Genomic analysis of myeloproliferative neoplasms in chronic and acute phases

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Supplemental Data

Supplemental methods

DNA sequencing was performed on a MiSeq (Illumina) with 2x150-bp, paired-end reads according to the manufacturer's instructions. The read sequencing was aligned to human reference genome (UCSC hg19) (http://hgdownload.cse.ucsc.edu/) using Burrows-Wheeler Aligner.[1] Samples were sequenced at about 700 x coverage which allowed the identification of missense, nonsense, splicing, frameshift and nonframeshift mutations with quantitative data on variant allele frequency (VAF). Bam files were processed according to the workflow recommended for variant analysis with GATK. Briefly, reads groups were added with PICARD tools version 1.91(1451) (http://broadinstitute.github.io/picard/). Local realignment and score recalibration were done using GATK version 2.5-2-gf57256b. SNVs calling was done with FreeBayes version 0.9.9 [2] with a minimal alternate variant frequency and coverage set at 2% and 10. Indel calling was done using GATK haplotype caller version 2.5-2-gf57256b [3] with default parameters. The variants, i.e SNVs and Indels, were annotated with RefSeq annotation, dbsnp129, dbsn138NonFlagged, 1000 Genome and ESP6500 population frequencies, COSMIC V68, Clinvar, and predicted effects score on the protein using the Annotate Variation Software (ANNOVAR, version 2013-11-12). Mutations predicted as "neutral" were excluded. SNVs were further filtered. Known variants found in dbsnp129 and dbsnp137 with a MAF > 1% (1000g or ESP6500) or suspected (according data literature and VAF percentage) to be germline were removed. Finally, low frequency SNVs and indels suspected to be false positive were systematically inspected with IGV version 2.3.32. [4]

In addition, JAK2V617F was determined by real-time quantitative PCR; MPLW515 was detected by Sanger sequencing and *CALR* exon 9 mutations were determined according to standard protocols and as previously described [5] by fragment analysis techniques followed by Sanger sequencing.

Supplemental Table 1 Characteristics of MPN and post-MPN AML cohorts.

	sMPNs (n=40)	MPN/AML paired samples (n=17)		Total post-MPN AMLs (n=38) including 17 post-tMPN AMLs
		tMPNs	Post-tMPN AMLs	
MPN diagnosis and mutated genes driver (*JAK2* / *CALR* / *MPL*)	ET (n=30) *JAK2* n= 11 *CALR* n= 12 *MPL* = 1 TN n= 6	ET (n=5) *JAK2* n= 4 *MPL* n= 1	ET (n=5) *JAK2* n= 3 *MPL* n= 1 TN n= 1	ET (n=14) *JAK2* n= 5 *CALR* n= 3 *MPL* n= 1 TN n= 5
	PV (n=5) *JAK2* = 5	PV (n=3) *JAK2* = 3	PV (n=2) *JAK2* = 2	PV (n=6) JAK2 = 6
	PMF (n=4) *JAK2* n= 3 *CALR* n= 1	PMF (n=4) *JAK2* n= 4	PMF (n=4) *JAK2* n= 3 TN n= 1	PMF (n=7) *JAK2* n= 4 *CALR* n= 1 TN n= 2
	post-ET MF (n=1) *CALR* n= 1	post-ET MF (n=3) *JAK2* n= 1 TN n= 2	post-ET MF (n=3) *JAK2* n= 1 TN n= 2	post-ET MF (n=5) *JAK2* n= 2 *CALR* n= 1 TN n= 2
		post-PV MF (n=2) *JAK2* n= 2	post-PV MF (n=3) *JAK2* n= 3	post-PV MF (n=6) *JAK2* n= 5 TN n= 1
Average age at diagnosis (min-max), years	46.6 (10-71)	60.4 (25-78)	68.9 (37-82)	70.5 (37-90)
	51 (10-78)			
Median time from diagnosis to sample (min-max), years	12.9 (2-24)	5.7 (0-25)	0 (0-0.5)	0 (0-0.7)
	10.8 (0-25)			
	Average time to diagnosis (min-max), years	**Median time to transformation (min-max), years**		
	17.9 (11-28)	8.5 (1-29)		10.6 (0.4-29)

sMPNs, “steady” MPNs; tMPNs, “transformed” MPNs; ET, essential thrombocytemia; PV, polycythemia vera; PMF, primary myelofibrosis; AML, acute myeloid leukemia; TN, Triple-negative.

