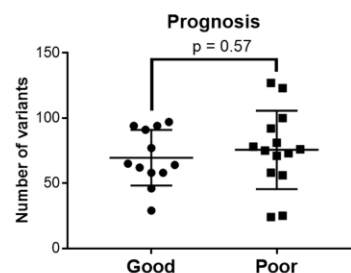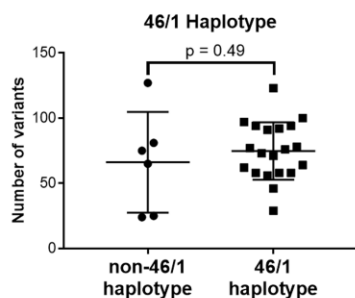# Supplementary Materials

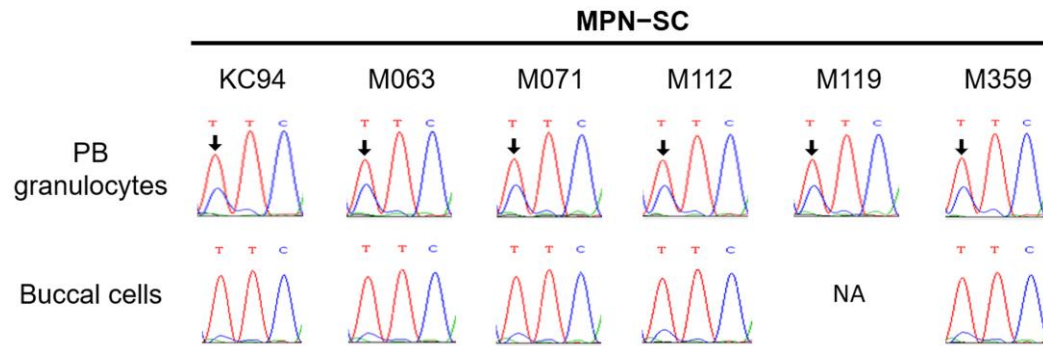**The Genomic Landscape in Philadelphia-Negative Myeloproliferative Neoplasm Patients with Second Cancers**

Hsu *et al*.

Supplementary Figures and Tables

Supplementary Figure S1. Genes with variants in MPN with SC stratified by 46/1 haplotype or by the prognosis of the SC subtypes. (A-B) Comparative illustration of genes with variants in individual MPN-SC patients stratified either by the presence or absence of 46/1 haplotype (A) or by the prognosis of the SC subtypes (B). Each stratification was represented by a corresponding color shown at the bottom. Prognosis-driven subcategorization of SC types was based on the stratification proposed by Marchetti M *et. al.*, as the "poor prognosis" SC (PPSC) encompassed a variety of aggressive cancers that occur in the stomach, esophagus, liver, pancreas, lung, ovary, nervous system, and so on [1].

Supplementary Figure S2. Assessing the nature of the *KRT6A* c.745T>C mutation in MPN patients. DNA from PB granulocytes and buccal mucosal cells (germline DNA) was extracted and subjected to Sanger sequencing respectively. The mutation was detected in granulocytes but not in buccal cells. The result confirms this mutation being somatic in nature. Arrows indicate the positions of the mutation.

Supplementary Table 1. Clinical profiles of enrolled MPN patients with second cancer

ID	Age[1]	Sex	Dx	Driver[2]	SC Type[3]	Origin	Disease sequence[4]
1	84.1	F	PMF	V617F	SqCC (Skin)	Ectoderm	MPN first
2	70.1	M	ET	*CALR*	BCC (Skin)	Ectoderm	MPN first
3	45.5	M	ET	V617F	BCC (Skin)	Ectoderm	MPN first
4	43.1	F	PV	V617F	DCIS (Breast)	Ectoderm	MPN first
5	67.4	M	ET	V617F	Colon	Endoderm	MPN first
6	57.6	F	PV	V617F	Acral Melanoma	Ectoderm	MPN first
7	78.5	M	PrePMF	V617F	DLBCL	Mesoderm	Concurrent
8	78.0	M	PMF	TN	MUO		Concurrent
9	92.9	M	PMF	V617F	HCC	Endoderm	Concurrent
10	75.9	M	PMF	V617F	Grade II Glioma	Ectoderm	Concurrent
11	80.2	M	ET	V617F	HCC	Endoderm	MPN first
12	65.5	M	PV	V617F	Lung	Endoderm	Concurrent
13	71.8	M	PV	V617F	Rectum	Endoderm	SC first
14	54.0	M	PV	V617F	HCC + Cholangio Ca	Endoderm	MPN first
15	67.3	F	ET	TN	Lung	Endoderm	MPN first
16	62.9	F	ET	V617F	Lung	Endoderm	MPN first
17	74.0	F	ET	V617F	Lung	Endoderm	MPN first
18	80.3	F	ET	V617F	Clear cell carcinoma	Mesoderm	MPN first
19	87.6	F	ET	V617F	HCC	Endoderm	MPN first
20	74.6	M	ET	*CALR*	TCC	Mesoderm	MPN first
21	70.6	M	ET	*CALR*	RCC	Mesoderm	SC first
22	45.9	M	ET	V617F	Lung	Endoderm	MPN first
23	88.7	M	ET	V617F	Ampulla Vater	Endoderm	Concurrent
24	89.7	M	ET	V617F	Pancreas	Endoderm	Concurrent
25[5]	80.8	F	ET	*CALR*	RCC	Mesoderm	MPN first
26	68.9	F	ET	TN	Lung	Endoderm	SC first
27	40.5	F	PV	Exon 12	Cervix	Mesoderm	SC first

