Positive impact of molecular analysis on prognostic scores in essential thrombocythemia: a single center prospective cohort experience

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Supplemental methods

Molecular Analysis

DNA was isolated from total blood with a QiaAmp DNA extraction kit and the QiaCube robot (Qiagen, Germantown, MD) and quantitated by Nanodrop spectrophotometry (Agilent, Santa Clara, CA). DNA samples were stored at diagnosis (n = 112, 58.9%) or during the first years of follow-up (median time period between diagnosis and sample collection = 2.28 years). All patients were first tested for *JAK2* (allele specific PCR), *MPL* (Sanger sequencing) and *CALR* (Sanger sequencing) mutations using different methods.

NGS was performed at Henri Mondor hospital between September 2016 and October 2017, by a 16-genes panel (*ASXL1*, *EZH2*, *DNMT3A*, *TET2*, *IDH1*, *IDH2*, *TP53*, *SF3B1*, *SRSF2*, *CBL*, *NRAS*, *KRAS, JAK2*, *MPL*, *SH2B3*, *IKZF1*). For NGS, we used MiSeq-Illumina technology with a TruSeq Custom Amplicon (TSCA) library from Illumina. For TSCA, 250ng of DNA were used to generate amplicons covering the regions of interest, designed with the Design Studio software (Illumina), with a median length of 425bp, the strategy being based on PCR multiplexing without fragmentation. TSCA libraries were run on MiSeq instruments (Illumina) for reversible terminator-based sequencing by synthesis chemistry in paired-end 250bp cycle sequencing.

Sequencing data were first aligned on hg19 genome using BaseSpace (Illumina). Variant calling and annotation were done with a custom pipeline using Varscan2 for SNP and short Indels calling and Annovar for databases annotation (Cosmicv81, dbSNP138, Exac03, 1000genomes, ESP6500, clinvar). For mutation calling, minimum coverage of called regions was set to 50 and the minimum variant allele frequency (VAF) was set to 5%. The mutation JAK2V617F was manually inspected on BAM file using IGV.

Only exonic nonsynonymous mutations were retained and putative somatic mutations were obtained by removal of known polymorphisms with a population frequency > 0.01 using the 1000 genomes, Exome Sequencing Project and the Exome Aggregation Consortium (ExAC) resources. The functional effects of mutations were predicted using Polyphen2 and SIFT algorithms. Integrative Genomics Viewer (IGV, Cambridge, United Kingdom) was used to visualize the reads alignment and confirm the variant calls. Mutations were classified according to their putative impact in three groups: A: Mutations described as somatic and functional in hematological malignancies or non-sense/frameshift mutations (Pathogenic variants), B: Mutations supposed to be functional according to Polyphen2 or SIFT alogorithms but functionally not tested in the literature or described in COSMIC without confirmation of the somatic status (Likely pathogenic variants) and C: Mutations of unknown significance in the databases (variants of unknown significance). The filtering, the validation by visualization on IGV and the classification of the mutations were performed independently by 2 molecular biologists and all discordant cases were then reviewed by all authors.

Statistics

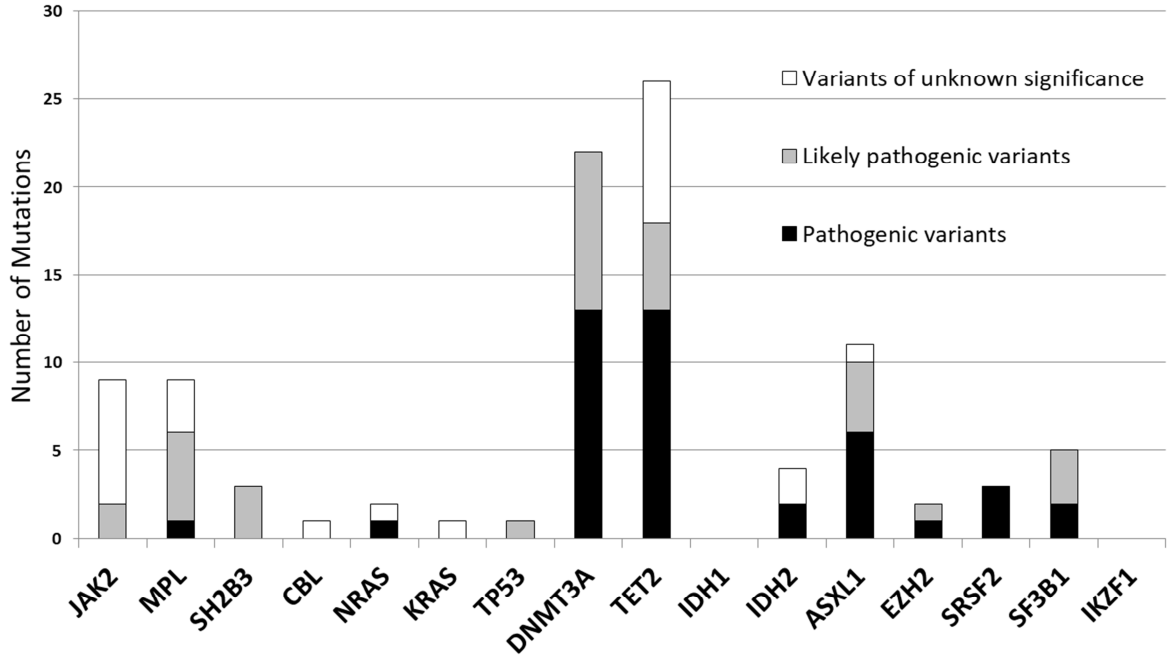
Variables were summarized as frequencies and percentages or means ± standard deviation, or median and Inter-Quartile Range, as appropriate. Comparisons were performed using Fisher exact test, or Student t-test or Mann-Whitney test, as appropriate. Survival curves were obtained with the Kaplan-Meier method. Cox proportional hazard regression models were performed for all events, PFS, thrombosis and transformation with results presented as hazard ratios (HRs) and 95% confidence intervals (CIs). Characteristics associated with significant results (type-one error rate at 0.25) in univariate analysis, performed with a cox-model, were included in the multivariate Cox proportional hazard regression models. Then a step by step manual backward selection was performed. All models were tested for assumptions and were found to be valid unless otherwise indicated. All tests were 2-sided with p-values smaller than

0.05 considered statistically significant. Statistical analyses were performed with R version 3.4.2 (R Core Team, Vienna, Austria).