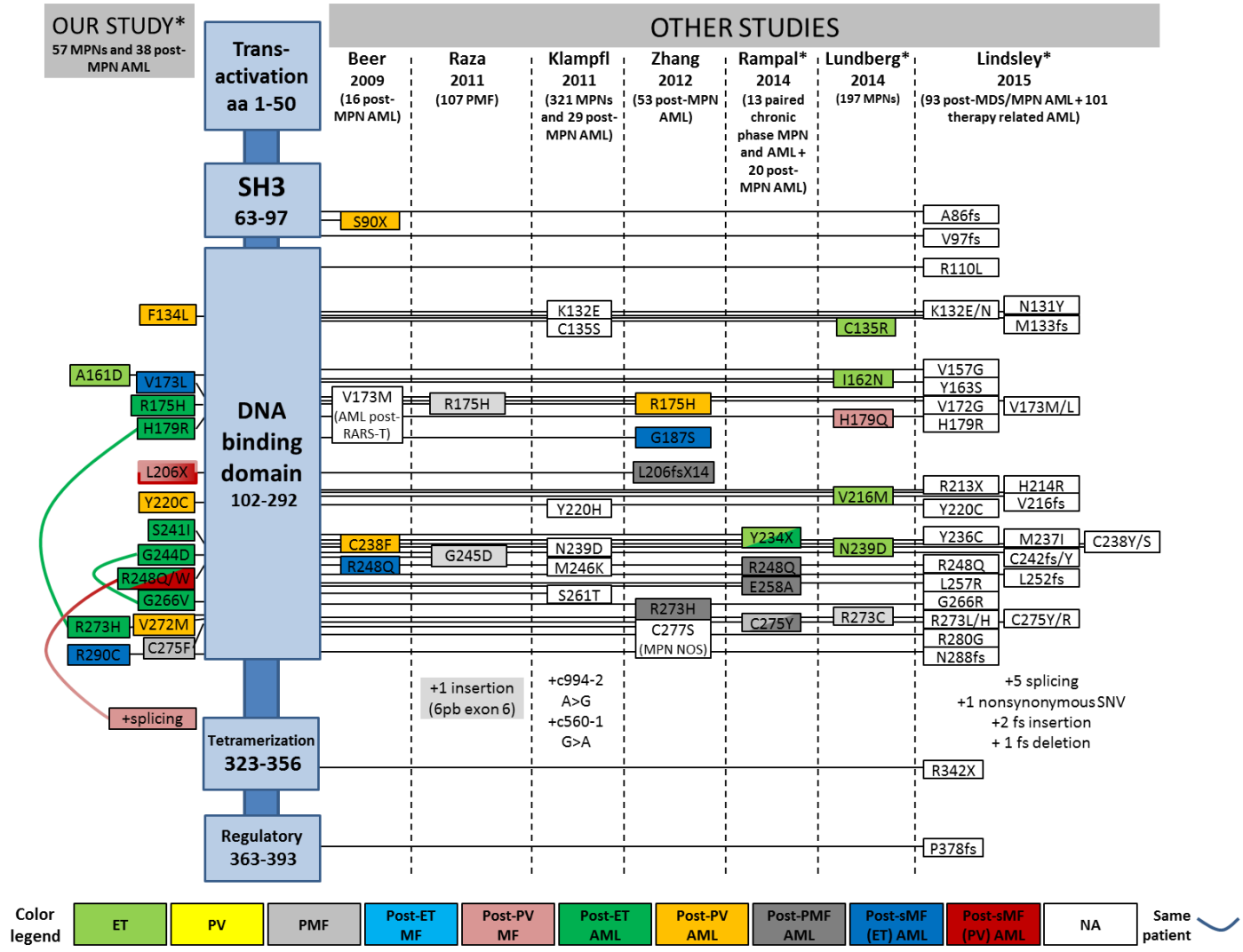
Supplemental Table 2 Disease story, previous therapies, characteristics of samples, cytogenetics and molecular results in 95 MPNs and post-MPN AML