[1] Age: Age in years

[2] Driver: Driver mutation: V617F: *JAK2*V617F; *CALR*: *CALR* Exon 9; TN: Triple negative; Exon 12: *JAK2* Exon 12

[3] SC: Second cancer: SqCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; DCIS: Ductal carcinoma in situ; DLBCL: Diffuse large B cell lymphoma; MUO: Metastasis of unknown origin; HCC: Hepatocellular carcinoma; Cholangio Ca: Cholangiocarcinoma; TCC: Transitional cell carcinoma; RCC: Renal cell carcinoma

[4] Disease sequence: We considered MPN and SC as concurrent diseases if the diagnoses were made within six months of each other.

[5] The DNA sample of this case failed quality control scrutinization and was excluded from WES analysis.

Supplementary Table 2. Comparison between tumor origins and either MPN subtypes or driver mutations

	Endoderm	Mesoderm	Ectoderm	*p*-value
MPN subtype				0.276
PV	3	1	2	
ET	10	4	2	
PMF/PrePMF	1	1	2	
Driver mutation				0.054*
JAK2	12	3	5	
CALR		3	1	
TN	2			

* *JAK2* vs. *CALR* mutation: *p* = 0.027

Supplementary Table 3. Baseline characteristics of MPN patients enrolled in the current study

Variables	With second cancer (n=26)	MPN Control (n=26)	*p*-value
Age[#], years	69.8 ± 14.8	71.7 ± 12.6	0.632
Male (%)	16 (61.5%)	16 (61.5%)	1.0
Diagnosis			1.0
PV	6	6	
ET	15	15	
PrePMF	4	4	
PMF	1	1	
Driver mutation			1.0
*JAK2*V617F	19	19	
JAK2 Exon 12	1	1	
CALR Exon 9	3	3	
MPL	0	0	
Triple negative	3	3	
*JAK2*V617F AB[^]	57.9% ± 33.4%	56.1% ± 30.2%	0.867
MF transform, No. (%)	2 (7.7%)	1 (3.8%)	1.0
Hydroxyurea exposure			0.579
Yes	12	14	
No	14	12	
Anagrelide exposure			1.000
Yes	7	7	
No	19	19	
Ruxolitinib exposure			0.638
Yes	2	3	
No	24	23	
HU exposure time*	58.4 ± 65.1	60.5 ± 57.3	0.930
Follow-up duration*	84.6 ± 70.6	75.8 ± 64.9	0.644

[#] Age at diagnosis of MPN; mean ± standard deviation

[^] AB: Allele burden

* Time in months; mean ± standard deviation; HU: hydroxyurea

Supplementary Table 4. Variants in *SYNE2*, *ACAN*, and *PDZD7* genes identified in our MPN patients with second cancers

