET
A_064	59	F	150	837	10.9	7.3	1.1	4.0	N	N	Y	ET	unclear	/	unclear
A_065	40	F	135	812	5.0	3.1	1.0	2.4	N	N	N	No MPN	No MPN	*TET2* N767D (47%)	No MPN
B_003	18	M	153	1017	8.5	5.0	0.8	NR	N	N	Y	No MPN	not reviewed	/	No MPN
B_004	18	F	107	980	9.4	6.3	1.0	NR	Y	N	N	ET	ET	***MPL* P200A (36%)**	ET
B_005	20	M	158	1444	7.2	4.7	0.9	2.0	N	N	Y	No MPN	not reviewed	/	No MPN

B_007	23	F	136	550	10.7	7.8	0.7	NR	NR	N	Y	ET	ET	/	ET
B_008	25	F	141	919	8.5	6.0	0.9	NR	N	N	Y	No MPN	ET	*ASXL1* S846N (51%)	ET
B_009	26	M	160	1484	8.5	5.1	NR	NR	Y	N	Y	ET	not reviewed	/	ET
B_012	27	F	127	520	13.9	8.38	0.7	NR	N	N	N	No MPN	No MPN	/	No MPN
B_013	28	F	132	489	7,0	4.2	0.7	NR	N	N	N	ET	ET	/	ET
B_014	28	F	110	606	15.1	9.3	0.8	2.0	N	N	N	No MPN	No MPN	/	No MPN
B_015	32	F	135	522	6.8	4.5	0.9	NR	N	N	Y	ET	ET	/	ET
B_017	34	F	138	509	7.3	6.0	1.3	NR	N	N	N	No MPN	No MPN	/	No MPN
B_018	35	F	126	1342	9.7	6.4	1.0	2.0	N	N	Y	No MPN	No MPN	/	No MPN
B_020	35	F	129	1040	9.1	6.6	0.0	2.0	N	N	N	ET	ET	*CBL* V904I (47%)	ET
B_023	43	F	131	966	8.9	6.8	1.3	NR	N	N	Y	ET	pMF	*BCOR* S1223L (52%)	pMF
B_024	47	M	157	526	11.4	8.7	0.9	NR	N	Y	Y	No MPN	No MPN	/	No MPN
B_025	47	M	169	555	9.5	6.4	0.7	3.0	N	N	Y	No MPN	No MPN	/	No MPN
B_026	47	F	144	1544	8.2	5.7	1.2	NR	N	N	Y	unclear	not reviewed	/	unclear
B_027	48	F	136	536	9.8	5.8	NR	2.0	N	N	N	ET	ET	*SH2B3* S213R (54%)	ET
B_028	48	F	140	544	9.6	7.8	0.9	1.5	N	N	N	No MPN	No MPN	/	No MPN
B_029	48	F	125	580	8.8	5.3	0.8	NR	N	N	Y	unclear	not reviewed	/	unclear
B_030	51	M	150	521	10.8	8.7	0.9	4,0	N	N	N	No MPN	No MPN	/	No MPN
B_032	54	M	154	557	4.9	2.7	0.8	NR	N	N	N	No MPN	No MPN	/	No MPN
B_033	55	M	111	773	13.6	11.8	1.6	NR	N	N	Y	No MPN	No MPN	/	No MPN
B_034	55	F	128	622	8.5	5.1	0.9	NR	N	N	Y	ET	ET	/	ET
B_035	55	F	141	477	7.4	4.7	0.8	NR	N	N	N	No MPN	No MPN	/	No MPN
B_036	56	F	126	472	4.9	2.7	1	NR	N	N	Y	No MPN	No MPN	/	No MPN
B_038	57	M	144	586	5.5	3.2	0.9	NR	N	Y	N	No MPN	ET	*SH2B3* S186I (54%)	ET
B_042	58	F	140	1286	7.4	4.4	1	NR	N	N	Y	ET	ET	/	ET
B_043	59	M	134	917	8.1	4.4	0.9	5.0	N	N	Y	ET	ET	/	ET
B_044	59	F	138	922	9.8	7.6	1.4	NR	N	N	Y	No MPN	ET	/	ET
B_045	60	F	145	636	9.6	5.7	1.0	4.0	N	N	Y	No MPN	No MPN	/	No MPN
B_046	61	M	126	1150	10.3	9.2	2	NR	Y	Y	Y	ET	not reviewed	/	ET
B_048	62	M	118	600	NR	10.8	NR	NR	N	Y	N	No MPN	No MPN	***DNMT3A* R882K (21%)**	MPN
B_049	62	F	137	537	4.7	2.7	1.0	3.0	N	N	N	unclear	unclear	/	unclear
B_050	62	M	146	741	7.6	4.2	1.0	3.0	N	Y	Y	ET	ET	***DNMT3A* S663* (4%)**	ET