Supplemental Table 3 List of the 79 genes in panel sequencing

Genes								
MPN driver	Tumor suppressor	DNA methylation	RNA splicing	Cohesin complex	Chromatin modifier	Transcription factor	Signaling	Others
CALR	NPM1	DNMT3A	PRPF8	RAD21	ASXL1	CEBPA	BRAF	BARD1
JAK2	RB1	IDH1	SF3B1	SMC1A	BCOR	CREBBP	CBL	BRCC3
MPL	TP53	IDH2	SRSF2	SMC3	BCORL1	CUX1	CBLB	C7ORF55
		TET2	U2AF1	STAG1	EED	DAXX	CSF3R	C7ORF55-LUC7L2
			ZRSR2	STAG2	EZH2	ETV6	DOK1	
					KDM6A	FBXW7	DOK2	COPA
					KMT2A	GATA1	FLT3	KLHL6
					PHF6	GATA2	GNAS	LAMB4
					SETBP1	IKZF1	GNB1	LUC7L2
					SUZ12	IRF1	IL7R	MAML1
						NFE2	KIT	MFSD11
						NOTCH1	KRAS	chr15:65845419-65845525
						NOTCH2	MYD88	
						PAX5	NCSTN	chr16:820183-820277
						RUNX1	NF1	
						WT1	NRAS	chr17:74732532-74732630
							PDGFRA	
							PTEN	chr4:153258807-153259248
							PTPN11	
							PTPRT	chr7:139102209-139112272
							RIT1	
							SCRIB	chr8:144895127-144895212
							SH2B3	