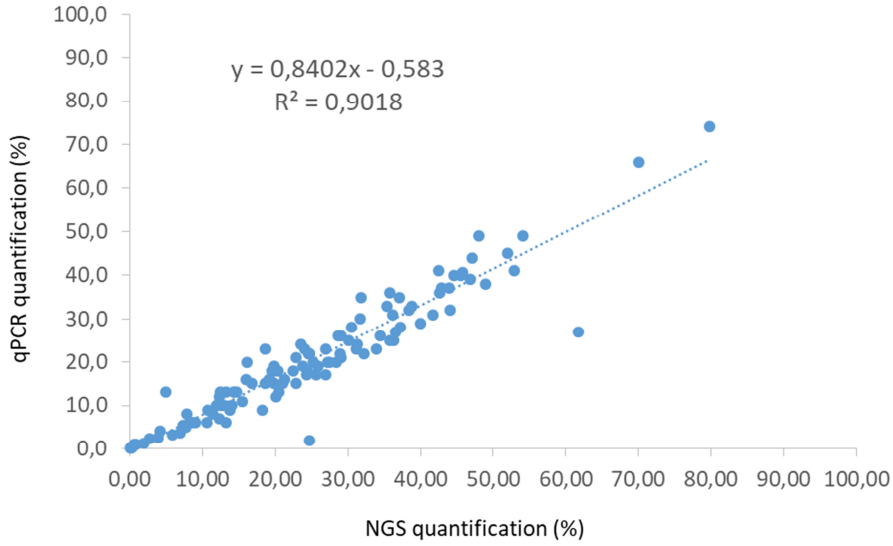
Ethic consideration

Patients have provided written informed consent for the use of remnant DNA for investigational purposes. This non-interventional research was made in accordance with the MR03 french methodology for biomedical research.

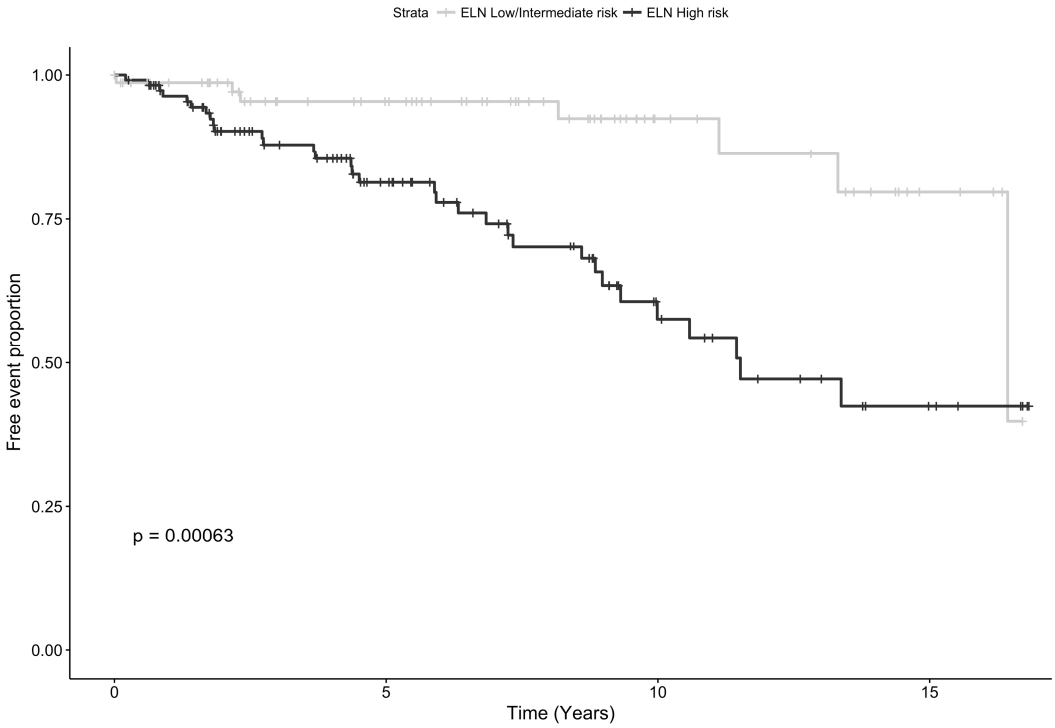
Supplemental data



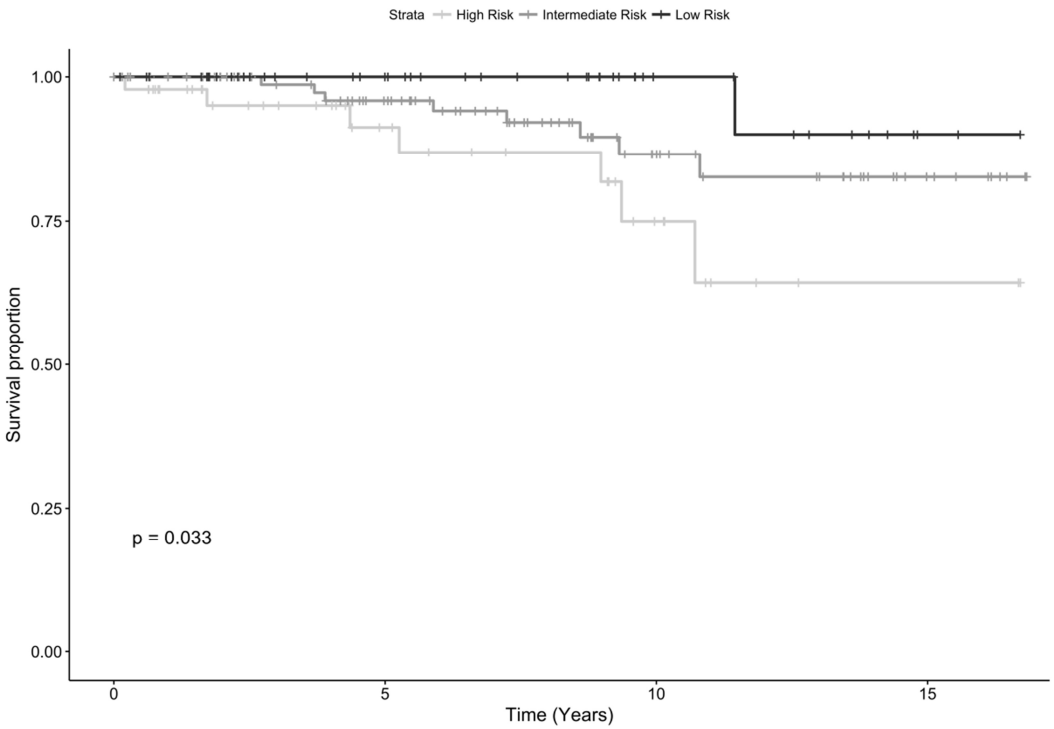
Supplemental Figure S1: Repartition of additional mutations among the sequenced genes according to their pathogenic classification



