DECIPHERING THE IMMUNOPHENOTYPIC HETEROGENEITY OF NPM1 MUTATED AML THROUGH A GENOTYPIC LENS

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Introduction

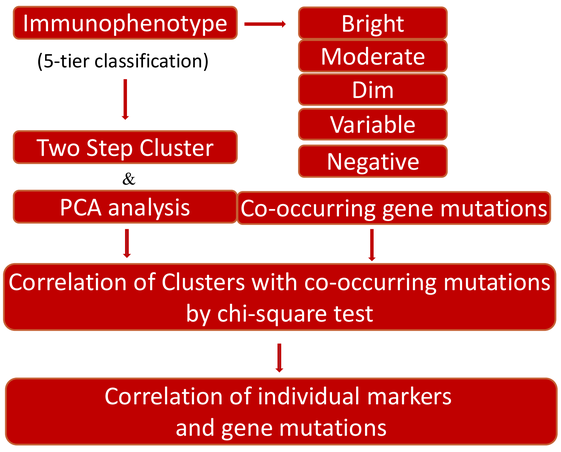
Acute myeloid leukaemia (AML) with mutated *NPM1* (NPM1m+) is a prevalent subtype, known for typical immunophenotypic features including low or absent CD34 and decreased or absent HLADR expression. However, blasts in NPM1m+ AML exhibit substantial heterogeneity, reflecting diverse differentiation patterns such as immature, neutrophil, and monocytic lineages. Deciphering these complex genotype-phenotype correlations is vital for understanding disease biology and potentially guiding clinical decisions. In this study, we aimed to look at the genotype and marker correlations in NPM1m+ AML.

Objectives

1. To assess the patterns of immunophenotypic heterogeneity in NPM1m+ AML
2. To assess common co-occurring genetic alterations in NPM1m+ AML
3. To check for associations between the above observed immunophenotypic heterogeneity and co-occurring genetic alterations

Methods

All cases of NPM1m+ AML diagnosed at our centre between April 2021 and December 2024 with baseline myeloid NGS results were included in the study



All statistical analyses were performed on SPSS version 29.

Results

- N = 60 (with baseline molecular details)
- M:F = 2.3:1
- Median blast/equivalent percentage = 55

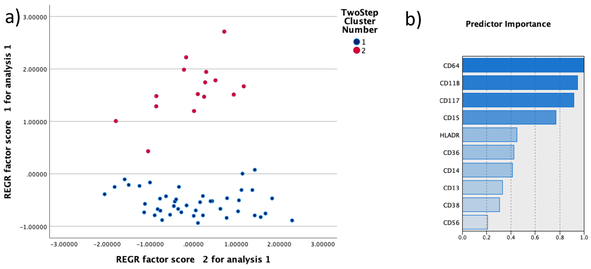


Figure 1: a) PCA plot showing two distinct clusters of NPM1m+ AML based on immunophenotype. b) Markers with descending order of predictor importance.

Parameter	Myeloid cluster	Myelomonocytic cluster
N	44	16
Median blast/equivalent percentage	52	56
Percentage of CD34 positivity	30	25
Percentage of HLADR positivity	52	31

Table 1: Comparison of myeloid and myelomonocytic clusters.

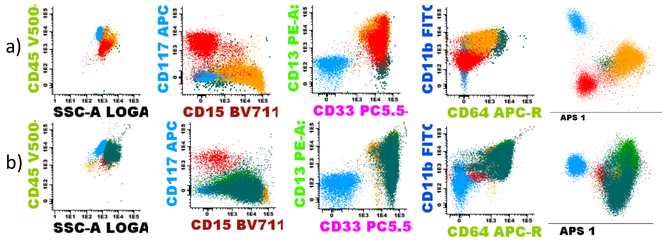


Figure 2: a) Scatter plots and Automated Population Separator (APS) showing the myeloid immunophenotype of NPM1m+ AML. b) Scatter plots and APS showing myelomonocytic immunophenotype of NPM1m+ AML.

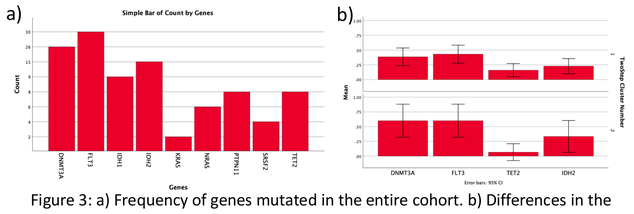


Figure 3: a) Frequency of genes mutated in the entire cohort. b) Differences in the frequencies of genes mutated between the two clusters.

Myeloid Cluster

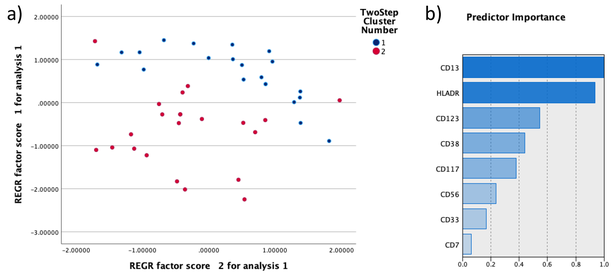


Figure 4: a) Two fairly different sub-clusters within the myeloid cluster. b) Markers with descending order of predictor importance in separating these sub-clusters.

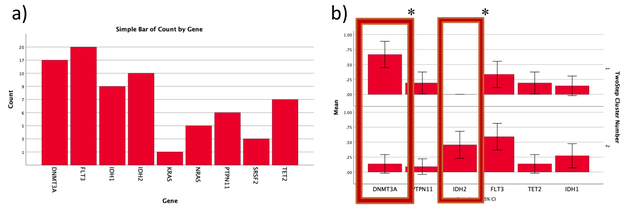


Figure 5: a) Frequencies of genes mutated in the myeloid cluster. b) Differences in the frequencies of genes mutated between the two sub-clusters of the myeloid cluster. Significant differences were found in the frequencies of *DNMT3A* and *IDH2*.

Marker	*DNMT3A* mutation	*IDH2* mutation
	p-value	p-value
Sub-clusters	0.031	0.048
CD13	0.005	0.001
HLADR	0.27	0.018

Table 2: Table showing significant association of *DNMT3A* and *IDH2* with the sub-clusters, CD13 and HLADR expression. CD13 and HLADR are positively associated with *DNMT3A* and negatively associated with *IDH2*.

Myelomonocytic Cluster

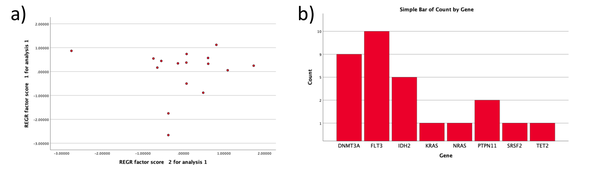


Figure 6: a) Shows the PCA plot of myelomonocytic cluster showing no distinct sub-groups. b) Frequencies of genes mutated showing absent *IDH1* mutations and reduced *TET2* mutations in the myelomonocytic cluster compared to the myeloid cluster.

Marker	*DNMT3A* mutation	*IDH2* mutation
	p-value	p-value
CD13	0.012	<0.001
CD123	0.031	0.151
CD38	0.037	0.104

Table 3: Shows significant associations between markers and *DNMT3A* and *IDH2* in the entire cohort.

References

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