Supplemental Table 4 Description of all mutations found in the 79 selected genes

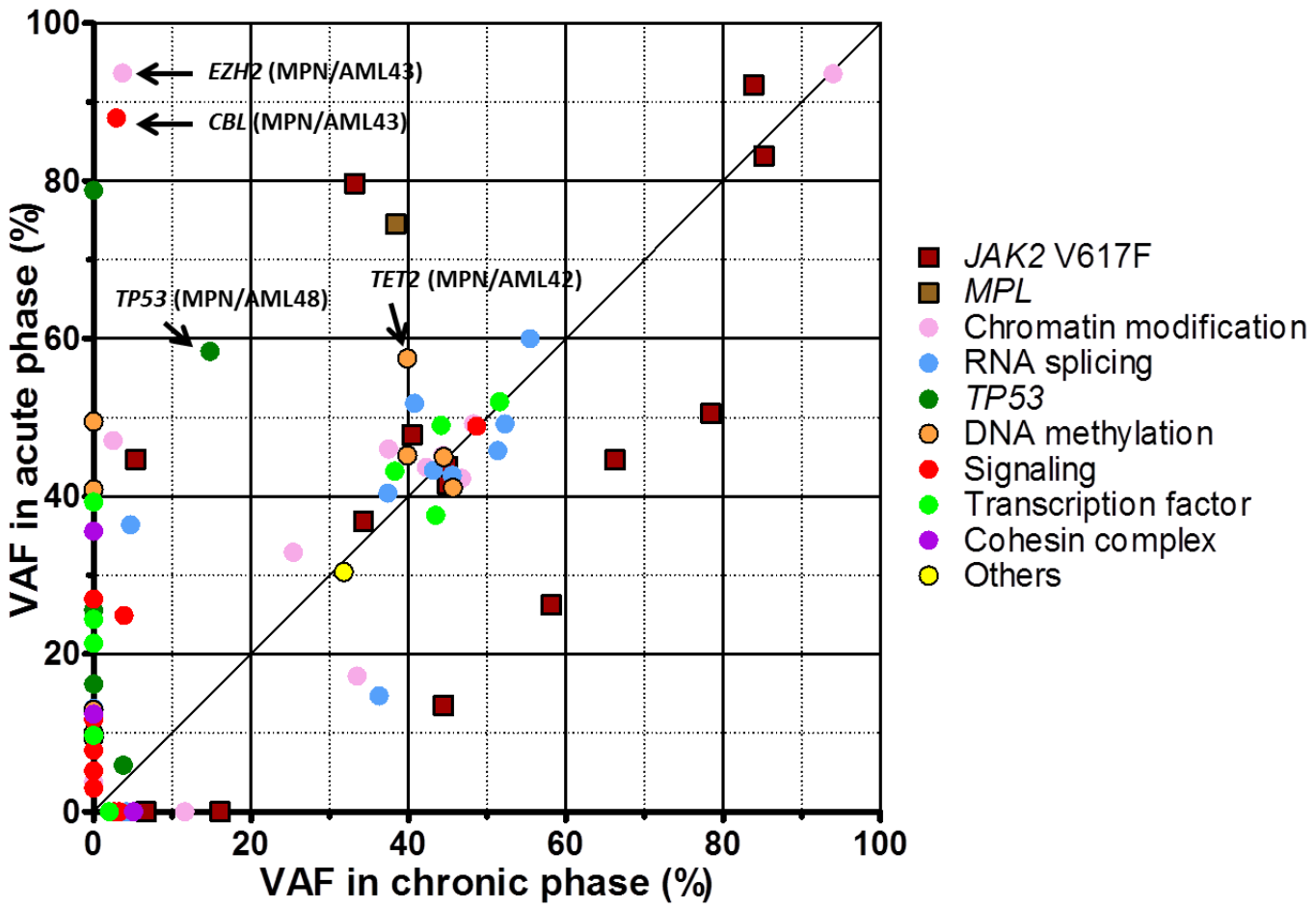
Supplemental Figure 1



Supplemental Figure 1 *TP53* mutations in MPNs and post-MPN AMLs at the protein level. The vast majority of missense mutations affect the central DNA-binding domain of TP53. *TP53* mutations were associated with ET, PV, post-ET/PV and post-ET/PV AML but rarely observed in PMF and post-PMF AML. This observation is in agreement with previous studies.[6-12]

sMF = secondary myelofibrosis; NA = not available. *NGS technology.

