A comprehensive genomic profiling approach to detect functional translocations and genomic alterations in a single tube workflow

#2208

Cynthie Wong[1] • Brad Thomas[2] • Yanglong Mou[2] • Christophe Magnan[3] • Segun Jung[1] • Tibor Gyuris[3] • Francys Alarcon[1] • Eve Shinbrot[4] • Fei Ye[3] • Ryan Bender[1,3] • Sally Agersborg[1] • Lawrence M. Weiss[1] • Vincent A. Funari[1]

1 NeoGenomics Laboratories, Aliso Viejo, CA – 2 NeoGenomics Laboratories, Houston, TX – 3 NeoGenomics Laboratories, Carlsbad, CA – 4 NeoGenomics Laboratories, La Jolla, CA

Background

To comprehensively profile mutations in hematologic malignancies, we developed a targeted multimodal NGS assay that can detect SNVs, InDels, fusions, gene expression, and copy number variations from total nucleic acid (TNA) in a single tube workflow. While FISH and cytogenetics are the current standard of care, they are limited in resolution and can not reveal the functional significance. This CGP assay complements those approaches.

Methods

TNA was extracted from peripheral blood and bone marrow specimens from patients with hematologic cancers. TNA was used to prepare libraries then sequenced on a NovaSeq 6000. DNA variants were compared to results from DNA NGS assays and RNA fusions were compared to FISH and RT-PCR.

Results

Our multimodal NGS assay can efficiently use TNA to detect mutations simultaneously within the DNA and RNA in a single tube workflow. From 100 fusion positive samples, we detected fusions in all samples and >25 different fusions were detected. Our NGS assay was 100% concordant with the BCR-ABL1 qRT-PCR assay in samples with an IS value of >0.5, 92.7% concordant with the ArcherDX Heme NGS assay, and 100% sensitive in detecting high-confidence fusions.

In 5 samples previously tested for BCR-ABL1 translocations, we confirmed the RNA expression as well as detected pathogenic DNA variants, including JAK2 p.V617F, U2AF1 p.S34F, ASXL1 p.E635Rfs*15, BRCA p.S1982Rfs*22, and DNMT3A p.S708Vfs*71. In another patient, we found multiple pathogenic mutations (ASXL1 and JAK2), in addition to a BCR-FGFR1 fusion.

Two PML(e4)-RARA and PML(e6)-RARA isoforms were detected and confirmed in one sample, illustrating the high resolution that could be used to help monitor the patient. Three fusions involving CXCR4 (CXCR4-FOSL2, CXCR4-DDX5, and ARID5A-CXCR4), a receptor known to promote proliferation, migration and resistance to chemotherapy were also detected in addition to CXCR4 over-expression in three patients. In another patient, we confirmed a KMKT2A-ARHGEF12/del(11)(q23q23) aberration by NGS that was missed by cytogenetics. In one sample, we confirmed expression of 3 out of 4 different MYC fusions (MYC-BCL6, MYC-IgH, IgH-MYC); importantly only NGS could identify the fusion orientation, illustrating the high resolution of NGS over FISH. In several patients with IgH-BCL1 translocations, we expected BCL1 overexpression. Although DNA PCR results were mostly negative, we discovered increased expression in a subset of these samples. This suggests that despite the detection of the fusion by FISH, a subset may lack gene expression which may suggest a different biological or clinical significance.

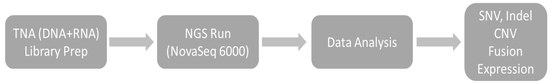

Figure 1. Multimodal NGS workflow.

Fusions	Expected	Detected	Fusions	Expected	Detected
BCR-ABL1	33	32	TCF3-PBX1	4	4
CRLF2-P2RY8	10	10	ZNF384-CREBBP	1	1
JAK2-PAX5	1	1	ZNF384-EP300	1	1
KMT2A-AFF1	3	3	ZNF384-SYNRG	1	1
KMT2A-MLLT3	1	1	ABL1-NUP214	1	1
KMT2A--UBE4A	1	1	ABL1-SRP9	1	1
MEF2D-HNRNPUL1	1	1	BCR-HBA2	1	0
MLLT10-PICALM	3	3	ETV6-PRKAR1A	1	0
PDGFRB-ATF7IP	2	2	RUNX1-ETS2	1	0
RUNX1-RUNX1T1	1	1	TAL1-SLC6A9	1	0

Table 1. Extensive orthogonal validation of fusions. Sixty-nine samples with a list of 20 fusions, previously reported by ArcherDX, were tested by NGS resulting 92.5% (64/69) concordance.

