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Molecular Profiling of Myeloid Neoplasm by Next-Generation Sequencing

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Abstract: We performed an integrative analysis of molecular profiles of 52 patients with Myeloid malignancies from a tertiary care teaching hospital in Jaipur. Targeted next-generation sequencing of 80 genes revealed 23 genes mutated in over 5% of patients. Mutational profiles differed considerably from previous studies. *FLT3, DNMT, NPM1, N-RAS, ASXL1, CEBPA* and *RUNX1* mutations were more frequent in AML patients, while *CBL, JAK2, GATA2, SF3B1, WT1, KRAS, MPL, TP53* and *ZRSR2* were less frequent. Additionally, we identified ABL gene in 2 CML, *DNMT* in 1 MDS, and *IDH1, JAK2, MPL, EZH2, PDGFRA in MPN/MPN-PV.*

Keywords: *Next-Generation Sequencing, Myeloid Plasma, Oncomine, Bioinformatics, Biomarkers.*

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I. INTRODUCTION

Genomic mutations, when used in conjunction with morphologic, peripheral blood, and clinical findings, play a crucial role in risk stratification, monitoring, and targeted therapeutic decision-making for patients with myeloid neoplasms (MN). In 2022, the International Consensus Classification (ICC), World Health Organization (WHO), and European Leukemia Network (ELN) released updated guidelines for the diagnosis and management of myeloid malignancies. These updates reaffirm the importance of cytogenetics but also place a growing emphasis on somatic mutations as drivers of disease (Duncavage et al., 2022). On the other hand, there are essential molecular markers in adult acute myeloid leukemia (AML), myelodysplastic syndromes/neoplasms (MDS), and myeloproliferative neoplasms (MPN), and their prognostic significance (Tran and Siddon, 2023). While there are conventional genomic methods, viz. Chromosomal microarrays (CMA), FISH, GISH, etc., the next-Generation Sequencing (NGS), approaches that are also known as massive parallel sequencing or high-throughput sequencing have over the years, been used to identify genomic abnormalities. With NGS being highly scalable and can be coupled with enrichment technologies to interrogate a small subset of key genes (targeted gene panels), up to thousands of genes or genomic regions (whole-exome sequencing [WES]), or can be used without enrichment to detect full genome (whole-genome sequencing [WGS]) or transcriptome-wide (whole-transcriptome shotgun sequencing [WTSS] or RNA sequencing [RNA-seq]) genomic abnormalities (Mukherjee et al., 2017; Kahraman et al. 2022). Depending on the design of the assay, NGS can be used to study the full range of genomic variation, including Single Nucleotide Variations (SNVs), small insertions and deletions (indels), structural changes (i.e., Copy Number Alterations [CNAs]), gene fusions or chromosomal translocations, gene expression, and DNA methylation. NGS may be used for both initial diagnosis and monitoring purposes (Miller et al., 2018).

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The MNs including Chronic Myeloid Leukemia (CML), are the most common adult leukemias, with enormous challenges remaining in diagnosis and treatment, particularly due to cost and access to care. An annual incidence of CML in India contributes to 0.8 to 2.2 per 100,000 population, but these estimates may not be entirely accurate due to data limitations. Other myeloproliferative neoplasms (MPN) like Polycythemia Vera (PV), Primary Myelofibrosis (PMF), and Essential Thrombocythemia (ET) are also seen in India. In this work, we sought to underpin the MNs across Indian sub-population and discern candidate mutations and genes responsible for various phenotypes. At the end of the analyses, we propose a list of hotspot genes associated with these and develop a protein-protein interaction network and pathway analyses.