Supplemental Figure 2

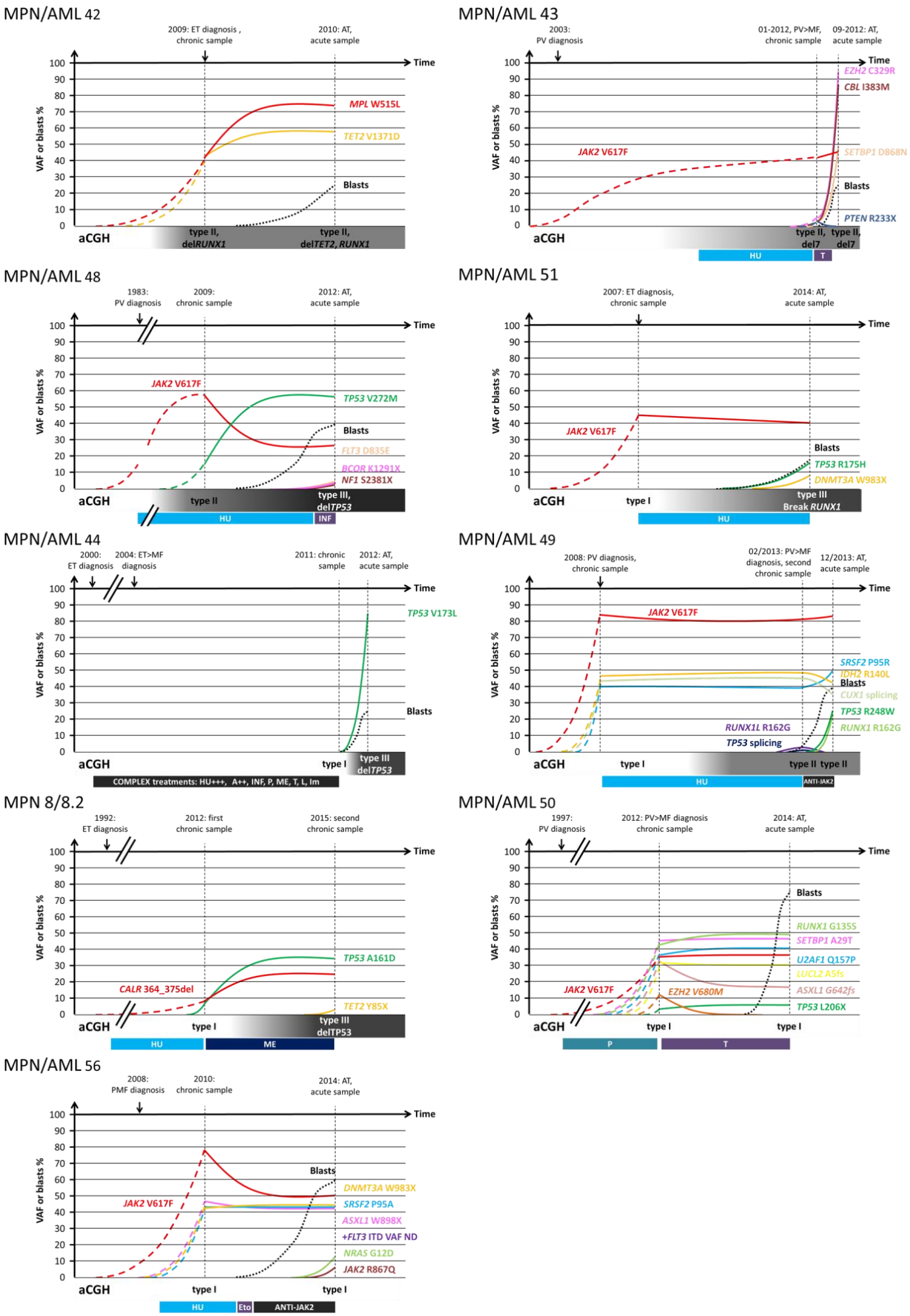


Supplemental Figure 2 Variant allele frequency (VAF) of gene mutations in chronic phase and acute phase of 17 matched samples. Squares correspond to driver mutations; red squares correspond to JAK2V617F mutation; brown squares correspond to MPLW515 mutation. Each circle corresponds to one mutation associated with cellular function represented by different colors; diagonal line represents x = y, points over this line represent mutations with increase VAF at acute phase, points below this line represent mutations with decreased VAF at acute phase, and points on this line represent mutations with steady VAF. Points on the left edge represent mutations detected only at acute phase and points on the bottom edge represent mutations not detected any more at acute phase.

For the most part of genes, there was no increase in VAF in acute phases (diagonal line) except for *JAK2*, *MPL*, *TET2*, *TP53*, *EZH2* and *CBL* mutations (VAF elevation was coupled with the loss of the second allele for *TET2*, *TP53* and *EZH2* genes).

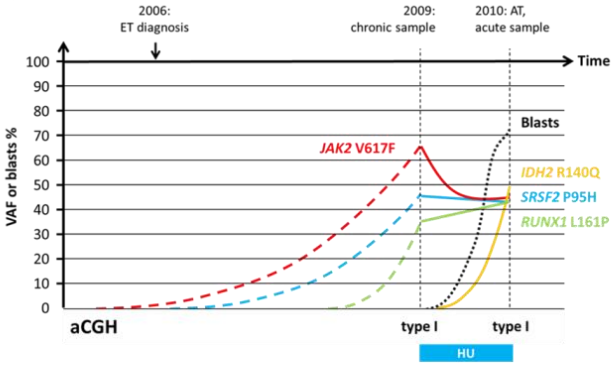
Three mutations (appeared at acute phase) but could not be included in this figure because VAF was not evaluable (*FLT3* ITD (AML56); *LAMB4* G451fs (AML54); *RB1* P776fs (AML53)).