B_051	63	F	143	468	9.5	5.7	1.0	NR	N	Y	Y	ET	No MPN	***ASXL1* G646Wfs*12 (10%)**	MPN
B_053	64	M	136	622	7.2	4.0	1.1	NR	N	Y	Y	ET	ET	/	ET
B_054	65	F	119	728	14.3	10.6	0.9	NR	N	N	Y	ET	No MPN	***DNMT3A* N797D (37%)**	MPN
B_055	65	F	124	605	6.9	4.5	1.1	4.0	N	Y	Y	No MPN	No MPN	/	No MPN
B_056	67	M	126	559	10.5	7.7	0.7	NR	N	N	Y	No MPN	No MPN	*KRAS* M189L (52%)	No MPN
B_058	69	F	150	707	7.6	5.9	1.1	3.0	N	N	Y	ET	ET	/	ET
B_060	71	M	136	634	6.1	3.7	0.9	NR	N	N	Y	No MPN	not reviewed	/	No MPN
B_061	73	F	134	577	10.3	7.1	0.8	NR	N	Y	Y	No MPN	No MPN	*ASXL1* E484G (47%) / ***SH2B3* E400K (33%)**	MPN
B_063	74	M	132	755	10,0	8.1	1.0	14.0	N	N	Y	ET	ET	/	ET
B_066	76	F	111	622	6.1	4.6	1.0	NR	N	N	Y	No MPN	No MPN	/	No MPN
B_067	78	F	130	626	11.4	9.7	1.2	NR	N	N	Y	No MPN	No MPN	/	No MPN
B_069	82	F	163	454	8,0	5.6	1.0	3.0	N	N	Y	ET	ET	/	ET
B_070	82	F	155	550	23.8	22.4	1.1	NR	N	N	Y	unclear	unclear	/	unclear
B_344	52	M	156	454	13.9	10.2	1.1	3.5	N	N	N	No MPN	No MPN	/	No MPN
B_345	64	M	130	488	16,0	14.3	NR	1,0	N	Y	Y	No MPN	No MPN	/	No MPN
B_346	32	F	140	590	9.7	5.9	1.0	2,0	N	N	N	No MPN	No MPN	/	No MPN
B_349	40	F	128	1068	8.3	5.5	NR	NR	N	N	Y	ET	not reviewed	/	ET
B_350	18	F	134	681	9.2	6.5	1.1	1.0	N	N	N	ET	not reviewed	***MPL* P70R (21%)**	ET
B_351	18	F	147	785	16.0	11.7	0.0	NR	N	N	Y	ET	not reviewed	/	ET
B_352	62	F	141	444	6.1	3.5	1.2	0.5	N	N	N	unclear	not reviewed	***CBL* L380P (3%)** / *ASXL1* Q977R (48%)	MPN
B_356	56	F	135	511	6.9	4.8	1.3	NR	NR	NR	Y	ET	not reviewed	/	ET
B_357	39	F	143	442	6.8	4.7	0.9	1,0	N	N	N	ET	No MPN	/	No MPN
B_360	67	F	135	509	11.7	7.8	1.3	NR	N	N	Y	ET	unclear	/	unclear
B_361	62	F	123	678	10.2	4.75	1.6	NR	N	N	N	No MPN	No MPN	/	No MPN
B_362	41	F	135	1081	7.5	4.5	0.5	NR	N	N	N	ET	ET	/	ET
B_363	78	F	138	470	10.7	6.9	0.9	1,0	N	Y	Y	No MPN	No MPN	***ASXL* G646Wfs*12 (21%) / *ASXL1* P808Lfs*10 (7%)**	MPN
B_364	36	F	137	472	10,0	4.9	1	NR	NR	N	N	unclear	unclear	*STAG2* R1/12L (43%)	unclear
B_365	23	F	134	714	10.2	5.3	1	6.0	N	N	N	ET	No MPN	/	No MPN
A_005	24	M	170	511	5.97	3.5	1.3	2.2	Y	N	Y	ET	MPN	*JAK2* T875N (49%)	Constitutional T
A_036	27	F	131	668	7.47	4.8	0.9	NR	N	N	N	ET	not reviewed	*JAK2* D873E (53%)	Constitutional T
A_054	45	F	141	788	9.79	8.3	NR	7.4	Y	N	N	ET	ET	*JAK2* P97A (46%)	Constitutional T

B_016	32	F	144	512	8.5	5.7	0.9	4.0	N	N	N	ET	No MPN	*MPL* R123Q (47%)	Constitutional T
B_019	35	F	135	580	13.9	10.5	0.7	2.5	N	N	Y	No MPN	ET	*JAK2* E846D (55%)	Constitutional T
B_021	39	F	131	530	11.4	6.8	0.7	3.0	N	N	N	ET	No MPN	*MPL* G144R (51%)	Constitutional T
B_022	41	F	140	588	7.9	4.7	0.8	NR	N	N	Y	No MPN	No MPN	*JAK2* Y613C (49%) / ***TP53* P278A (22%)**	Constitutional T
B_039	57	M	157	468	6.8	4.3	1.1	NR	N	N	Y	No MPN	ET	*JAK2* R1063H (51%)	Constitutional T
B_040	58	F	148	503	10.8	7.9	0.9	3.0	N	N	N	No MPN	No MPN	*MPL* L510Cfs*4 (50%)	Constitutional T
B_059	71	F	146	511	13.1	8.9	1.1	2,0	N	Y	Y	ET	ET	*JAK2* Y613C (46%)	Constitutional T
B_347	38	M	139	647	8.5	4.8	1.2	NR	N	Y	Y	ET	ET	*JAK2* R867Q (50%)	Constitutional T
B_358	43	F	139	517	13,0	9.1	1.1	1.5	N	N	Y	ET	ET	*JAK2* R1063H (51%)	Constitutional T

Table S2: Demographic, biological (Hgb: hemoglobin, Neutro: neutrophils) and clinical characteristics at diagnosis, diagnosis according to each classification and variant description for each patient included in the present study (TN group). Initial diagnosis based on local bone marrow biopsy conclusions was re-evaluated by experts of the GEBOM and diagnosis was reanalysed with NGS results: ET: essential thrombocytemia ; pMF : pre-myelofibrosis; MPN : myeloproliferative neoplasm (unclassifiable); no MPN : not in favour of MPN; Constitutional T: Constitutional thrombocythemia. Variants are typed in bold for pathogenic or likely pathogenic variants and underlined for germline or likely germline variants. VAF: variant allele frequency.