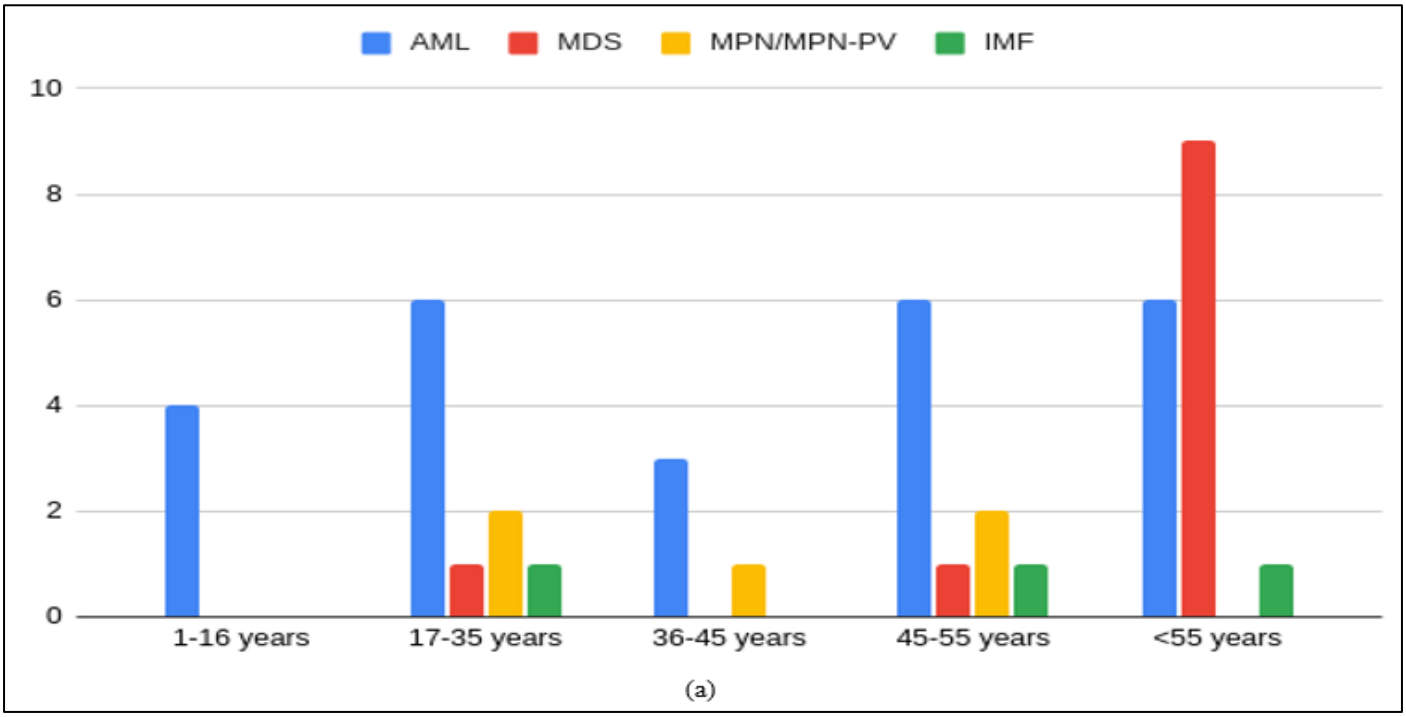
II. MATERIALS AND METHODS

We conducted an observational study which included 52 patients who underwent myeloid panel testing for myeloid malignancies during 2023-'24. This study received ethical approval from the Institutional Ethics Committee with written informed consent obtained from all participants. Oncomine Myeloid Assay is a one single assay for identifying relevant myeloid mutations to discern hematological malignancies that often involve multiple sequential tests and laborious workflows (Park et al. 2020). While NGS approaches are often looked into, discerning multiple relevant driver genes in associated myeloid malignancies with a single test is the need of the hour. In this work, we employed the Ion Torrent Oncomine Myeloid NGS assay to identify relevant DNA mutations associated with myeloid disorders, *viz.* AML, MDS and MPN (Valent, 2019; Schejbel et al. 2022) The variants were annotated by integrated Oncomine Knowledge base reporting software that links variants to relevant diseases, labels, guidelines, and global clinical trials.