Supplemental Figure 3A

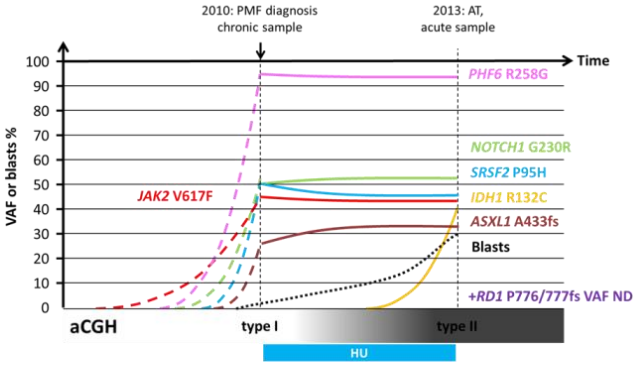


Supplemental Figure 3B

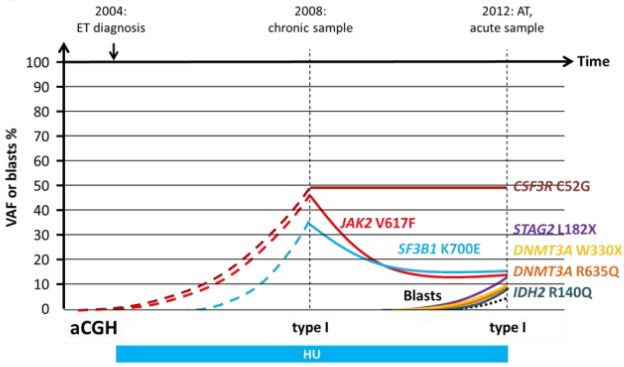
MPN/AML 41



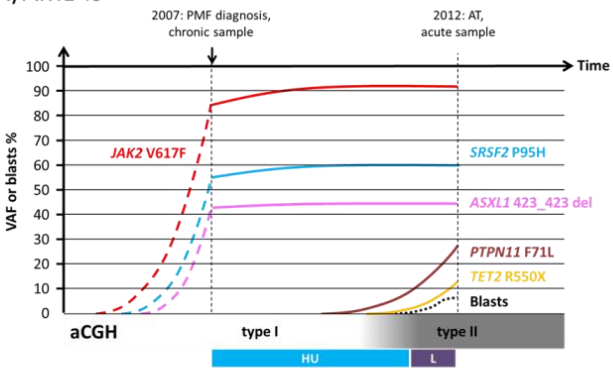
MPN/AML 53



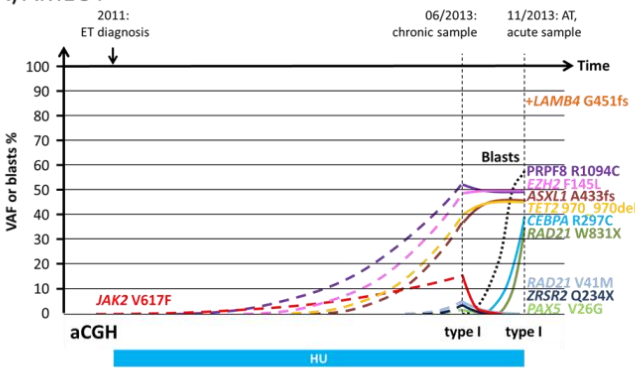
MPN/AML 55



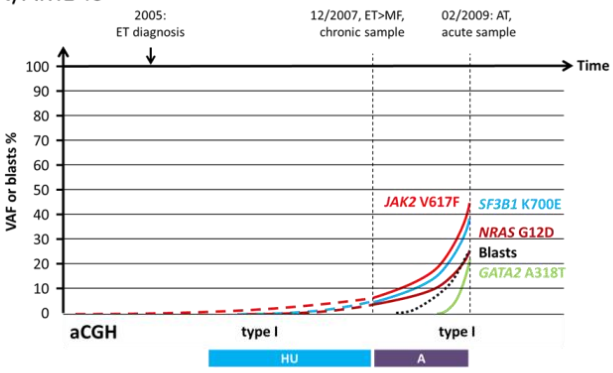
MPN/AML 45



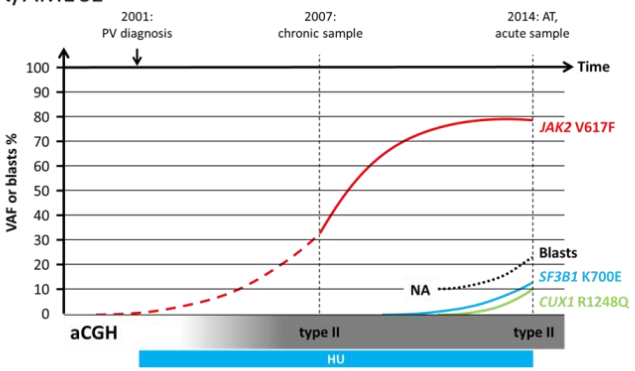
MPN/AML 54



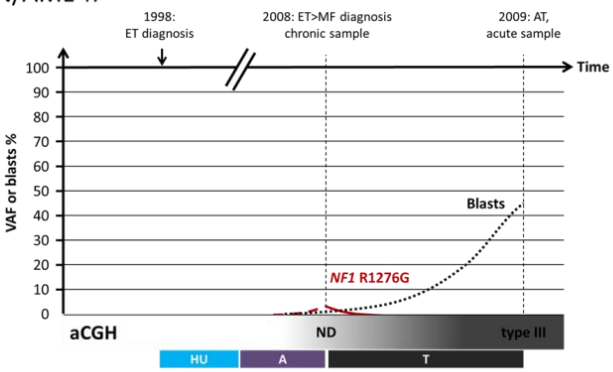
MPN/AML 46



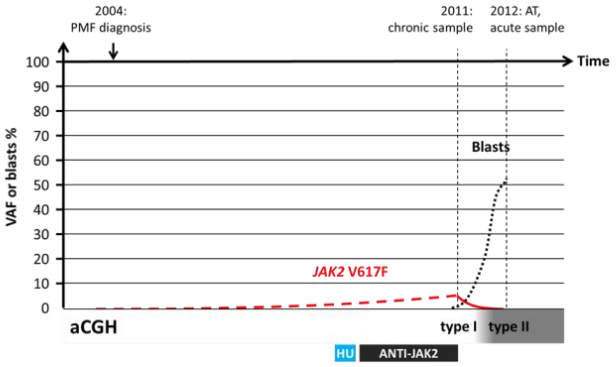
MPN/AML 52



MPN/AML 47



MPN/AML 57



Supplemental Figure 3 Evolution of VAF mutation in 17 matched paired samples (MPN/AML cases) and one MPN in chronic phase (sMPN) with sequential samples. Blast percentage evolution and different therapies used are represented. Vertical stippled lines represent samples obtained and analyzed.
A: For most patients, we could observe the apparition or increase of VAF mutation associated with the evolution to acute phase or to a long disease progression (MPN8/8.2). VAF elevation was coupled with the loss of the second allele for *TET2*, *TP53* and *EZH2* genes. Therapeutic intervention could provide potent selective pressure for the expansion of resistant variants (*TP53* mutations and JAK2R867Q).
B: 9 other matched paired samples; only two cases (MPN/AML47 and 57) were unmutated in acute phase.
VAF, Variant allele frequency**;** A, anagrelid; INF, interferon; ME, melphalan; HU, hydroxyurea; P, pipobroman; T, thalidomide; Eto, etoposide; L, lenalidomide; Im, imatinib; ET, essential thrombocytemia; PV, polycythemia vera; PMF, primitive myelofibrosis; PV>MF, post-PV myelofibrosis; ET>MF, post-ET myelofibrosis, AT, acute transformation.

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