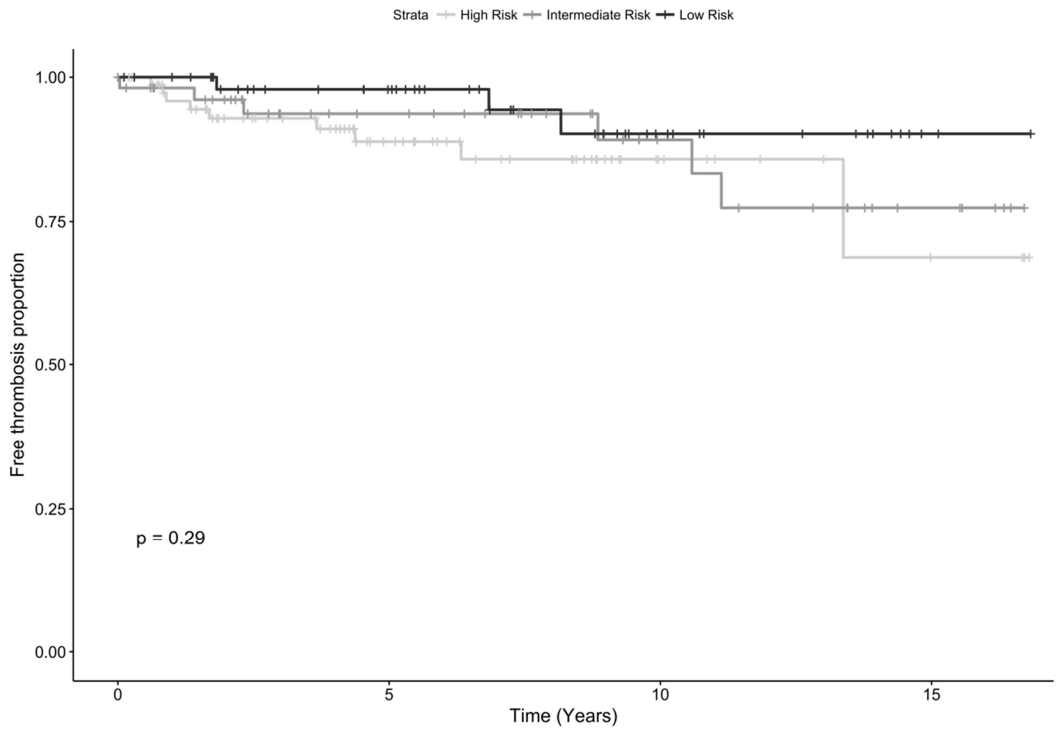
Supplemental Figure S2: Correlation between quantitative PCR and NGS for JAK2V617F allele burden measurement



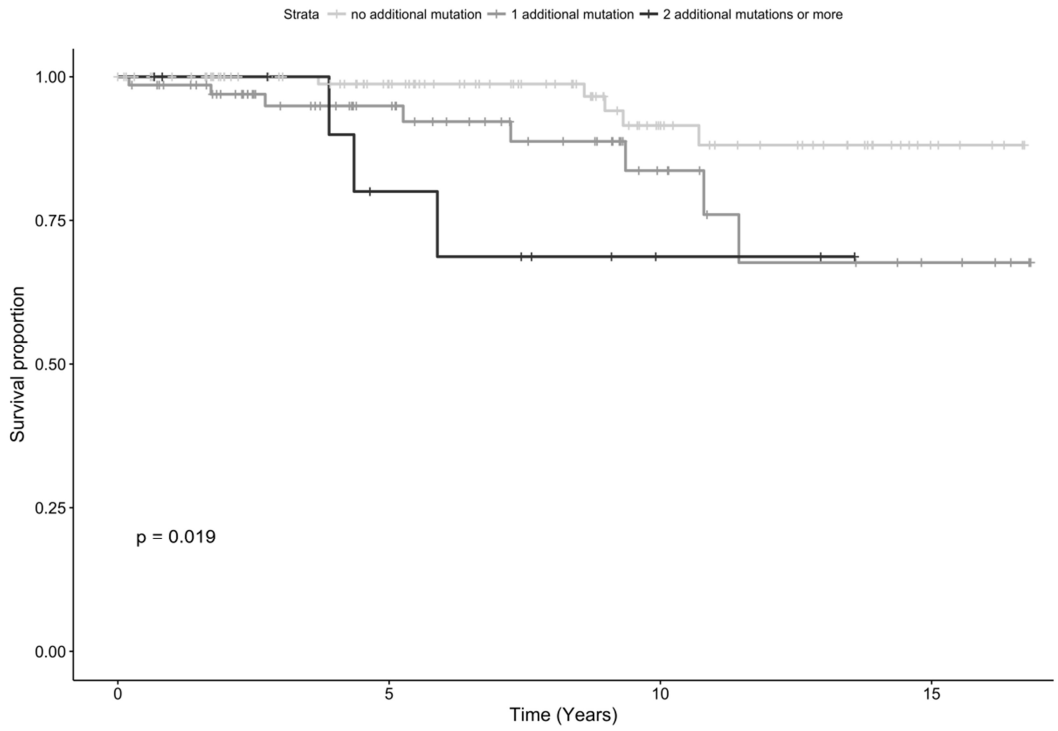
Supplemental Figure S3: Event free survival (death, transformation or thrombosis) according to the ELN prognostic score