	Coding sequence mutation	Amino acid mutation	Case number	Cancer type*
SYNE2	c.7243A>G	p.Met2415Val	1	HCC
	c.10802A>G	p.Gln3601Arg	1	RCC
	c.12001_12002delTGinsCA	p.Trp4001Gln	1	Melanoma
	c.14939A>C	p.Asp4980Ala	1	DLBCL
	c.15740C>T	p.Thr5247Ile	1	Lung cancer
	c.18509C>T	p.Thr6170Met	1	Lung cancer
	c.19582C>A	p.Gln6528Lys	1	TCC
ACAN	c.151_152insTAAAGATAAG	p.Cys51fs*43	1	Lung cancer
	c.1666A>T	p.Arg556Trp	1	MUO
	c.2153C>A	p.Thr718Lys	1	Rectal cancer
	c.2842G>C	p.Ala948Pro	1	Glioma
	c.4150_4151insCTGCCCCTGGAGTAGAGGACATCAGCGGGCTTCCTTCTG	p.Ala1384_Ala1385insAlaProGlyValGluAspIleSerGlyLeuProSerGlyGluValLeuGluThrThr	1	Lung cancer
	c.4229_4230insTGCCTAGGAGTGAATGCACTAAAGCAGGGTTTTTCAAA	p.Ser1411fs*1	1	BCC
PDZD7	c.715T>A	p.Ser239Thr	1	HCC
	c.880T>G	p.Tyr294Asp	1	Lung cancer
	c.1267G>A	p.Ala423Thr	1	Melanoma
	c.1529G>A	p.Gly510Asp	1	Rectal cancer
	c.2126A>C	p.His709Pro	1	DCIS (Breast)

* Cancer type: the subtype of SC in our enrolled MPN patients. HCC: Hepatocellular carcinoma; RCC: Renal cell carcinoma; DLBCL: Diffuse large B cell lymphoma; TCC: Transitional cell carcinoma; MUO: Metastasis of unknown origin; BCC: Basal cell carcinoma; DCIS: Ductal carcinoma in situ.

Supplementary Table 5. Information on PCR primers used in this study

Primer	Sequence (5'-3')
h_SYNE2_M2415V_F	GTCTATAATCCAAGTAAACAGAAGAAACT
h_SYNE2_M2415V_R	GACTTTTCACTCTGTACAAGCAAGT
h_SYNE2_Q3601R_F	CTATTCAGGCATTTCATTAATTGTTGCA
h_SYNE2_Q3601R_R	TGGATACAAGACTGTGGGCCT
h_SYNE2_W4001Q_F	GCATATTTGCTGTGAGTGATTAAAACT
h_SYNE2_W4001Q_R	CACTGCACCACTAGAAAGGCT
h_SYNE2_E4695A_F	GATAACTATGTGTACTTCTCTGACTTA
h_SYNE2_E4695A_R	CTGTGTACCTGGCTCTTAGCAT
h_SYNE2_D4980A_F	TTAGGACTGATCAACATAGGAGGA
h_SYNE2_D4980A_R	CATTCGCCCAAGTCTTACCC
h_SYNE2_T6170M_F	CGGGTGTCAGGGGATAAAAGA
h_SYNE2_T6170M_R	GCTGGCTACTTAGAGGAAGTCA
h_ACAN_T692P_F	AAGGACGGGTCACTGGTAAGA
h_ACAN_T692P_R	GTAGGAAGACAGGGGTATGCA
h_ACAN_R556W_F	GTGCATCTACCAGCCCCTG
h_ACAN_R556W_R	CTTTGTATCGCCCTGTCAGCT
h_CEP164_Q525R_F	CACACACTGTACTCCCCAGTT
h_CEP164_Q525R_R	TACACACAGAGCAGCCACCA
h_PDZD7_G510D_F	CTCCTGAACCCCATTTTCAGACT
h_PDZD7_G510D_R	CAGTATGCACCCTCATCTGC
h_PDZD7_S239T_F	GTGGTGGGTGGCAGTGGA
h_PDZD7_S239T_R	TGCACCAGTCAAGGTCCTTGA
h_PDZD7_Y294D_F	GAAGTTGAAAGTTTACTCCCATCCT
h_PDZD7_Y294D_R	CGAGGTCTACCTATATGGCCAA
h_PDZD7_A423T_F	CTAAGCCCAAGGGTGGTCT
h_PDZD7_A423T_R	GATGTGAGACAGGTTTTGGACCA
h_KRT6A_239_249_F	GGTGCGGTTCCTGGAGCA
h_KRT6A_239_249_R	GTGCTCTTCATTTCCACGGACAT

Reference

1. Marchetti, M.; Ghirardi, A.; Masciulli, A.; Carobbio, A.; Palandri, F.; Vianelli, N.; Rossi, E.; Betti, S.; Di Veroli, A.; Iurlo, A.; et al. Second cancers in MPN: Survival analysis from an international study. *Am J Hematol* **2020**, *95*, 295-301, doi:10.1002/ajh.25700.