Sample	BCR-ABL1 qRT-PCR Result	BCR-ABL1 NGS Result	DNA NGS Result
P1	Not Detected	Not Detected	BRCA2: p.S1982Rfs*22
P2	Not Detected	Not Detected	JAK2: p.V617F
P3	Not Detected	Not Detected	ASXL1: p.E635Rfs*15 JAK2: p.V617F
P4	Not Detected	Not Detected	DNMT3A: p.S708Vfs*71
P5	Pos (IS 83.868 %)	Detected	U2AF1: p.S34F

Sample	FISH Result	RNA NGS Result	DNA NGS Result
P6	FGFR1 gene rearrangement	BCR-FGFR1	ASXL1: p.S846Qfs*5 JAK2: p.K558N

Tables 2 and 3. Additional pathogenic DNA variants detected by NGS. RNA translocations detected by qRT-PCR or FISH methodologies were confirmed by NGS. Additionally within the same patient sample, pathogenic variants were detected from the DNA.

Sample	FISH Result	NGS Result				
		Expression outlier value	CCND1	FGFR3	MAF	MAFB
PCE1	Gain in CCND1, MAFB locus; loss of IGH locus	6651	7442	94	177	3265
PCE2	Gain CCND1	7052	6193	96	38	548
PCE3	CCND1/IgH fusion, IGH rearrangement, dup(1q), del(13q)	7061	14577	163	43	4239
PCE4	Dup(1q), Del(13q), atypical FGFR3/IGH	7061	965	32	30	2023
PCE5	IGH rearrangement (not FGFR3, CCND1, MAF, or MAFB)	6408	2695	47	139	4778
PCE7	CCND1/ IGH , gain in FGFR3, MAF, MAFB	2260	129066	13	98	162
PCE8	gain in the CCND1, Del MAF locus	5618	29298	79	38	875

Figure 2. Differential BCL1 (CCND1) expression in patient samples with IgH-BCL1 translocations. NGS detected increased expression of CCND1 (supporting reads in pink), as expected in samples with the IgH-BCL1 translocation or gain in the CCND1 gene. However, a subset did not show increased expression (supporting reads in blue).

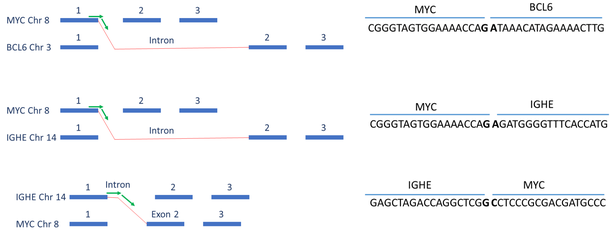

Figure 3. Multiple MYC fusions detected in one patient sample by NGS. Rearrangements in BCL6, MYC, and a t(8;14) IGH-MYC fusion were detected by FISH. NGS detected these abnormalities, found three MYC fusions, and differentiated the orientation of the fusions which were confirmed by Sanger Sequencing.

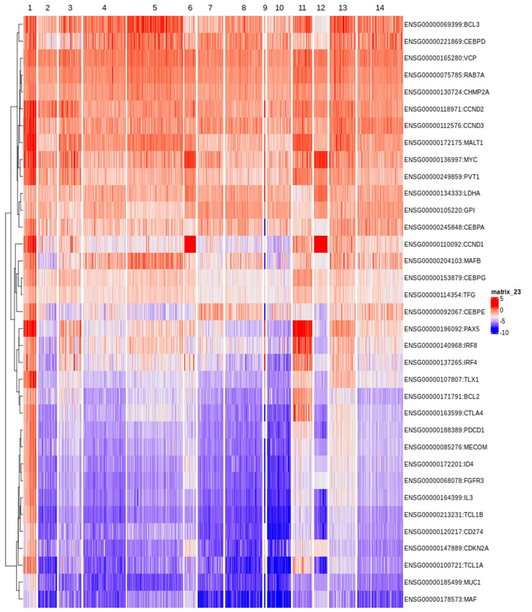

Figure 4. Distinct gene expression patterns among hematologic diseases. Gene expression profiles from over 900 samples tested with our Heme NGS panel were assessed by K-means clustering. Clusters 6, 11 and 12 show expression profiles dominated by overexpression of CCND1, MYC, PAX5, IRF8 which are known to have clinical significance in Heme Malignancies. Roughly 54% of patients screened for CLL showed a B-cell signature (cluster 11).

Conclusion

- Comprehensive profile utilizing DNA and RNA with NGS provides improved diagnostic testing of hematologic disease.
- NGS has high resolution and by targeting RNA we can verify the functional significance unlike traditional DNA approaches.
- Combination of FISH and NGS results can provide a complete picture to improve detection and characterization of various hematologic diseases.
- The simultaneous detection of clinical significant gene fusions, along with genomic mutations are critical to characterizing the disease.
- Gene expression profiles that reveal upregulation of pathways may be important in helping refine the diagnosis and prognosis of patient