The presence of ASXL1 mutations as well as a total number of Myeloid Driver Mutations higher than two is strongly associated with the diagnosis of Primary Myelofibrosis as opposed to Essential Thrombocythemia.

Primary Myelofibrosis (PMF) and Essential Thrombocythemia (ET) are myeloproliferative neoplasms with similar genetic backgrounds. Both diseases are characterized, at the molecular level, by mutations in the genes JAK2, MPL and CALR. In addition recurring mutations is several other genes have been described in myeloid malignancies in general. Although the differential diagnosis between PMF and ET may be straight forward in most cases, there is a significant clinical and pathologic overlap between these two conditions, making the differential diagnosis difficult sometimes, mostly between early PMF and ET. With the goal of utilizing genomic information to better differentiate ET from PMF we decided to identify and compare all genomic alterations present in patients with ET and PMF, through whole exome / genome sequencing of paired granulocytes and skin. A total of 84 patients with either PMF (N=48) or ET (N=36) were analyzed. Whole-exome targeted capture was carried out using the Agilent SureSelect Kit The exome library was sequenced in Illumina HiSeq2000. Somatic variants calls were generated by combining the output of Somatic Sniper (Washington University), Mutect (Broad Institute) and Pindel (Washington University). The combined output of these 3 softwares was further filtered by in-house criteria in order to reduce false-positive calls. For this work, other myeloid driver mutations were defined as mutations occurring recurrently in myeloid malignancies in the medical literature. Fisher´s exact test was used for statistical comparisons. The most common mutated genes after JAK2 and CALR were ASXL1 (n=16), TET2 (n=9) and DNMT3A (n=9).

1 - The presence of additional myeloid driver mutations concomitant with either CALR or JAK2 mutations was strongly associated with a diagnosis of PMF. (Table 1 and 2):

2 – The presence of 3 or more total myeloid driver mutations was strongly associated with a diagnosis of PMF

3 – The presence of ASXL1 mutations was strongly associated with a diagnosis of PMF (Table 4)

In order to validate our findings in an independent cohort of patients, we performed the same analysis using data from 2 published studies using myeloid multi-gene panels in ET and PMF (Nangalia J, NEJM 2013) (Lundberg P, Blood, 2014). We pooled together all patients with ET (N=117) and PMF (N=56) from both studies and repeated the four previous analyses (Table 5 – 8)

We have demonstrated that ASXL1 mutations as well as a number of myeloid driver mutations higher than two is strongly associated with PMF. This information may be useful in the near future to improve the differential diagnosis between ET and PMF.

Table 1.

	CALR_Single	CALR_plus
ET	6	0
PMF	5	9

P=0.014

Table 2.

	JAK2_Single	JAK2_Plus
ET	17	5
PMF	7	20

P=0.0005

Table 3.

	mut<3	mut>2
TE	28	2
PMF	25	21

P= 0.0002

Table 4.

	ASXL1+	ASXL1-
TE	1	35
PMF	15	33

P=0.0007

Table 5.

	CALR_Single	CALR_plus
ET	29	5
PMF	8	7

P= 0.028

Table 6.

	JAK2_Single	JAK2_Plus
ET	53	23
PMF	16	19

P=0.020

Table 7.

	ASXL1+	ASXL1-
TE	4	113
PMF	14	42

P=3.9E-05

Table 8.

	mut<3	mut>2
TE	110	6
PMF	42	14

p=0.0005