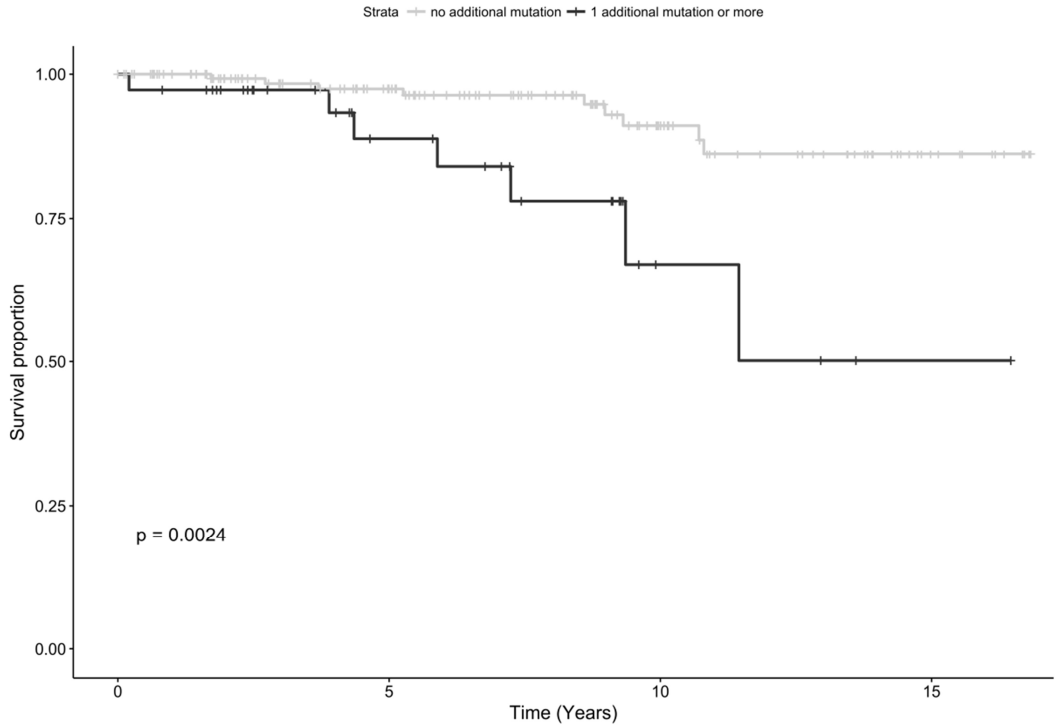
Supplemental Figure S4: Overall survival according to the IPSET-survival prognostic score



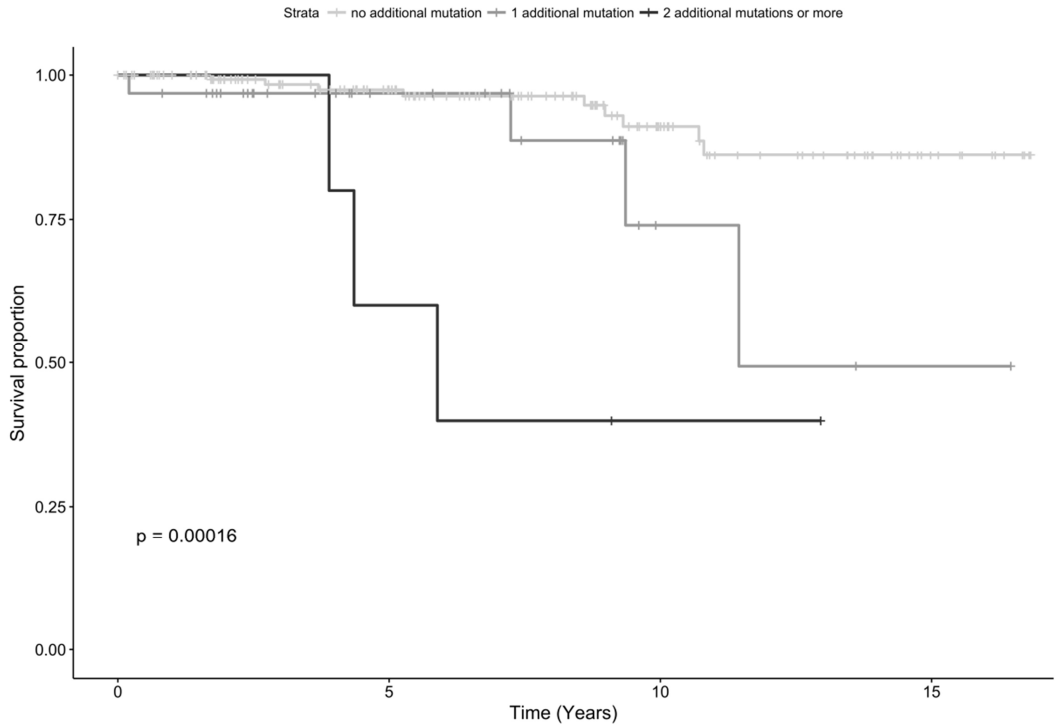
Supplemental Figure S5: Thrombosis free survival according to the IPSET-thrombosis prognostic score



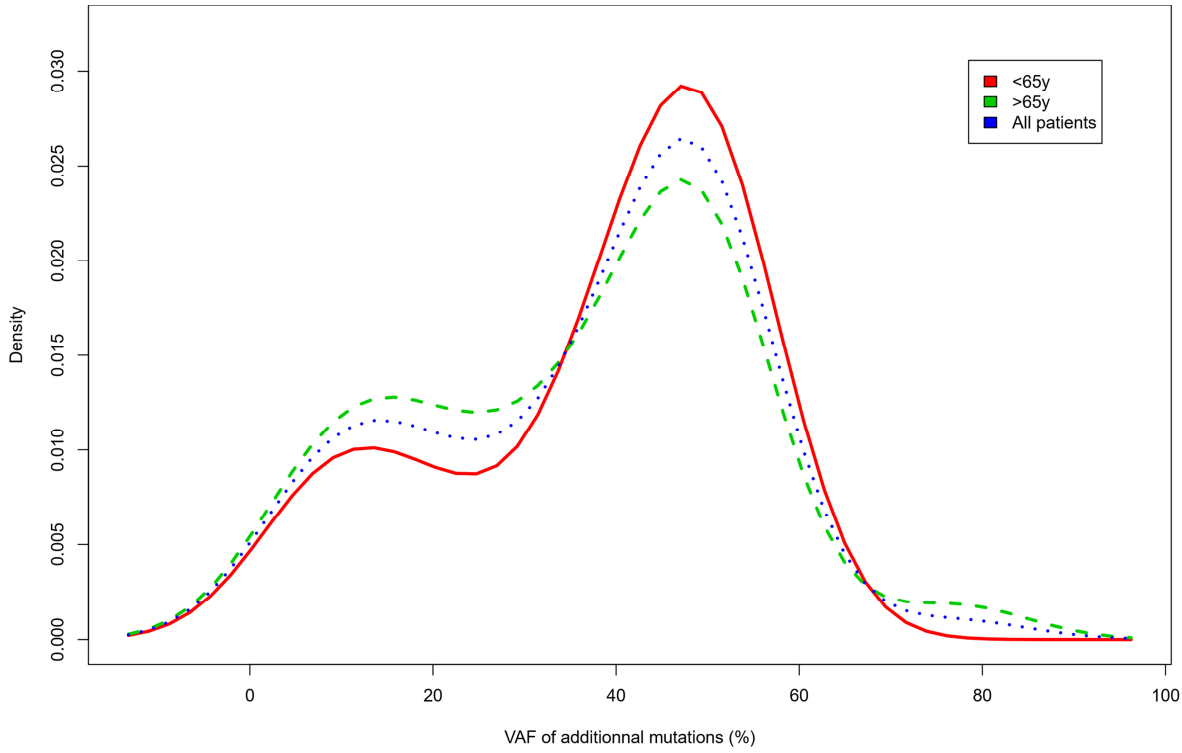
Supplemental Figure S6: Overall free survival according to the number of additional mutations (all mutations were considered, "ABC" classification)



Supplemental Figure S7: Overall free survival according to the presence of at least one additional mutation (only pathogenic mutations were considered, "A" classification).



