Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic disorders characterized by the increased production of erythroid, megakaryocytic, or granulocytic cells. BCR-ABL1-negative MPNs primarily include Essential Thrombocytemia (ET), Polycythemia Vera (PV), and Primary Myelofibrosis (PMF). These classical MPNs harbor oncogenic driver mutations in JAK2, CALR, and MPL genes and the reported frequencies of these mutations are approximately 95%, 0%, and 0% in PV; 45%, 20%, and 3% in ET; and 60%, 25%, and 7% in PMF. MPN driver mutations result in the myeloproliferative phenotype due to intracellular JAK-STAT signaling and their downstream effectors. Mutational profiling is therefore a primary component of routine clinical diagnostic evaluation. However, there is a subset of MPNs that lack alterations in these three genes which are referred to as “triple-negative” MPNs.

Materials and Methods

Specimens: 2157 blood or bone marrow samples previously tested in our laboratory by Next-generation sequencing (NGS) were included in this study. Samples selected on the basis of an MPN or suspected MPN diagnosis and for which the Onksoft MPN panel was ordered. Illumina NextSeq: A real-time method of analyzing DNA using a sequencer. Coded and non-coding regions of the selected genes were enriched and subsequently sequenced on an Illumina MiSeq instrument (San Diego, CA) with paired-end, 186 base pair reads. Following mapping of the read data to the human genome (reference build GRCh37/38), single nucleotide variants, insertions and deletions with an allele frequency greater than 3% were detected utilizing a customized bioinformatic analytical pipeline. Reported variants include known disease associated mutations and unclear variants with little or no literature support. Benign population polymorphisms are not included in the report. The mutation hotspots of the following genes were interrogated by this test: ABL1, AKT1, ALK, CALR, CBL, CSF3R, DNM3TA, EZH2, DNT1, IDH1, IDH2, JAK2, MPL, SETBP1, SRSF2, TP53, U2AF1, SF3B1, KRAS, and CML3 (Figure 1).

Results

- 2157 MPN cases were analyzed, of which 36% were abnormal (n=776) and the remaining 1381 (64%) were normal i.e. negative for all the 17 genes interrogated in this panel (Figure 2).
- 71% (n=552) of the abnormal cases were positive for either JAK2, MPL, or CALR alteration (Figure 3a). Consistent with published literature, JAK2 alterations were most prevalent (73%), followed by CALR (20%) and MPL (7%). Interestingly, although generally considered mutually exclusive, co-occurrence of mutations in JAK2, CALR, and/or MPL were noted among 17% of total abnormal cases (Figure 3b).
- Among the triple-negative MPNs studied (29% of remaining abnormal cases, n=225), pathological mutations were most frequently identified in DNMT3A (27%), ASXL1 (23%), SF3B1 (16%), TP53 (17%), SRSF2 (16%), SETBP1 (6%) and IDH2 (5%) (Figure 4).
- Alterations in DNMT3A and ASXL1 were most prevalent among triple negative cases submitted for evaluation of erythrocytosis (49% and 44% of the 41 total triple-negative cases submitted). On the other hand, in the limited number of confirmed triple negative ET, with frequent somatic alterations (31% of ET cases, n=26). On the other hand, in the limited number of definitive triple-negative PMF cases, SRSF2 (33%) alterations were most common (Figure 5).
- Unclassified MPN had a similar mutation profile as polychromatophilic or confirmed ET cases (Figure 4), however, limited follow-up information is available on these.

Conclusions

This data illustrates the clinical utility of extended myeloid mutational profiling using NGS to further define the molecular profile of triple-negative MPNs.

TAT from time of initial sample receipt for this assay averages 4-5 days.

Alterations in genes that are part of the splicosome and involved in epigenetic regulation such as ASXL1, DNMT3A and SRSF2 are generally considered to be adverse prognostic markers in various myeloid neoplasms.

Our findings demonstrate that alterations in these genes are recurrent in triple-negative MPNs and therefore, may contribute to the worse prognosis of this class of MPNs. One previous study identified CALR-ASXL1 and ‘triple-negative’ as high-risk molecular signatures in PMF.

Overall, NGS panel testing for MPNs beyond JAK2, CALR and MPL may serve as a useful diagnostic adjunct to further inform clinical prognostication and treatment decisions in patients with triple-negative MPNs.

References