

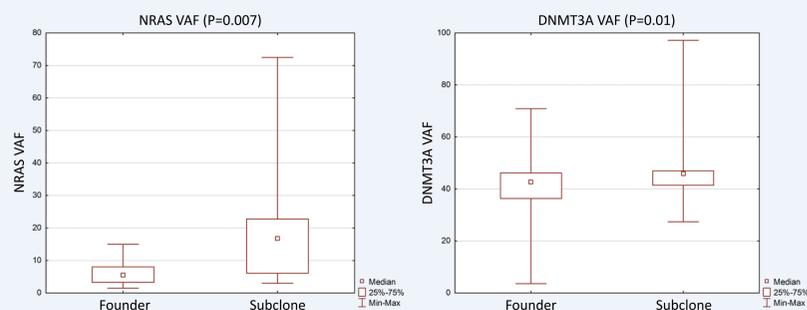
Introduction

The receptor tyrosine kinase *FLT3* gene is commonly mutated in acute myeloid leukemia (AML) and its mutation is associated with significantly more aggressive disease and poor outcome. However, in the co-presence of *NPM1* mutation, the disease is less aggressive and is associated with better outcome. *FLT3* inhibitor midostaurin (Rydapt; Novartis Pharmaceuticals, Inc.) was recently approved for patients with *FLT3* mutations. While the presence of a *FLT3* mutation is commonly associated with AML, it can develop in less acute myeloid neoplasms, such as MDS and CMML, and implies transformation into more acute phase. In this context, the *FLT3* mutation is typically detected in a subclone of the original chronic myeloid clone. The prevalence of subclonal *FLT3* mutations is unknown, as is the clinical relevance of subclonal *FLT3*, especially when treated with *FLT3* inhibitors. We used variant allele frequency (VAF) and next generation sequencing (NGS) to determine if *FLT3* mutations are founding or secondary mutations and to characterize the co-mutational background in large number of consecutive patients who presented with a clinical impression of myeloid neoplasm.

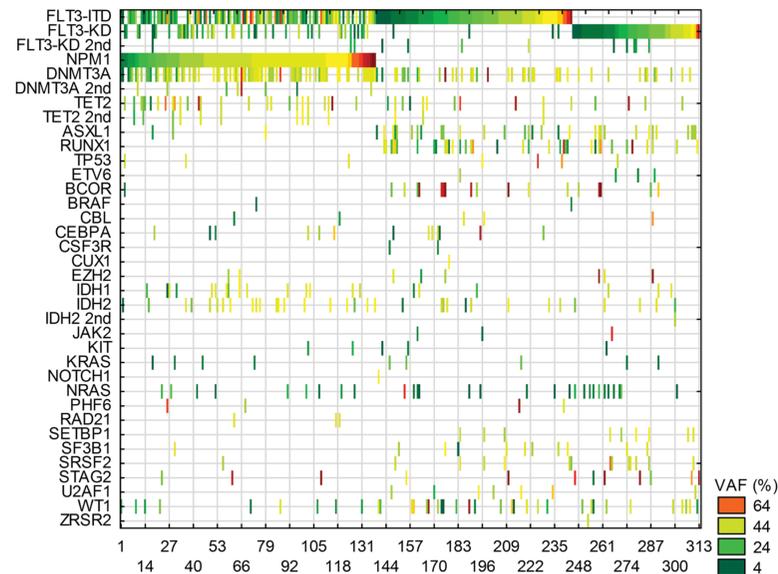
Methods

A total of 6390 consecutive bone marrow aspirate samples or peripheral blood samples were submitted with clinical impression of AML, MDS, or MPN between January 2014 and mid 2017 were tested for mutations in myeloid genes using NGS. We used the TruSight Myeloid panel (Illumina, San Diego, CA) for detecting missense mutations and fragment length analysis (FLA) for detecting internal tandem repeat (ITD) in *FLT3*. DNA was extracted from samples using the QIAamp DNA Mini Kit. This NGS testing covers mutations in 54 myeloid-related genes. The average depth of sequencing was 10,000x.

Higher Variant Allele Frequency in NRAS and DNMT3A in FLT3 Progressor



Variant Allele Frequency of AML, MDS, and MPN Cohort



Mutation profile in the 313 patients with *FLT3* mutation. Each column represents one of the 313 samples with the detected mutations. Each bar represents a mutation and the color the bar reflects the VAF of that mutation. The scale of the VAF is shown on the side.

