JAK2 or *CALR* mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes

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Supplemental Methods and Tables

Supplemental Methods

Diagnosis of *JAK2* mutated polycythemia vera according to the criteria of the British Committee for Standards in Hematology

Diagnosis of *JAK2* mutated polycythemia vera requires both of the following 2 criteria to be present: 1) high hematocrit (>0.52 in men, >0.48 in women) or raised red cell mass (>25% above predicted); 2) mutation in *JAK2*, i.e., *JAK2* (V617F) or mutation of exon 12. These criteria are very similar to the major criteria of the WHO classification, which considers hemoglobin level (>18.5 g/dL in men, >16.5 g/dL in women, or other evidence of increased red cell mass) instead of hematocrit. The only difference is that the WHO classification requires, in addition to the two major criteria, at least one minor criterion (bone marrow biopsy showing hypercellularity, serum erythropoietin below the normal range, or endogenous erythroid colony formation in vitro).

In order to assess polycythemic transformation of essential thrombocythemia, we adopted the criteria of the British Committee for Standards in Hematology since sequential evaluations of bone marrow biopsy were not available in most patients.

References

1. McMullin MF, Bareford D, Campbell P, et al. Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. *Br J Haematol*. 2005;130(2):174-195.

2. McMullin MF, Reilly JT, Campbell P, et al. Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis. *Br J Haematol*. 2007;138(6):821-822.

Sanger sequencing of *CALR* exon 9

The following M13-tagged primers were used for PCR

- Forward 5'-tgtaaaacgacggccagtAAGCAAGGGCTATCGGGTAT-3'
- Reverse 5'-caggaaacagctatgaccAACCAAAATCCACCCCAAAT-3'

and for sequencing

- Forward 5'-TGTAAAACGACGGCCAGT-3'.

Supplemental Table 1. Patients studied in the present work in the analyses of genotype/phenotype relationships and clinical outcomes.

Myeloproliferative neoplasm	*JAK2* (V617F) mutated (%)	*CALR* exon 9 mutated (%)	*JAK2/MPL/CALR* wild-type (%)	All genotypes
Patients belonging to the initial patient population				
Essential thrombocythemia	466	176	75	717
Polycythemia vera	468	-	-	468
All patients with PV or ET				1185
Patients with secondary myelofibrosis evaluated for mutant allele burden				
Post-ET myelofibrosis	18	10		28
Post-PV myelofibrosis	55			55
All patients with secondary myelofibrosis				83
Total no.				*1268*

Supplemental Table 2. Type and frequency of *CALR* mutations in patients with essential thrombocythemia with nonmutated *JAK2* and *MPL*. The nomenclature adopted here is that of Klampfl et al [N Engl J Med. 2013 Dec 19;369(25):2379-90. Epub 2013 Dec 10].

CALR	Change		Frequency	
mutation	Nucleotide	Aminoacid*	No.	%
Type 1	c.1092_1143del	AAEKQMKDKQDEEQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	81	46.0
Type 2	c.1154_1155insTTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDNCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	67	38.1
Type 3	c.1095_1140del	AAEKQMKDKQDEEQRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	2	1.1
Type 4	c.1102_1135del	AAEKQMKDKQDEEQRLRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	4	2.3
Type 5	c.1091_1142del	AAEKQMKDKQDEGQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	2	1.1
Type 6	c.1094_1139del	AAEKQMKDKQDEERRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 8	c.1104_1137del	AAEKQMKDKQDEEQRLKRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 9	c.1140del	AAEKQMKDKQDEEQRLKEEEEDKKRKEEERQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	2	1.1
Type 10	c.1154delinsTGTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDMCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 13	c.1100_1134delinsA	AAEKQMKDKQDEEQRQRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 16	c.1102_1137delinsCA	AAEKQMKDKQDEEQRLQRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 18	c.1104_1155del	AAEKQMKDKQDEEQRLKRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 20	c.1118_1136del	AAEKQMKDKQDEEQRLKEEEEGRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 21	c.1118_1145delinsCGTTTA	AAEKQMKDKQDEEQRLKEEEEALRGQGG-	1	0.6
Type 22	c.1120_1123del	AAEKQMKDKQDEEQRLKEEEEDNAKRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 28	c.1131_1152del	AAEKQMKDKQDEEQRLKEEEEDKKRKRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 32	c.1153_1154delinsTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 34	c.1154_delinsCTTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDTCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 36	c.1155_1156insTGTCG	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDKCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	2	1.1
Type 37§	c.1091-1124del	AAEKQMKDKQDEDAKRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 38§	c.1101-1152del	AAEKQMKDKQDEEQRLRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 39§	c.1109-1145del	AAEKQMKDKQDEEQRLKEERTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Type 40§	c.1113A>C;1122-1140del	AAEKQMKDKQDEEQRLKEEDEDKRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-	1	0.6
Total			176	100
Wild-type reference sequence		AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDKEDDEDKDEDEEDEEDKEEDEEEDVPGQAKDEL-		

* The aminoacid sequence starting from codon A352 is reported. Acid and basic residues are in red and blue, respectively.

§ Novel *CALR* mutations with respect to *CALR* abnormalities described in the original report by Klampfl et al [N Engl J Med. 2013 Dec 19;369(25):2379-90. Epub 2013 Dec 10].