DNA isolated from peripheral Blood/Bone marrow samples was used for NGS Library preparation. RNA was used for myeloid RNA library preparation. In the process quality controls were determined for the prepared library. The libraries were sequenced at minimum mean depth: >1000x on Ion Torrent next generation sequencing platform. Clinically relevant mutations were identified and annotated using published variants in literature and a set of databases. The effect of the non-synonymous variant was calculated using multiple prediction algorithms such as PolyPhen, SIFT, Mutation Taster2. The genetic test results were reported based on the recommendations of AMP-ASCO-CAP guidelines.

III. RESULTS AND DISCUSSION

A total of 49 patients which had SNPs were included in the study, the median age of the entire cohort was 21.23 ± 45.59; and 33 (67.35%) were male and 16 (32.65%) were female (Supplementary Table 1). Twenty-five patients had a diagnosis of acute myeloid leukemia, 8 had myelodysplastic syndromes, 7 had a myelodysplastic/ myeloproliferative neoplasm (MDS/MPN), 4 had Chronic myeloid leukemia, 2 had Idiopathic Myelofibrosis, 1 had Biphenotypic AL, 1 with pancytopenia/MDS and 1 with Myeloid sarcoma (Supplementary Table 2). Targeted next-generation sequencing of 80 genes revealed 23 genes that were recurrently mutated in >5% of patients. Considerable differences were observed in the mutational profiles compared with previous studies, as *FLT3,DNMT, NPM1, N-RAS, ASXL1, CEBPA* and *RUNX1* mutations were detected at a higher frequency in AML patients, whereas CBL, JAK2, GATA2, SF3B1, WT1,KRAS, MPL, TP53 and ZRSR2 were less frequently mutated in AML. Further, ABL gene was identified in 2 CML, *DNMT* in 1 MDS, *IDH1, JAK2, MPL, EZH2, PDGFRA* in MPN/MPN-PV (Figure 1).



