P984 MUTATIONAL PATTERNS IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN)

**Topic:** 15. Myeloproliferative neoplasms - Biology & Translational Research

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**Background:** According to the 2016 WHO classification, Ph-negative MPN include essential thrombocythemia (ET), polycythemia vera (PV), prefibrotic myelofibrosis (prePMF) and overt fibrotic myelofibrosis (PMF). The molecular basis of MPN was clarified with the discovery of driver mutations in JAK2, MPL and CALR genes, but the biological landscape is more complex than initially assumed. Subclonal somatic mutations, also found in other myeloid neoplasms (MN), have been described and are associated with disease progression and poor prognosis. Those mutations target epigenetic regulators of DNA (DNMT3A, TET2, IDH1/IDH2), genes involved in the chromatin structure (ASXL1, EZH2), spliceosome genes (SF3B1, SRSF2), transcription factors and tumor suppressor genes (RUNX1, TP53).

**Aims:**

To correlate driver mutations, subclonal variants, clinical data and diagnosis in 509 MPN patients (236 ET, 91 prePMF, 182 PMF) and to define a diagnostic and prognostic role of these variants.

**Methods:**

We studied DNA variants with the Illumina Nextera Rapid Capture Custom Enrichment Kit and HiSeq2500 platform. We selected a panel of 81 genes based on prior implication in the pathogenesis of MN. Inclusion criteria were (i) a diagnosis of ET, prePMF or overt PMF according to the 2016 WHO criteria, (ii) a peripheral blood sampling at diagnosis or before therapy administration.

**Results:** Overall, we detected 598 additional somatic variants. PMF showed a larger proportion of patients (pts) with at least one additional variant compared with prePMF and ET (86.3% vs 42.9% vs 34.8%, p<0.001) and a higher average number of variants per pts. These findings suggest that PMF is not an advanced stage of prePMF, but a different clinical entity with high tendency in variants accumulation.

Considering the driver mutation, the number of pts with at least one additional variant was significantly different among the molecular subgroups (CALR 46.5%, JAK2 58.9%, MPL 82.1% TN 31.1%, p<0.001).

Somatic mutations were grouped based on their role in the cell cycle. In ET the most commonly mutated genes belong to the DNA methylation regulation and the most frequently involved gene was TET2. In PMF, chromatin structure, RNA splicing and DNA methylation were the most recurrently involved pathway and ASXL1 and TET2 were the most frequently mutated genes. In prePMF most of the variants impaired DNA methylation and RNA splicing and TET2 and SF3B1 were the most frequently mutated genes. RNA splicing and DNA methylation are often involved in myelodisplastic syndromes (MDS). Thus, PMF and prePMF share molecular signatures with MDS, suggesting that MN are part of a continuum spectrum of diseases.

There was no association between driver mutations and additional variants, except for the spliceosome genes. The rate of splicing factors alterations in CALR mutants was significantly lower compared with JAK2 or MPL mutants (3.9% vs 22.3% vs 35.7%, p<0.001).

We finally correlated additional mutations and clinical data (blood count, splenomegaly, LDH, CD34+ circulating...
cells), overall survival (OS) and progression to blast phase (BP). We focused on the most commonly mutated genes, defined as mutations detected in at least 10 pts (>5%): ASXL1, DNMT3A, TET2, EZH2, SRSF2. ASXL1 was associated with anemia and splenomegaly, a higher risk of leukemic evolution (sHR=4.5, 95%CI:1.7-11.8, p=0.003) and shorter OS (HR=6.2 (4.3-9.0) p<0.001); SRSF2 had an impact on BP evolution and OS, while EZH2 showed an impact on OS.

Summary/Conclusion: We suggest that not only histological and clinical criteria, but also distinct mutational patterns might differentiate ET, prePMF and overt PMF.