Supplemental Figure S8: Overall free survival according to the number of additional mutations (only pathogenic mutations were considered, "A" classification)



Supplemental Figure S9: Density plot showing distribution of variant allele frequencies (VAF) of additional mutations for all patients, patients > 65 years and patients < 65 years.

Supplemental Table S1: Patients characteristics (n=190)

Parameter		Entire cohort
Sex ratio (M/F)		0.4 (76/114)
Age (median[IQ range])		61 [19;93]
Hemoglobin (g/dL) (median [IQ range])		14.2 [9.1;17.1]
Platelets (10^9/L) median [IQ range]		774 [484;1895]
Leukocytes (10^9/L) median [IQ range]		9.8 [2.3;26.4]
Thrombosis history before diagnosis n (%)		35 (18)
CV risk history n (%)		65 (34)
Driver mutations n (%)	*JAK2*V617F	116 (61%)
	CALR	27 (14%)
	*MPL*W515	4 (2%)
	Triple negative	43 (23%)
Events during follow-up n (%)	Deaths	16 (8.4)
	Hematological transformation	11 (5.7)
	Thrombosis	18 (9.4)
First line therapy	No cytoreductive drug	20 (11%)
	Hydroxycarbamide	159 (84%)
	Peg-Interferon	10 (5.5%)
	Pipobroman	1 (0.5%)
ASA treatment		151/159 (95%)

Supplemental Table S2: Description of the *CALR* mutations others than type 1 and 2 (NM_004343.3)

Patient	cDNA	Protein change
5002	c.1100_1145del	p.(Leu367Glnfs*48)
4599	c.1099_1132del	p.(Leu367Argfs*52)
2665	c.1147_1154delinsTGTC	p.(Glu383Cysfs*46)
3666	c.1121_1139del	p.(Lys374Argfs*50)

Supplemental Table S3: All additional mutations detected using NGS sequencing.

Gène	Mutation	VAF (%)	Depth (X)	COSMIC reference	dbSNP rs	POLYPHEN2	SIFT	Pathogenic classification	N° patient
ASXL1	ASXL1:NM_015338:exon12:c.C1762T:p.Q588X	7	1563	COSM110707	-	.	.	A	445
ASXL1	ASXL1:NM_015338:exon12:c.C3965G:p.P1322R	49	736	COSM36204	rs141930107	-	-	B	2296
ASXL1	ASXL1:NM_015338:exon12:c.C1774T:p.Q592X	48	3249	COSM96400	-	.	D	A	2654
ASXL1	ASXL1:NM_015338:exon12:c.1888_1910del:p.H630fs	21	508	-	-	-	-	A	3288
ASXL1	ASXL1:NM_015338:exon12:c.T1747C:p.W583R	48	2015	-	-	D	T	B	3590
ASXL1	ASXL1:NM_015338:exon12:c.C3935T:p.A1312V	51	875	COSM97046	rs148144203	B	T	B	3658
ASXL1	ASXL1:NM_015338:exon11:c.C1534T:p.Q512X	31	254	COSM3799427;COSM443634	-	-	-	A	4417
ASXL1	ASXL1:NM_015338:exon12:c.2497delA:p.S833fs	10	1634	COSM97036	-	-	-	A	5454
ASXL1	ASXL1:NM_015338:exon12:c.G4099A:p.V1367I	56	283	COSM723114	rs147456014	B	T	C	6189
ASXL1	ASXL1:NM_015338:exon11:c.C1636T:p.Q546X	51	1349	-	-	-	-	A	6301
ASXL1	ASXL1:NM_015338:exon11:c.A1654G:p.I552V	50	2433	-	rs143952412	P	T	B	B2(80)
CBL	CBL:NM_005188:exon15:c.G2269A:p.A757T	52	402	COSM3687124	rs146517083	B	T	C	3705
DNMT3A	DNMT3A:NM_175629:exon22:c.2481delC:p.F827fs	12	1831	COSM53096	rs769831202	-	-	A	1258
DNMT3A	DNMT3A:NM_175629:exon19:c.C2245T:p.R749C	8	2089	COSM219133	-	D	D	A	1416
DNMT3A	DNMT3A:NM_175629:exon22:c.2498_2510del:p.I833fs	49	1811	-	-	-	-	A	1670
DNMT3A	DNMT3A:NM_175629:exon22:c.2577dupA:p.W860fs	15	6322	-	-	-	-	A	1713
DNMT3A	DNMT3A:NM_175629:exon23:c.G2645A:p.R882H	39	526	COSM52944;COSM442676	rs147001633	B	D	A	2230
DNMT3A	DNMT3A:NM_175629:exon23:c.G2645A:p.R882H	37	253	COSM52944;COSM442676	rs147001633	P	D	A	2493
DNMT3A	DNMT3A:NM_175629:exon14:c.T1640A:p.L547H	39	1287	COSM231556		D	D	B	2550
DNMT3A	DNMT3A:NM_175629:exon18:c.C2099T:p.P700L	6	502	-	-	D	D	B	2641
DNMT3A	DNMT3A:NM_175629:exon9:c.C1103T:p.A368V	27	45	-	-	D	D	B	2891
DNMT3A	DNMT3A:NM_175629:exon14:c.G1627T:p.G543C	21	1340	COSM87002	-	D	D	A	3267
DNMT3A	DNMT3A:NM_175629:exon19:c.T2210G:p.L737R	37	1137	COSM87008	-	D	D	A	3705
DNMT3A	DNMT3A:NM_175629:exon19:c.C2206T:p.R736C	18	1662	COSM231560	-	D	D	B	3904

