Genomic Profiling can Provide Utility in Cases of Suspected Myeloid Neoplasia

Matt Hiemenz, Yelena Kemel, James Weisberger, Robert D. Daber
GenPath, Bio-Reference Laboratories, Inc. Elmwood Park, NJ

Introduction

Myelodysplastic syndromes (MDS) largely result from recurrent mutations in a small number of genes with diverse functions including splicing, DNA methylation, transcriptional regulation and signaling. Morphologic assessment of dysplasia can be challenging and additional genetic and cytogenetic abnormalities may aid in diagnosis under certain circumstances. Next-Generation Sequencing (NGS) can serve as a useful aid in cases where traditional methods show no definitive evidence of disease.

Methods

Mutational hotspots, surrounding exon regions, and, where appropriate, full length genes were captured using the Illumina TruSeq Custom Amplicon library preparation method.

LIQUID TUMOR PANELS

OnkoSight Myeloid Malignancies Panel (37 genes)								
ABL1	ASXL1	BCOR	BCORL1	BRAF	CALR	CBL	CDKN2A	CSF3R
DNMT3A	ETV6	EZH2	FBXW7	FLT3	GATA2	HRAS	IDH1	IDH2
JAK2	KIT	KRAS	MPL	MYD88	NPM1	NRAS	PHF6	PTEN
PTPN11	RUNX1	SETBP1	SF3B1	SRSF2	TET2	TP53	U2AF1	WT1
ZRSR2								

OnkoSight MPN Panel (17 genes)								
ABL1	ASXL1	CALR	CBL	CSF3R	DNMT3A	EZH2	IDH1	IDH2
JAK2	MPL	SETBP1	SRSF2	TP53	U2AF1	SF3B1	KIT	

OnkoSight MDS Panel (18 genes)								
ASXL1	BCOR	CBL	DNMT3A	ETV6	EZH2	JAK2	KRAS	NRAS
PTPN11	RUNX1	SETBP1	SF3B1	SRSF2	TET2	TP53	U2AF1	ZRSR2

OnkoSight AML Panel (17 genes)								
ASXL1	BCOR	DNMT3A	EZH2	FLT3	IDH1	IDH2	KIT	KRAS
NPM1	NRAS	PHF6	PTPN11	RUNX1	TET2	TP53	WT1	

Samples were sequenced on an Illumina Miseq with 2 by 186 base pair reads. Sample data was then processed with an in-house suite of tools to identify mutation presence or absence as well as allelic burden. Peripheral blood or bone marrow specimens were submitted with a variety of diagnoses including one or more cytopenias, suspected myeloid neoplasm, and/or a history of a myelodysplastic syndrome, myeloproliferative neoplasm, or leukemia. Samples were defined as abnormal if genomic analysis revealed one or more disease-associated mutations at 4% allele frequency (AF) or greater. Samples were defined as variant if they had a variant present with a population frequency less than 0.05% or if no\limited information was available regarding the clinical significance of the alteration. Normal samples had neither disease-associated mutations nor unclear variants.

Results

A subset of samples (n=218) with concomitant flow cytometry and/or bone marrow morphology showing no definitive evidence of disease was included for analysis.

Number of Cases with No Definitive Evidence of Disease by traditional assessment methods	NGS Result Category
151 (70%)	Normal
23 (11%)	Variant
41 (19%)	Abnormal

Of the 41 cases containing an alteration, 19 had mutations present at allele frequencies (AF) greater than 25% suggesting a disease burden of 25% or greater.

Sample Number	Genes with Mutations (#) present at > 4% AF	Genes with Mutations (#) present at >25% AF
1	TET2 (4)	TET2 (2)
2	TET2 (2)	TET2 (2)
3	DNMT3A, TET2 (2)	
4	CBL	
5	DNMT3A	
6	ASXL1, CBL, RUNX1	ASXL1, CBL, RUNX1
7	TET2 (2)	TET2 (2)
8	NRAS, SRSF2, TET2 (2)	SRSF2, TET2 (2)
9	JAK2	JAK2
10	TP53	
11	TET2 (2)	TET2 (2)
12	U2AF1	
13	TP53	
14	TET2 (4)	TET2
15	DNMT3A	
16	SF3B1	SF3B1
17	DNMT3A, SRSF2, TET2 (2)	
18	TET2 (2)	TET2 (2)
19	TET2	
20	DNMT3A	
21	SRSF2, TET2	SRSF2, TET2
22	RUNX1, SETBP1	RUNX1, SETBP1
23	SF3B1, SRSF2	SF3B1
24	DNMT3A	
25	JAK2	
26	SRSF2	SRSF2
27	ASXL1, SF3B1, TET2	ASXL1, SF3B1, TET2
28	CALR	
29	TET2	
30	U2AF1	
31	DNMT3A	
32	TET2, U2AF1	
33	ASXL1, RUNX1, SRSF2, TET2 (2)	ASXL1, RUNX1, SRSF2, TET2 (2)
34	TET2	
35	SRSF2, WT1	SRSF2, WT1
36	ASXL1	ASXL1
37	ASXL1	
38	DNMT3A	
39	SF3B1, TET2 (3)	SF3B1
40	DNMT3A	
41	TET2, ZRSR2	

The most commonly mutated genes included TET2 (n=18), DNTM3A (n=9), SRSF2 (n=7), SF3B1 (n=4), RUNX1 (n=3), and U2AF1 (n=3). In several instances, the presence of these alterations supported the diagnosis of myelodysplastic syndrome.

Example Case: Sample 27

- 83 year-old female with pancytopenia
- Bone marrow morphology showed mildly hypercellular marrow with erythroid hyperplasia and megakaryocytic atypia
- Flow cytometry detected no phenotypic evidence of abnormal myeloid maturation, increased blasts, or lymphoproliferative disorder
- A normal female karyotype was observed (46, XX[20])
- FISH showed no evidence of: deletion of 5q or monosomy 5, deletion of 7q or monosomy 7, trisomy 8, or deletion of 20q12.
- OnkoSight MDS panel revealed the following mutations: ASXL1 p.Pro722Leufs*3 (36.46% AF), SF3B1 p.Glu592Lys (35.04% AF), TET2 p.Gln1548* (37.79% AF)
- Final integrated diagnosis was low grade myelodsyplastic syndrome, most compatible with refractory cytopenia with multilineage dysplasia (RCMD)
- ASXL1 mutations correlate with decreased overall survival and increased risk of leukemic transformation in MDS

Conclusions

- OnkoSight liquid tumor panels detected recurrent mutations in a number of samples where traditional methods showed no definitive evidence of disease
- In addition to useful prognostic information, NGS may contribute to the diagnosis of MDS and MPNs
- Further studies are needed to define the positive predictive value of recurrent mutations in the diagnosis of MDS with respect to allele frequency and number of genes mutated