Poster #3176

Using Next Generation Sequencing of Peripheral Blood cfDNA As a Clinical Test in Screening for Hematologic Neoplasms

Vincent Funari, PhD, Wanlong Ma, MS, Maya Thangavelu, PhD, Ivan De Dios, and Maher Albitar, MD

NeoGenomics Laboratories, Inc

INTRODUCTION

Recent advances in molecular techniques and the adaptation of next generation sequencing (NGS) in routine clinical testing increased our ability to use molecular approaches in the diagnosis and classification of most hematologic diseases. Bone marrow aspiration and biopsy remain necessary for initial confirmation of diagnosis of neoplastic processes in bone marrow, but recently screening or monitoring patients by testing peripheral blood cell-free DNA (cfDNA) might be a reliable alternative to marrow biopsy and might reduce the need for a painful bone marrow procedure. Here we report the results of routine clinical testing of cfDNA that is ordered by practicing hematologists in the context of the presence or the suspicion of the presence of hematologic neoplasm.

METHODS

*DNA from 227 peripheral blood samples was extracted using NucliSenS EasyMAG automated platform

*cfDNA was screened using a 54 gene focused myeloid panel (Illumina TruSight Myeloid Sequencing Panel) and an average sequencing depth of >10,000X with Illumina NextSeq Sequencers (Illumina; San Diego, CA).

Table 1. Referring diagnosis (~71 years)

Referring Diagnosis	Cases
Cytopenia	0.5%
Myeloma	0.5%
Megablastic Anemia	1.3%
Lymphoma	2.2%
Polycythemia	1.3%
Neoplasm, Other	4.0%
Leukemia	8.0%
Other	8.4%
Pancytopenia	8.9%
Thrombocytopenia	11.6%
MDS	12.5%
Decreased/Increases White Blood	16.0%
Anemia	24.9%

- The patients' average age was 71 (18-96) years.
- The reason for submitting samples was ruling out MDS in 199 and ruling out AML or other hematologic neoplasms in 28 samples.
- Of these samples, 12 patients had a follow up testing of bone marrow aspiration sample. Table 1 shows the distribution of patients tested by original referring diagnosis.

RESULTS

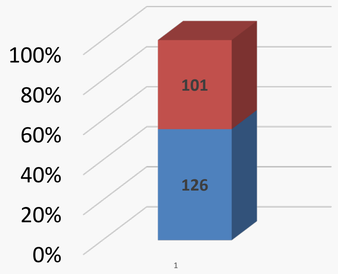

Figure 1. Percent of Patients with a mutation.
101 patients had mutations (Red).
126 had no mutations (Blue).

- **Figure 1. Percent of Patients with a mutation.**
  - 126 showed no evidence of mutation in any of the tested genes. Based on previous data (see 2016 ASH Poster 3902), this suggests that MDS can be ruled out and bone marrow biopsy could be avoided.
  - 101 (45%) had mutations in one or more genes.
- **Table 2. Percent of referring diagnoses that tested positive/negative for mutations in cfDNA.**
  - MDS could be ruled out in most cases.
  - However, 7% of positive cases were confirmed for MDS.
  - Patients with benign referring diagnoses like anemia or changes in WBC mostly tested negative.
  - Patients with a leukemia diagnosis tested positive (22% versus 2%).

Table 2. Samples' referring diagnosis and percent of cases that were POS (positive) or NEG (negative) for testing

CANCER	POS(%)	NEG(%)
Lymphoma	2.90%	1.92%
Leukemia	21.74%	1.92%
Neoplasm, Other		5.77%
Myeloma		0.64%
MDS	7.25%	14.74%
BENIGN		
Megaloblastic Anemia	4.35%	
Polycythemia		1.92%
Pancytopenia	11.59%	8.33%
Thrombocytopenia	11.59%	11.54%
Decrease/Increases WBC	11.59%	17.95%
Anemia	14.49%	29.49%
Other	14.49%	5.77%

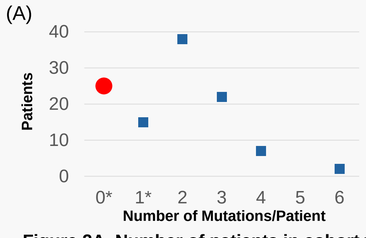

Figure 2A. Number of patients in cohort with number of mutations per patient.