DNMT3A	DNMT3A:NM_175629:exon7:c.G682T:p.E228X	6	157	COSM5989188;COSM5989189	-	-	-	A	4751
DNMT3A	DNMT3A:NM_175629:exon23:c.G2645A:p.R882H	41	911	COSM52944;COSM442676	rs147001633	P	D	A	4924
DNMT3A	DNMT3A:NM_175629:exon14:c.1603_1623dup:p.Y528_Q534dup	40	626	-	-	-	-	B	5428
DNMT3A	DNMT3A:NM_175629:exon23:c.C2695T:p.R899C	13	388	COSM5075581	-	D	D	B	5653
DNMT3A	DNMT3A:NM_175629:exon23:c.A2716G:p.K906E	28	489	-	-	D	D	B	5784
DNMT3A	DNMT3A:NM_175629:exon19:c.C2311T:p.R771X	20	353	COSM231563	-	-	-	A	5828
DNMT3A	DNMT3A:NM_175629:exon19:c.T2264C:p.F755S	35	382	-	rs536841393	D	D	B	5839
DNMT3A	DNMT3A:NM_175629:exon14:c.G1628T:p.G543V	8	4876	COSM249135	-	D	D	B	5928
DNMT3A	DNMT3A:NM_175629:exon23:c.A2716T:p.K906X	56	249	-	-	-	-	A	B2 1351
DNMT3A	DNMT3A:NM_175629:exon22:c.C2536T:p.Q846X	7	4703	-	-	-	-	A	B2 1521
EZH2	EZH2:NM_004456:exon16:c.A1927G:p.I643V	49	2559	-	-	P	T	B	2748
EZH2	EZH2:NM_004456:exon9:c.A965G:p.N322S	52	2639	COSM53031	rs151023145	B	T	A	3705
IDH2	IDH2:NM_002168:exon4:c.G419A:p.R140Q	21	47	COSM41590	rs121913502	D	D	A	1084
IDH2	IDH2:NM_002168:exon11:c.C1304T:p.T435M	50	160	-	rs118053940	B	T	C	2524
IDH2	IDH2:NM_002168:exon4:c.G419A:p.R140Q	17	507	COSM41590	rs121913502	D	D	A	5428
IDH2	IDH2:NM_002168:exon11:c.C1304T:p.T435M	52	156	-	rs118053940	B	T	C	B2 1471
JAK2	JAK2:NM_004972:exon24:c.G3188A:p.R1063H	46	1170	-	rs41316003	B	T	C	861
JAK2	JAK2:NM_004972:exon18:c.C2390G:p.S797C	45	4309	COSM2777217	rs201992086	B	T	C	965
JAK2	JAK2:NM_004972:exon5:c.C464T:p.A155V	49	5870	-	-	P	D	B	1416
JAK2	JAK2:NM_004972:exon24:c.G3188A:p.R1063H	49	966	-	rs41316003	B	T	C	3005
JAK2	JAK2:NM_004972:exon9:c.C1127G:p.T376S	50	719	-	-	D	P	B	3631
JAK2	JAK2:NM_004972:exon19:c.G2538C:p.E846D	56		-	rs150221602	B	D	C	4958
JAK2	JAK2:NM_004972:exon25:c.A3323G:p.N1108S	50	925	COSM33708	rs142269166	B	T	C	5555
JAK2	JAK2:NM_004972:exon24:c.G3188A:p.R1063H	47	601	-	rs41316003	B	T	C	6117
JAK2	JAK2:NM_004972:exon25:c.A3323G:p.N1108S	48	1866	COSM33708	rs142269166	B	T	C	B2 1310
KRAS	KRAS:NM_033360:exon5:c.G504T:p.L168F	50	2728	-	-	B	T	C	B2 1351
MPL	MPL:NM_005373:exon12:c.T1771G:p.Y591D	53	344	COSM28997	-	D	D	A	1006
MPL	MPL:NM_005373:exon4:c.C601A:p.H201N	37	1734	-	-	B	T	C	1006
MPL	MPL:NM_005373:exon3:c.T313C:p.F105L	52	455	-	rs145313814	B	T	C	2426

MPL	MPL:NM_005373:exon11:c.G1610A:p.R537Q	78	676	-	rs3820551	D	T	B	4823
MPL	MPL:NM_005373:exon12:c.G1896C:p.W632C	17	269	-	-	D	D	B	5241
MPL	MPL:NM_005373:exon5:c.G775A:p.E259K	41	110	-	rs528834914	B	T	B	6136
MPL	MPL:NM_005373:exon4:c.C626T:p.S209F	48	1073	-	-	P	T	B	6188
MPL	MPL:NM_005373:exon12:c.G1896C:p.W632C	13	122	-	-	D	D	B	6235
MPL	MPL:NM_005373:exon7:c.C1051T:p.R351C	49	575	-	rs201998783	D	D	B	B2 384
MPL	MPL:NM_005373:exon4:c.A530G:p.K177R	50	5388	-	-	B	T	C	B2(80)
NRAS	NRAS:NM_002524:exon2:c.G38A:p.G13D	36	6717	COSM573	rs121434596	B	D	A	1006
NRAS	NRAS:NM_002524:exon3:c.G189C:p.E63D	27	1085	-	-	B	T	C	4362
SF3B1	SF3B1:NM_012433:exon14:c.G1998T:p.K666N	38,00	2555	COSM131557	-	D	D	A	3542
SF3B1	SF3B1:NM_012433:exon15:c.A2098G:p.K700E	42	2320	COSM84677	rs559063155	D	D	A	4669
SF3B1	SF3B1:NM_012433:exon21:c.T3047C:p.L1016P	5	197	-	-	D	D	B	5504
SF3B1	SF3B1:NM_012433:exon21:c.G3127A:p.A1043T	8	123	-	-	D	D	B	5725
SF3B1	SF3B1:NM_012433:exon21:c.A3037T:p.I1013F	7	98	-	-	D	D	B	B2(80)
SH2B3	SH2B3:NM_005475:exon4:c.G841T:p.D281Y	6	187	-	-	D	D	B	796
SH2B3	SH2B3:NM_005475:exon6:c.G1175A:p.R392Q	76	42	COSM5840118;COSM5840119	-	D	D	B	2414
SRSF2	SRSF2:NM_003016.4:exon1:C284A:p.P95H	45	29	COSM146288	-	D	D	A	2680
SRSF2	SRSF2:NM_003016.4:exon1:C285A:p.P95T	45	571	COSM307353	-	D	D	A	4216
SRSF2	SRSF2:NM_003016.4:exon1:C284T:p.P95L	17	82		-	D	D	A	6235
TET2	TET2:NM_001127208:exon11:c.T5366C:p.M1789T	5,7	262	-	-	B	T	C	187
TET2	TET2:NM_001127208:exon11:c.G5152T:p.V1718L	50	1091	COSM41742	rs142312318	B	T	C	632
TET2	TET2:NM_001127208:exon11:c.G5103A:p.M1701I	52	1155	-	rs62623390	B	T	C	652
TET2	TET2:NM_001127208:exon11:c.C4613T:p.P1538L	53	191	-	-	B	T	C	861
TET2	TET2:NM_001127208:exon11:c.C4854A:p.Y1618X	50	474	-	-	-	-	A	937
TET2	TET2:NM_001127208:exon3:c.A413T:p.Q138L	51	1461	-	rs138203452	P	D	B	1891
TET2	TET2:NM_001127208:exon11:c.T5890C:p.Y1964H	47	537	COSM4642835	106197557	D	T	B	2569
TET2	TET2:NM_001127208:exon9:c.T4129G:p.F1377V	47	240	-	-	D	D	B	2665
TET2	TET2:NM_001127208:exon5:c.3573_3794delinsCTAAGTGC:p.Q1191Hfs*2	31	3167	-	-	D	D	A	2680
TET2	TET2:NM_001127208:exon3:c.2217delT:p.P739fs	45,9	1727	-	-	-	-	A	2863

