Reliability of Liquid Biopsy and Next Generation Sequencing in Monitoring Residual Disease Post-Hematopoietic Stem Cell Transplant

Maher Albitar, Hong Zhang, Andrew Pecora, Andrew IP, Andre Goy, Jeffrey Estella, Ivan De Dios, Wanlong Ma, Sukhdeep Kaur, and Hyoung Suh, Michele Donato, Scott D. Rowley

Genomic Testing Cooperative, Irvine, CA and John Theurer Cancer Center, Hackensack Meridian Health

INTRODUCTION

Monitoring and early detection of neoplastic clones posttransplant is important for the management of patients, especially for making decision for immunotherapeutic interventions. However, currently there are no standards for such monitoring. Bone marrow biopsies and flow cytometry analysis have been used with various degrees of success. DNA testing and chimerism evaluation is frequently combined with flow cytometry in evaluating patients post transplant. Next generation sequencing (NGS) with its relative high sensitivity can monitor patients who have no fusion genes by monitoring the specific mutations in the neoplastic clone. Unlike flow cytometry, NGS detection of genomic abnormalities is not dependent on the blast cells and can detect abnormalities in mature cells, which may provide higher level of sensitivity. One of the advantages of using NGS is the ability of using this technology as liquid biopsy. In principle, circulating cell-free DNA (cfDNA) has numerous advantages over bone marrow aspiration. cfDNA reflects the entire body and not influenced by the potential patcheyness of the neoplastic cells. Furthermore, cfDNA is enriched by tumor-specific DNA due to the higher turnover of neoplastic cells as compared with normal cells.

AIM

We explored the ability of using plasma cell-free DNA (cfDNA) in monitoring patients after HSCT and evaluated the potential of using liquid biopsy as a replacement to bone marrow biopsy. Because NGS also introduces new complexity by its ability to detect multiple subclones, we attempted to define the clinical relevance of these subclones and distinguish between CHIP (clonal hematopoiesis of indeterminate potential) and the actual neoplastic clone that will lead to relapse.

METHOD

cfDNA was isolated from 204 peripheral blood samples obtained from 75 patients, collected at various time points ranging from 27 days to 650 days (median 178 days) post-transplant. DNA from 102 bone marrow (BM) samples was extracted and sequenced using the same panel and approach as cfDNA. Diagnoses included 30 acute myeloid leukemia (AML), 2 chronic myelogenous leukemia (CML), 5 chronic myelomonocytic leukemia (CMML), 4 lymphoma, 10 myelodysplastic syndrome (MDS), 2 multiple myeloma (MM), 9 myeloproliferative neoplasm (MPN), 1 aplastic anemia, and 11 acute lymphoblastic leukemia. cfDNA was sequenced by NGS using 177 gene panel on Illumina platform. Single primer extension (SPE) approach with UMI was used. Sequencing depth was increased to more than 2000X after removing duplicates. Low level mutations were confirmed by inspecting BAM file.

RESULTS

Patients Characteristics

Number of patients	75
Age at Transplant	Median: 58, range: 27-76
Donor's age	Median: 31, range: 9-63
DX	
AML	30
CML	2
CMML	5
Lymphoma	4
MDS	10
MM	2
MPN	9
AA	2
ALL	11
Sex	
Female	41 (55%)
Male	34 (34%)
HLA	
Match	46 (61%)
Haplo	29 (39%)

- 156 of the total 204 tested cfDNA samples (76%) had negative data. The negative samples were collected from 28 days to 650 days post-transplant (median, 277).
- 48 samples (24%) from 30 different patients were positive. The positive samples were collected from 27 days to 650 days post-transplant (median 188 days). One of these positive patients was in full clinical relapse at the time of testing.
- Five patients converted from negative to positive and 12 from positive to negative with subsequent testing. Three of these converted to positive patients developed clinical relapse.
- No negative patient who remained negative had clinical relapse.
- Patients who were positive without clinical relapse had median variant allele frequency (VAF) of 0.85% (range: 0.01-13.25) and typically one mutated gene.

