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EXTENDED MYELOID MUTATION PROFILING USING NGS IN TRIPLE-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: SINGLE INSTITUTION EXPERIENCE AT A HIGH VOLUME NATIONAL REFERENCE LABORATORY

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Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic disorders characterized by the increased production of erythroid, megakaryocytic, or granulocytic cells.[1] BCR-ABL1-negative MPNs primarily include Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Primary Myelofibrosis (PMF).[2] 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, 60%, 20%, and 3% in ET, and 60%, 25%, and 7% in PMF.[3] MPN driver mutations result in the myeloproliferative phenotype due to intracellular JAK-STAT signaling and their downstream effectors.[4] Mutational profiling is therefore a primary component of routine clinical diagnostic evaluation.[2,5] However, there is a subset of MPNs that lack alterations in these three genes are referred to as "triple-negative" MPNs.

Significant survival differences have been reported in triple-negative MPNs compared to the *JAK2, CALR* and *MPL* positive subtype.[6] In the setting of primary myelofibrosis (PMF), for example, triple negative mutation status has been established as a significant indicator of adverse clinical prognosis associated with inferior overall survival and progression to acute leukemia compared to the JAK2- or CALR-mutated subtype.[5,7] Studies specifically delineating the somatic mutational landscape among triple negative MPNs are relatively limited, however.[1] Herein, we report molecular profiling results among >2000 MPNs sequenced at our institution, including a large cohort of 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 were selected on the basis of an MPN or suspected MPN diagnosis and for which the Onkosight MPN panel was ordered.

Illumina NGS: Nucleic acid from the submitted specimen was subjected to a PCR-based, Amplicon target enrichment using the Illumina TruSeq Amplicon Assay (San Diego, CA). Coding 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/hg19), single nucleotide variants, insertions and deletions with an allele frequency greater than 5% 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, ASXL1, CALR, CBL, CSF3R, DNMT3A, EZH2, IDH1, IDH2, JAK2, MPL, SETBP1, SRSF2, TP53, U2AF1, SF3B1, KIT* (Figure 1).

FIGURE 1: ONKOSIGHT MPN PANEL (17 GENES)

ABL1	*ASXL1*	*CALR*	*CBL*	*CSF3R*	*DNMT3A*	*EZH2*	*IDH1*	*IDH2*
JAK2	*KIT*	*MPL*	*SETBP1*	SF3B1	*SRSF2*	*TP53*	*U2AF1*	

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 a *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 1% of total abnormal cases (Figure 3b).

FIGURE 2: RESULT STATUS OF ALL CASES ANALYZED

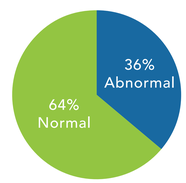


FIGURE 3A: PERCENTAGE OF TRIPLE-NEGATIVE V/S *JAK2, CALR* AND *MPL* POSITIVE CASES

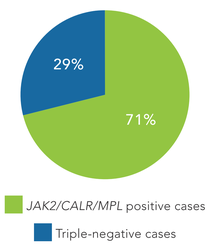
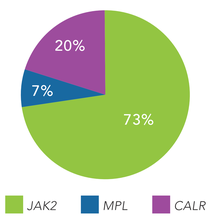
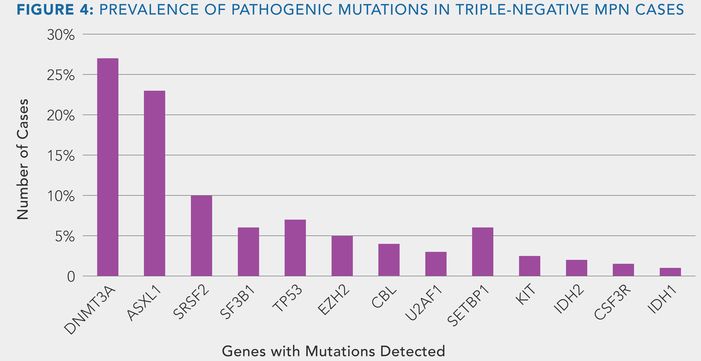


FIGURE 3B: BREAKDOWN OF *JAK2, MPL* AND *CALR* POSITIVE SAMPLES AMONG THE DRIVER MUTATION POSITIVE CASES



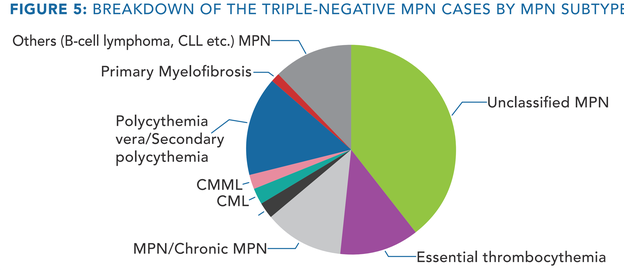
- Among the triple-negative MPNs studied (29% of remaining abnormal cases, n=225), pathogenic mutations were most frequently identified in *DNMT3A* (27%), *ASXL1* (23%), *SRSF2* (10%), *TP53* (7%), *SF3B1* (6%), *SETBP1* (6%) and *EZH2* (5%) (Figure 4).

FIGURE 4: PREVALENCE OF PATHOGENIC MUTATIONS IN TRIPLE-NEGATIVE MPN CASES



- 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 for evaluation of polycythemia, respectively). Interestingly, a similar pattern was seen among confirmed triple negative ET, with *ASXL1* and *DNMT3A* alterations each representing the most 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, 6).
- Unclassified MPNs had a similar mutation profile as polycythemia or confirmed ET cases (Figure 6), however, limited follow-up information is available on those.

FIGURE 5: BREAKDOWN OF THE TRIPLE-NEGATIVE MPN CASES BY MPN SUBTYPE



Results

FIGURE 6A: PREVALENCE OF PATHOGENIC MUTATIONS IN TRIPLE-NEGATIVE ESSENTIAL THROMBOCYTHEMIA

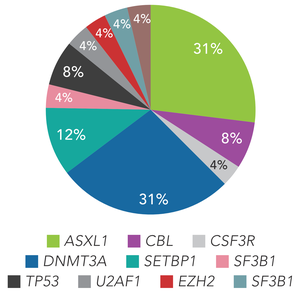


FIGURE 6B: PREVALENCE OF PATHOGENIC MUTATIONS IN TRIPLE-NEGATIVE ERYTHROCYTOSIS

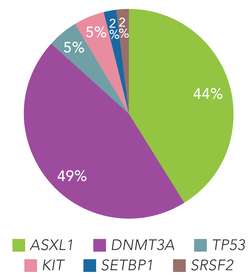
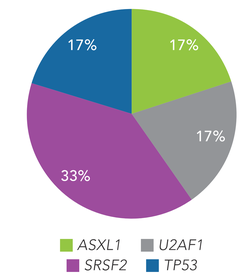


FIGURE 6C: PREVALENCE OF PATHOGENIC MUTATIONS IN TRIPLE-NEGATIVE PRIMARY MYELOFIBROSIS



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 spliceosome and involved in epigenetic regulation such as *ASXL1, DNMT3A* and *SRSF2* are generally considered to be adverse prognostic markers in various myeloid neoplasms.[8-10]
- 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 has identified 'CALR–ASXL1+' and 'triple-negative' as high-risk molecular signatures in PMF.[6]
- 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

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