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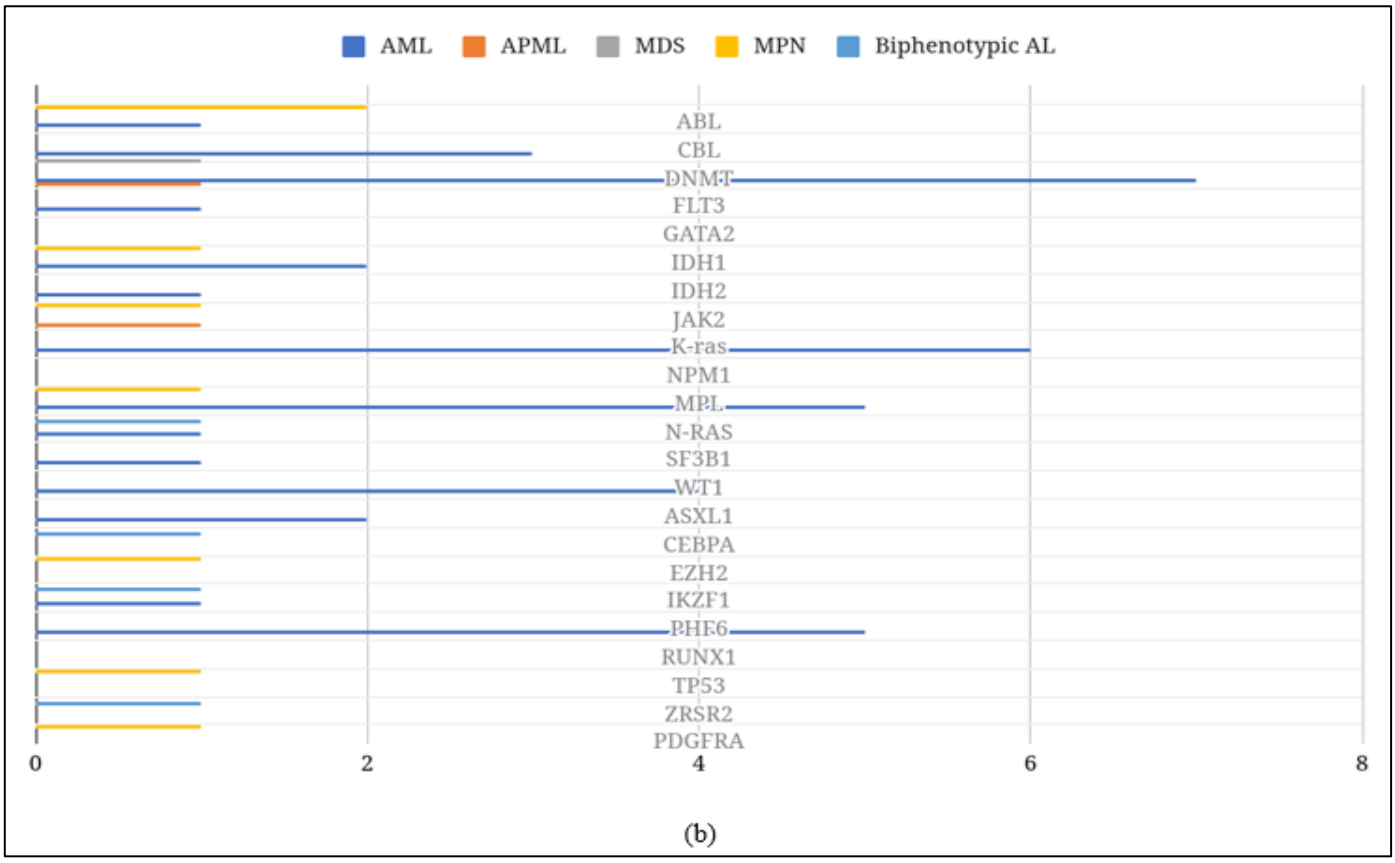
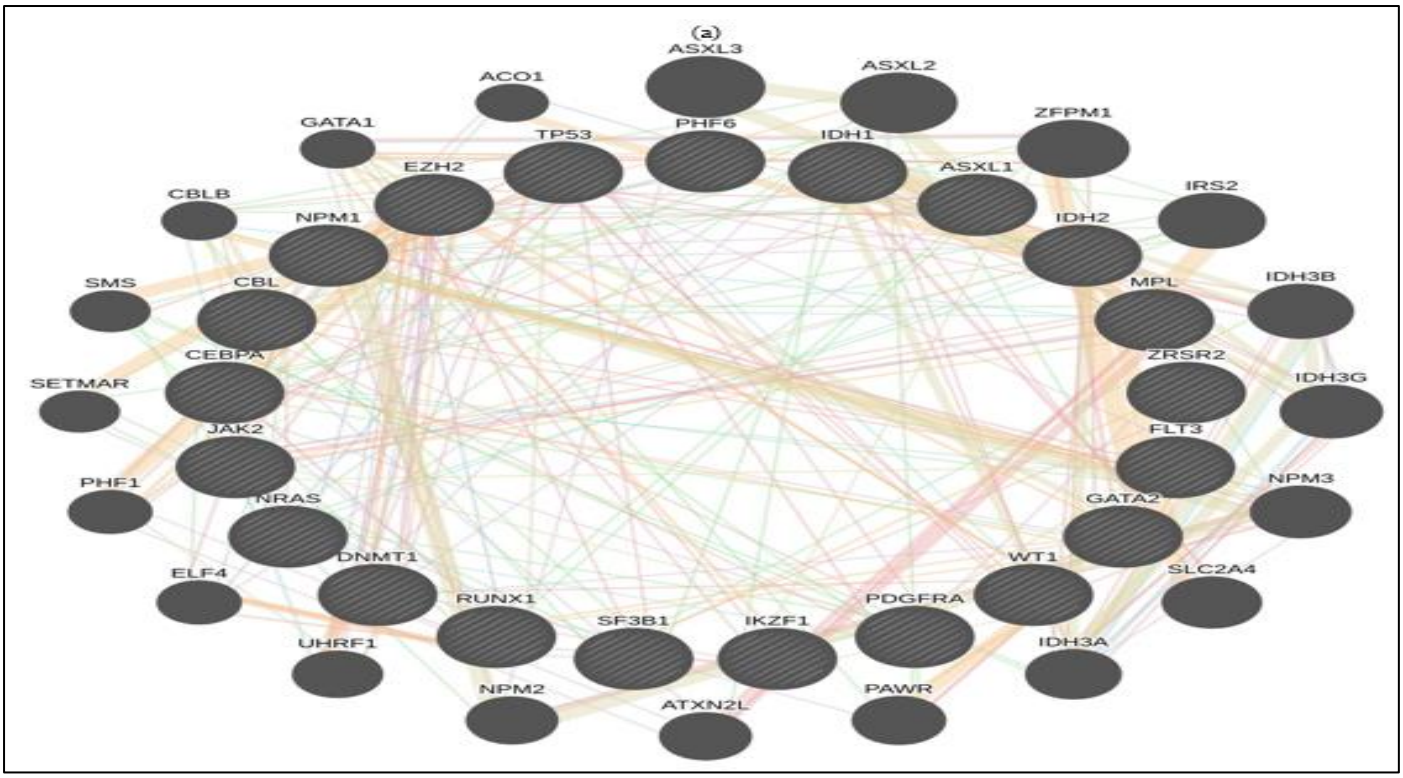