Mutations and VAF without overt relapse

Genes	Variant Allele frequency (VAF)
CEBPA	0.01
ASXL1	0.01
STAT3	0.15
STAT3	0.16
ASXL1	0.16
IDH2	0.27
EZH2	0.27
MLH1	0.32
JAK3	0.38
FLT4	0.39
SMC1A	0.45
PRKDC	0.48
PRKDC	0.53
DNMT3A	0.53
MPL	0.59
NRAS	0.61
CD79B	0.68
KMT2C	0.7
SMC1A	0.76
CDK4	0.78
NF1	0.78
TP53	0.79
JAK2 (V617F)	0.82
JAK2	0.87
IDH2	1.06
DNMT3A	1.35
SMC3	1.4
ASXL1	1.58
TET2	1.6
SOCS1	1.73
MPL	1.76
SF3B1	1.8
TET2	1.82
CBL	1.89
ERBB2	2.07
CD79B	2.53
CEBPA	2.56
GATA2	2.65
NF1	3
SOCS1	3.06
FBXW7	3.15
TET2	3.76
SMC1A	4.01
TAL1	4.35
EZH2	4.56
FLT3-ITD	4.71
CHEK2	4.76
SMC3	5.11
JAK3	5.15
CD79B	5.26
EZH2	5.33
TET2	5.38
KMT2C	5.94
MSH6	5.94
RNF43	5.97
SRC	6.3
MSH6	6.42
MSH6	6.42
U2AF2	6.9
SOCS1	6.98
CHEK2	7.17
SOCS1	7.51
MUTYH	7.56
FANCD2	7.69
MSH6	8.63
FANCD2	9.77
FANCD2	10.27
TCF3	10.71
MUTYH	12.35
TCF3	12.74
TCF3	12.82
TCF3	13.02
CHEK2	13.25
TCF3	13.64
TCF3	13.68

Mutations and VAF in relapsing patients

Genes	Variant Allele frequency (VAF)(%)
IDH2	0.4
ASXL1	0.91
U2AF1	1.08
KMT2A	1.31
U2AF1	1.64
TET2	1.99
NRAS	2.24
PMS2	2.35
TP53	2.99
SETBP1	3.5
TP53	4.61
U2AF1	6.45
PMS2	8.57
TET2	12.54
CEBPA	13.73
CEBPA	16.33
EZH2	16.75
ASXL1	19.08
FLT3-ITD	19.23
TET2	22.48
ASXL1	35.87
GATA2	37.27
SETBP1	38.57
NRAS	40.84
TP53	44.44
TP53	44.7
KMT2A	45.3
DNMT3A	46.4
TP53	47.14
U2AF1	47.65
DNMT3A	51.24
TP53	57.63

Correlation between Bone marrow and cfDNA

33 pairs of bone marrow and peripheral blood can be compared. They are collected within 120 days of each other.

- 10 with concordant positive results
- 17 with concordant negative results
- 5 pairs positive by cfDNA but negative by BM cells
- 1 pair positive by BM but negative by cfDNA

	Concordant Negative	Concordant Positiv	cfDNA pos Only	BM positive only
Number	17	10	5	1
Mutated genes (VAF)			DNMT3A (1.35%) CEBPA (2.56%) EZH2 (3.56%) ERBB2 (2.07%), CDK4 (0.78%) IDH2 (0.4%)	KDM6A (4.49%)

CONCLUSIONS

- Monitoring MRD after HSCT using cfDNA and NGS is reliable approach
- Peripheral blood cfDNA can be used as an alternative to performing bone marrow biopsy.
- Low level mutations should not be used as the sole criterion for determining relapse
- Variant allele frequency and the type of the mutated gene should be considered in evaluating actionable findings

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CONTACT INFORMATION

Maher Albitar, MD
Genomic Testing Cooperative, LCA.
175 Technology Dr. #100, Irvine, CA 92618 USA
Phone: 657-202-5950/FAX: 949-301-9719
Mobile: 949.275.7564
malbitar@genomictestingcooperative.com

Jennifer Varca
Genomic Testing Cooperative
175 Technology Drive, Suite 100
Irvine, Ca 92618
Phone: 714-401-3069
jvarca@genomictestingcooperative.com