TET2	TET2:NM_001127208:exon6:c.3729_3733del:p.K1243fs	17,5	644	-	-	-	-	A	2969
TET2	TET2:NM_001127208:exon3:c.C1852T:p.Q618X	12	1589	-	-	-	-	A	3005
TET2	TET2:NM_001127208:exon7:c.G3845A:p.G1282D	50	2453	COSM1737890		D	D	B	3005
TET2	TET2:NM_001127208:exon7:c.G3863A:p.G1288D	35	2063	-	-	D	D	B	3666
TET2	TET2:NM_001127208:exon10:c.C4210T:p.R1404X	20	3556	COSM42037	-	-	-	A	3904
TET2	TET2:NM_001127208:exon11:c.T4983G:p.Y1661X	22	473	-	-	-	-	A	4413
TET2	TET2:NM_001127208:exon9:c.4060_4061del:p.R1354fs	48	605	COSM1318617	-	-	-	A	4924
TET2	TET2:NM_001127208:exon3:c.C521A:p.P174H	48	5203	-	rs146031219	B	D	C	5169
TET2	TET2:NM_001127208:exon3:c.2551delC:p.P851fs	40	6320	-	-	-	-	A	5744
TET2	TET2:NM_001127208:exon10:c.C4481G:p.S1494X	32	1643	-	-	-	-	A	5979
TET2	TET2:NM_001127208:exon11:c.T5618A:p.I1873N	40	403	-	-	D	D	B	6434
TET2	TET2:NM_001127208:exon6:c.T3743C:p.L1248P	34	1923	COSM211718	rs372179780	D	D	A	4407
TET2	TET2:NM_017628:exon3:c.3231dupC:p.H1077fs	48	3897	-	-	D	D	A	4819
TET2	TET2:NM_001127208:exon3:c.A1330G:p.T444A	47	6870	-	-	B	T	C	B2 1280
TET2	TET2:NM_001127208:exon6:c.3730_3731del:p.L1244fs	48	1358	COSM3719516	-	-	-	A	B2 1351
TET2	TET2:NM_001127208:exon11:c.G5152T:p.V1718L	54	1314	COSM41742	rs142312318	B	T	C	B2 588
TET2	TET2:NM_001127208:exon3:c.C521A:p.P174H	50	2179	-	rs146031219	B	D	C	B2(485)
TP53	TP53:NM_000546:exon10:c.T1096G:p.S366A	38	1263	COSM44832	rs17881470	B	T	B	B2 384

Supplemental Table S4: Characteristics of ET patients according to the validated scoring systems ELN, IPSET-survival and IPSET-thrombosis. HR: High Risk; IR: Intermediate Risk; LR: Low Risk

Classification	Class	Sex ratio (M/F)	Age Median [IQ range]	Hemoglobin (g/dL) median [IQ range]	Platelets (109/L) median [IQ range]	Leukocytes (109/L) median [IQ range]	Thrombosis history before diagnosis n (%)	CV risk history n (%)	Driver mutation JAK2/CALR/MPL/ Triple negative n (%)
ELN	HR n=113	0.71	71 [35;93]	14 [10.3;17]	743 [514;1490]	9.9 [2.3;15]	37 (33)	62 (55)	72/15/4/22 (64/13/3.5/19.5)
	IR n=44	0.63	53 [42;59]	14.6 [9.1;17.1]	736 [484;1760]	8.9 [4.3;21]	0 (0)	2 (6)	23/4/0/8 (53/9/0/18)
	LR n=33	0.57	31 [19;40]	14.3 [10.8;16.9]	896 [525;1895]	11.1 [5.1;20.7]	0 (0)	1 (3)	15/7/0/11 (45/22/0/33)
IPSET-Survival	HR n=46	0.92	72.3 [61.2;92.6]	14.3 [11.5;17]	770 [514;1490]	11.6 [4.3;26.4]	20 (43.5)	29 (63)	34/5/2/5 (74/11/4/11)
	IR n=96	0.57	60.4 [18.8;91.2]	14.2 [9.1;17.1]	781 [488;1895]	10 [2.3;21]	17 (17.8)	33 (34.4)	56/13/2/25 (58/14/2/26)
	LR n=48	0.66	49.64 [18.9;81.3]	14.4 [10.9;16.3]	752 [484;1590]	8.3 [4.3;11]	0 (0)	3 (6.25)	26/9/0/13 (54/19/0/27)
IPSET-Thrombosis	HR n=79	0.76	71 [38.9;92.6]	14.6 [10.3;17]	734 [514;1376]	10.7 [4.3;26.4]	33 (41.8)	52 (65.8)	73/2/1/3 (92/3/1/4)
	IR n=55	0.67	49.1 [21.7;87.1]	14.6 [9.1;17.1]	751 [484;1760]	9.9 [5.1;21]	4 (7.3)	12 (21.8)	43/6/1/5 (78/11/2/9)
	LR n=56	0.56	56.3 [18.8;91.2]	13.4 [10.8;16.6]	908 [505;1895]	9 [2.3;19.9]	0 (0)	1 (1.8)	0/19/2/35 (0/34/4/62)

Supplemental Table S5: Univariate analysis for survival, thrombosis and transformation using the "ABC" and "A" classifications. Signaling mutations for "A" classification were not tested because only one patient had such mutation