Fig 1 (a) Comparison of AML, MDS, MPN and IMF Across Various Age Groups (b) Mutational Frequencies Observed Across our Observational Study in Individual MNs.

What we discerned from our study was important genetic markers associated with MNs. Our findings shed further light on the heterogeneity of AML and identify previously unappreciated alterations that may lead to improved molecular characterization and risk stratification in the AML cases. This is supported by the gene interaction networks revealing distinct genetic interactions (Figure 2).



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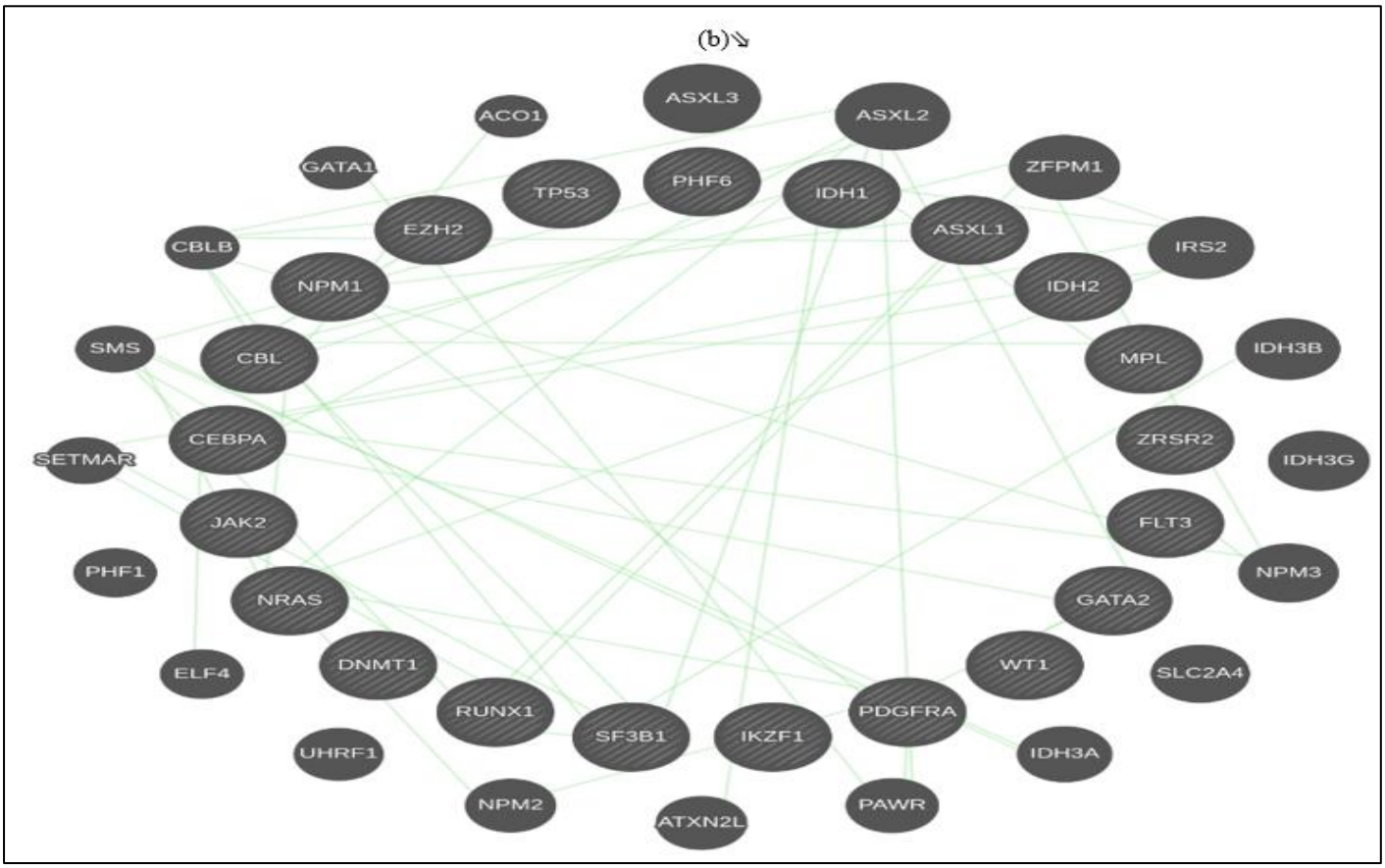

Fig 2 (a) Distinct Gene Interaction Ascertained from MNs and (b) Genetic Interactions seen with the Green Edges.

Our study identified a unique case of biphenotypic acute leukemia (BAL) in a patient who may also present with mixed-phenotype acute leukemia (MPAL). This particular case exhibited four gene aberrations. Given the rarity of this malignancy, we believe that hematopoietic stem cell transplantation (HSCT) could be a potential treatment option, if feasible. It is important to note a limitation of our current work. While the total number of patients assessed appears sufficient, the sample size becomes restricted when the cohort is further divided into subgroups based on specific diagnoses. Future research involving larger sample sizes would enable more conclusive comparisons between the identified mutations and distinct diagnoses.

IV. CONCLUSION

The routine clinical application of NGS panel tests has become common in recent years and may serve as a significant resource for clinicians for the genomic characterization of MNs (Levy et al. 2019). In this work, we analyzed molecular profiles of 49 patients having MNs by targeted sequencing of 80 genes wherein 23 genes were known to be mutated in over 5% of cases. The mutational profiles varied from prior studies with AML patients more frequently associated with a myriad number of mutations. We also found ABL gene mutations in 2 CML cases, DNMT in 1 MDS case, and IDH1, JAK2, MPL, EZH2, and PDGFRA in MPN/MPN-PV cases.

- *Legends to the Supplementary Tables:*

- *Supplementary Table 1:*
  Patients with SNPs included in the study

- *Supplementary Table 2:*
  Diagnostic landscape of patients associated with various phenotypes

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- **Funding: NA**

- **Competing interests:** None

- **Data availability:** The data is available upon request

AUTHORS' CONTRIBUTIONS

RMJ conceived the project and performed the analyses. NG provided the samples. SKP and PS performed systems genomics and bioinformatics analysis. RMK, SKP and PS wrote the first draft and edited the final draft. All authors approved the manuscript before communication.

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