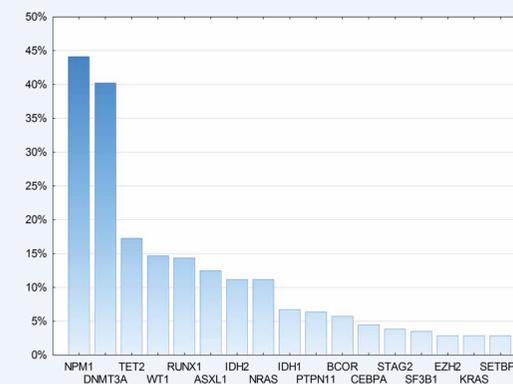
FLT3 Mutation as a Progressor versus Progressor Mutations

Abnormality	P-Value
Higher Number of mutated genes	<0.00001
More mutations in <i>TET2</i>	0.05
More mutations in <i>NRAS</i>	0.007
More mutations in <i>IDH2</i>	0.003
More mutations in <i>EZH2</i>	0.004
More mutations in <i>DNMT3A</i>	0.01
More mutations in <i>BCOR</i>	0.01

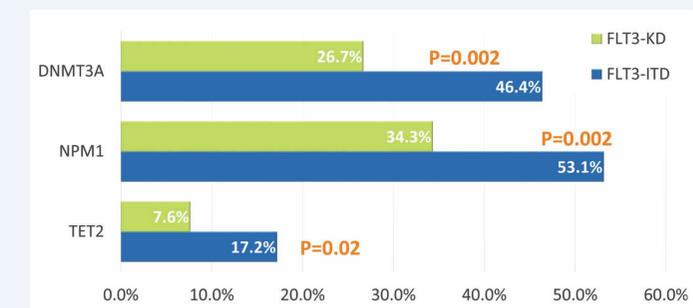
Results

A total of 2670 (42%) of the samples showed a mutation in one or more of the tested genes. Of the samples with mutations, *FLT3* ITD and kinase domain (KD) mutations were detected in 313 (12%) patients. Of these patients, 208 (66.5%) had ITD and 117 (37.4%) had KD mutations. Twelve patients (3.8%) had both ITD and KD mutations. *NPM1* mutation was detected in 138 (44%) of the patients. Most of *FLT3*-KD mutations were in codon 835, but mutations in codons 836, 839, 842 and 845 were also found. Additionally, missense mutations in codons 576, 592, and 593 at the site of ITD were detected. To distinguish a *FLT3* mutation as a founding mutation rather than a secondary mutation, we used a lower VAF of 10% as a cut-off. Using this approach, we found that 27% (115/313) of patients had secondary *FLT3* mutations. Of these subclonal *FLT3* mutants, *NPM1* co-mutation was detected in 61 (53%) patients, but was not statistically significant (P=0.06). Patients with *FLT3* mutation as a subclone had a significantly higher number of co-mutated genes (P-value <0.0001). This group of patients had higher (P<0.01) mutations in *TET2*, *DNMT3A*, *NRAS*, *IDH2*, *EZH2*, and *BCOR*, consistent with an MDS/CMML background. On the other hand, patients with both *FLT3* and *NPM1* mutations had a significantly lower number of co-mutant genes (P<0.0001) and particularly lower (P<0.01) mutation rate in *U2AF1*, *SF3B1*, *SRSF2*, *RUNX1*, *IDH1*, *BCOR*, and *AXL1*.

Most Commonly Genes Co-Mutated with FLT3



Difference between FLT3 ITD and KD in Co-Mutated Genes



Conclusion

FLT3 mutations are frequently (27%) a secondary mutation in a subclone of the neoplastic founding mutations. This appears to be more common when *FLT3* mutation is developing in a background of MDS/CMML. The co-presence of an *NPM1* mutation is more commonly associated with fewer co-mutations in MDS-related genes. This data suggests that in addition to detecting ITD by FLA, using NGS for profiling mutations in *FLT3*-KD and other myeloid genes should be considered whenever *FLT3* mutations are evaluated. Further, the clinical decision for combination therapy should consider the entire mutation profile and not only mutations in *FLT3*.