		Survival	Thrombosis	Transformation
Sex		**p=0.049**	p=0.56	**p=0.0047**
Age (> 60 years)		**p=0.00022**	p=0.21	p=0.25
History of thrombosis		p=0.72	p=0.18	p=0.49
Cardiovascular risk		p=0.5	**p=0.013**	p=0.47
Driver mutations	JAK2V617F	p=0.36	p=0.92	p=0.14
	CALR	p=0.14	p=0.34	p=0.77
	MPLW515	p=0.087	p=0.61	**p<0.0001**
Additional mutation ABC classification	Presence of at least one mutation	**p=0.012**	p=0.63	p=0.1
	Number of additional mutation (0, 1, ≥2)	**p=0.019**	p=0.45	p=0.26
	HMR mutations	p=0.18	p=0.8	p=0.77
	Signaling mutations	p=0.16	p=0.71	p=0.17
	Epigenetic mutation	p=0.12	p=0.48	p=0.62
	Splice mutation	**p=0.00051**	p=0.52	**p<0.0001**
Additional mutation A classification	Presence of at least one mutation	**p=0.0024**	p=0.98	p=0.054
	Number of additional mutation (0, 1, ≥2)	**p=0.00016**	p=0.49	p=0.11
	HMR mutations	**p=0.011**	p=0.29	p=0.68
	Epigenetic mutation	p=0.059	p=0.85	p=0.6
	Splice mutation	**p=0.00024**	p=0.57	**p<0.0001**

p values significant (ie < 0.05) indicated in bold

HMR (high-molecular risk) mutations: *ASXL1, EZH2, IDH1, IDH2* or *SRSF2* mutations (described by Vannuchi et al. in PMF)

Supplemental Table S6: Multivariate analysis of risk factors for all events (death+transformation+thrombosis) in the entire cohort (n=190) considering all additional mutations ("ABC" classification)

Parameter		Univariate analysis p value	Multivariate analysis HR [95% CI]	Multivariate analysis p value
Sex ratio (M/F)		0.27	-	-
ELN classification (High risk/ Low+Int risk)		0.00063	**3.72 [1.66-8.49]**	**0.0185**
Driver mutations (TN as reference)	*JAK2*V617F	0.25	**0.48 [0.24-0.98]**	**0.046**
	CALR	0.22	0.36 [0.13-1.03]	0.57
	*MPL*W515	0.0027	2.3 [0.5-10.7]	0.28
≥ 1 additional mutation (Yes/No)		0.038	**2 [1.1-3.8]**	**0.035**

TN: triple negative; P value significant (ie, <.05) indicated in bold.

Supplemental Table S7: Multivariate analysis of risk factors for all events (death+transformation+thrombosis) in the entire cohort (n=190) considering only pathogenic additional mutations ("A" classification)

Parameter		Univariate analysis p value	Multivariate analysis HR [95% CI]	Multivariate analysis p value
Sex ratio (M/F)		0.27	-	-
ELN classification (High risk/ Low+Int risk)		0.00063	**3.68 [1.61-8.4]**	**0.00198**
Driver mutations (TN as reference)	*JAK2*V617F	0.25	0.5 [0.24-1.01]	0.054
	CALR	0.22	0.39 [0.14-1.1]	0.54
	*MPL*W515	0.0027	1.9 [0.4-9.1]	0.42
≥ 1 additional mutation (Yes/No)		0.01	**2.2 [1.1-4.5]**	**0.03**

TN: triple negative; P value significant (ie, <.05) indicated in bold.

Supplemental Table S8: Multivariate analysis of risk factors for overall survival in the entire cohort (n=190) considering all additional mutations ("ABC" classification)

Parameter		Univariate analysis p value	Multivariate analysis HR [95% CI]	Multivariate analysis p value
Sex ratio (M/F)		0.049	-	-
ELN classification (High risk/Low+Int risk)		0.0038	**9.6 [1.26-73.3]**	**0.029**
Driver mutations (TN as reference)	*JAK2*V617F	0.36	0.39 [0.14-1.12]	0.08
	CALR	0.14	0.17 [0.02-1.4]	0.1
	*MPL*W515	0.087	1.1 [0.13-9.2]	0.93
≥ 1 additional mutation (Yes/No)		0.012	**3.5 [1.2-10.2]**	**0.022**

TN: triple negative; P value significant (ie, <.05) indicated in bold.

Supplemental Table S9: Multivariate analysis of risk factors for overall survival in the entire cohort (n=190) considering only pathogenic additional mutations ("A" classification)

Parameter		Univariate analysis p value	Multivariate analysis HR [95% CI]	Multivariate analysis p value
Sex ratio (M/F)		0.27	-	-
ELN classification (High risk/ Low+Int risk)		0.0038	**9.4 [1.24-71.9]**	**0.03**
Driver mutations (TN as reference)	*JAK2*V617F	0.36	0.39 [0.13-1.13]	0.08
	CALR	0.14	0.19 [0.02-1.6]	0.1
	*MPL*W515	0.087	1.1 [0.09-6.9]	0.82
≥ 1 additional mutation (Yes/No)		0.0024	**3.6 [1.3-10.2]**	**0.015**

TN: triple negative; P value significant (ie, <.05) indicated in bold.

Supplemental Table S10: Multivariate analysis of risk factors for hematological transformation in the entire cohort (n=190) considering all additional mutations ("ABC" classification)

Parameter		Univariate analysis p value	Multivariate analysis HR [95% CI]	Multivariate analysis p value
Sex ratio (M/F)		0.0047	**5.9 [1.2-29]**	**0.029**
ELN classification (High risk/Low+Int risk)		0.068	1.73 [0.42-7.2]	0.45
Driver mutations (TN as reference)	*JAK2*V617F	0.14	0.27 [0.05-1.3]	0.12
	CALR	0.77	0.38 [0.06-2.6]	0.33
	*MPL*W515	p<0.0001	2.98 [0.9-97.2]	0.088
Additional mutations	Signaling	0.17	5.8 [0.99-34.1]	0.052
	Epigenetic	0.62	-	-
	Splice	p<0.0001	4.8 [0.2-130]	0.34

TN: triple negative; P value significant (ie, <.05) indicated in bold.

Supplemental Table S11: Detail of prognostic scores for ET used in the manuscript

Score	ELN (Barbui et al., JCO 2011)	IPSET-SURVIVAL (Passamonti et al., Blood 2012)	IPSET-THROMBOSIS (Barbui et al., Blood 2012)
Risk factors for score calculation	Age ≥ 60y	Age ≥ 60y = 2 points	Age ≥ 60y = 1 point
	History of thrombosis	History of thrombosis = 1 point	History of thrombosis = 2 points
		WBC count ≥ 11 G/L = 1 point	
			Cardiovascular risk factor = 1 point
			*JAK2*V617F mutation = 2 points
High risk	Age ≥ 60y and/or History of thrombosis	Score equal to 0	Score equal to 0-1
Intermediate risk	-	Score equal to 1-2	Score equal to 2
Low risk	Age < 60y and no History of thrombosis	Score equal to 3-4	Score ≥ 3

y: years; WBC : white blood cell