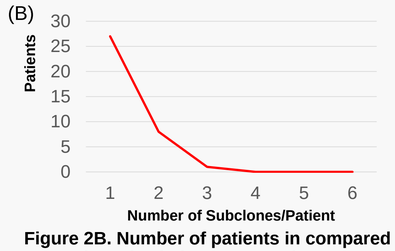

Figure 2B. Number of patients in compared to number of subclones per patient.

- **Figure 2A.** 25 (~12.8%) patients (🔴) with a single gene mutation and variant allele frequency (VAF) <20% (considered diagnostic for the presence of neoplasm).
  - Follow up was recommended.
- **Figure 2A.** 74 patients (33%) (🟦) with mutations in two or more genes or single gene (VAF ≥20%) (considered diagnostic for the presence of hematologic neoplasm)
  - Bone marrow morphologic evaluation was recommended.
- **Figure 2B.** shows most (34/74) contained disparate mutant allele frequencies (>10%)
  - This is consistent with multiple neoplastic subclones.

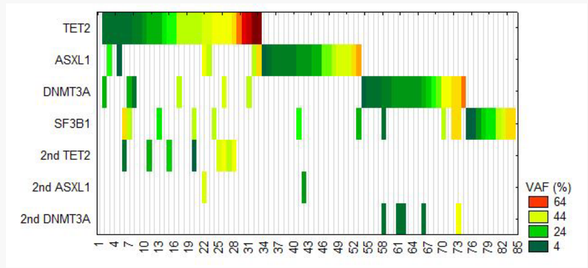

Figure 3. The four most frequently mutated genes and their mutation allele frequency (VAF) in positive liquid biopsies.

- The most commonly mutated gene was TET2, detected in 30 samples. ASXL1 and DNMT3A were mutated in 24 and 26 samples, respectively.
- Samples containing a TET2 mutation were more likely to have a second mutation in TET2 (8/32) or another gene. In contrast, other genes that were frequently mutated did not show this trend.
- P53 gene mutations were detected in 16 samples, 7 as a single abnormality with VAF <20%. These were reported as having unknown significance and it was recommended to rule out neoplasms in hematologic (lymphoid and myeloid) as well as solid tumors.
- SF3B1 gene mutations were detected in 19 samples and we recommended ruling out refractory anemia with ring sideroblasts (RARS).

CONCLUSIONS

- cfDNA testing is a reliable approach to screen for the presence of hematologic neoplasm and potentially could avoid the need for bone marrow biopsy in almost half the patients expected to have MDS or other hematopoietic neoplasms.
- Positive diagnosis can be confirmed in an additional 45% of patients and only 12.8% of patients will be reported with questionable results. Except for those with TP53 mutations, the rest of the 12.8% cases can be classified as Clonal Hematopoiesis of Indeterminate Potential (CHIP).
- Most cases (27) had evidence of only one subclone, but there were several cases which had evidence of multiple subclones and were suggestive of an advanced disease that should be monitored closely for changes/evolution of the subclones.
- Most patients with a referring diagnosis which included leukemia were positive, illustrating the sensitivity of the test, while most cases which were referred as anemia were negative as might be expected.
- Despite the small sampling (12 samples), follow up using cfDNA testing reliably recapitulated original bone marrow biopsy findings. In one patient, additional subclones were detected in cfDNA that were not detected in the bone marrow aspirate.
- **While bone marrow is still the gold standard, our real world experience shows liquid biopsies can be a sensitive and non-invasive approach to rule out MDS or other hematological diseases.**