A Novel NGS-Based Simultaneous Detection of DNA and RNA Biomarkers Using Total Nucleic Acid (TNA) for Acute Lymphocytic Leukemia (ALL)

#1875

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Background

Acute Lymphocytic Leukemia (ALL) is the most common childhood cancer and accounts for about a quarter of adult acute leukemias. Current NCCN recommendations for clinical testing for risk stratification and treatment guidance include karyotyping, FISH testing for translocations, and RT-PCR for gene fusions and sequencing for DNA mutations detection. Most NGS based approaches test DNA mutations and RNA fusions separately, thereby requiring higher input material and multiple workflows adding to the cost and turn-around-time. An NGS based assay for the detection of DNA variants (NeoGenomics Heme NGS assay) in heme malignancies using Total Nucleic Acid (TNA) is already available in our clinical laboratory and complements FISH based fusion detection and karyotyping but an integral assay to detect both DNA and RNA alterations with a simple workflow for ALL is needed.

Methods

We used TNA extracted from 93 bone marrow and peripheral blood samples from patients and healthy donors, and SeraSeq Myeloid Fusion RNA Mix (SeraCare Inc.) as control. DNA/RNA libraries were prepared using a custom amplicon based Multimodal NGS panel (Qiagen Inc.) targeting 297 genes and 213 genes for DNA and RNA fusion detection, respectively. The enriched dual indexed amplicon libraries were sequenced on an Illumina NovaSeq 6000. The sequence data was processed with a customized bioinformatic pipeline for DNA variant as well as a novel machine learning algorithm for RNA fusion detection (Figure 1). We analyzed sensitivity, specificity, accuracy, reproducibility, and repeatability for clinical use. The DNA variants were orthogonally confirmed using other NGS assays, and the RNA fusions were confirmed on an RNA-seq Archer assay or RT-Sanger confirmation assays.

Results

Here, we developed and validated a single tube comprehensive NGS panel using a custom multimodal chemistry that uses TNA as input for simultaneous dual detection of DNA and RNA abnormalities in ALL patients' samples. The fusion concordance was 96.3% for the RNA fusion panel. The assay detected significant gene fusions in ALL (Table 1).

The specificity was determined at 100% using a set of 42 fusion negative samples. The limit of detection (LOD) was analyzed using serial dilutions to up to 3 log reduction (LR) using a the SeraSeq Myeloid Fusion sample and were detected down to 1 LR. The reproducibility was tested using a positive fusion and SeraSeq samples across three runs and was reported at 100%.

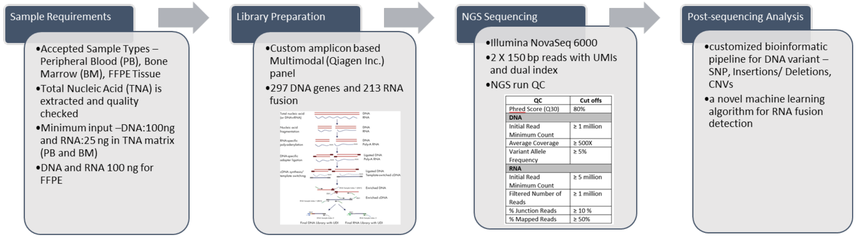

Figure 1. Multimodal NGS workflow.

Table 1. Results of Validation of Gene Fusions. Fifty two samples with known gene fusions confirmed by ArcherDx assay, were tested by the NeoHeme NGS assay resulting in 93.4% concordance.

Gene Fusion	Expected	Detected	Gene Fusion	Expected	Detected
AFF1-KMT2A	4	4	P2RY8-CRLF2	2	2
BCR-ABL1	18	18	PCM1-JAK2	1	1
ETV6-ABL1	2	2	PICALM-MLLT10	2	1
ETV6-NTRK3	6	6	PML-RARA	2	2
ETV6-RUNX1	2	1	RBPMS-NTRK3	1	1
FIP1L1-PDGFRA	2	2	RUNX1-NEB	1	1
KAT6A-CREBBP	1	1	RUNX1-RUNX1T1	4	4
KMT2A-AFF1	4	4	SET-NUP214	1	1
KMT2A-ARHGEF12	1	1	STIL-TAL1	1	1
KMT2A-MLLT1	1	1	TCF3-PBX1	3	3
LMNA-NTRK1	2	2	TFG-ADGRG7	2	2
NUP98-HOXA9	1	1	ZEB2-PTMA	1	1

Next, a small cohort of ALL samples (n=8) was included as part of this study to simultaneously evaluate DNA and RNA mutations. We detected pathogenic DNA variants in genes previously reported in ALL that included PTEN, FLT3, IKZF1, JAK1, JAK2, KRAS, PAX5,U2AF1, and TP53, and also RNA fusion BCR-ABL1, and the results were confirmed by an orthogonal NGS assay (NexCourse and RNA-Seqv1 for fusions).

One sample carrying a BCR-ABL1 fusion (detected by RNA panel) also harbored mutations in IKZF1 and KDM6A in DNA (detected by DNA panel) that is reported as unfavorable prognostic biomarker for Ph-Like ALL demonstrating comprehensive panel could identify multiple variants within the same sample, demonstrating the advantage DNA+RNA testing has over the classical single gene FISH/RT-PCR testing for the efficient risk stratification and treatment in ALL patients.

Table 1. Comprehensive profiling of ALL samples. The DNA variants and RNA fusion data from the NeoHeme NGS assay for ALL samples .

Sample	Gene	Variant type	DNA variant	Variant Allele Frequency	RNA fusion
P1	KDM6A	insertion	p.C760Rfs*21	56.40%	BCR-ABL1
	IKZF1	insertion	p.K463Mfs*3	5.60%	
P2	PAX5	deletion	p.R122Gfs*37	7.11%	None
P3	JAK2	snv	p.R683G	45.34%	None
P4	PTEN	snv	p.G127E	5.07%	None
	KRAS	snv	p.G13D	35.60%	
P5	FLT3	snv	p.D835E	3.75%	None
P6	JAK1	snv	p.L783F	28.50%	None
	CBLC	insertion	p.Q419Pfs*81	87.85%	
	U2AF1	snv	p.S34F	26.36%	

Conclusion

- In this study, we demonstrated that the single tube TNA based NeoGenomics NGS assay can simultaneously detect the DNA and RNA biomarkers associated with ALL for improved diagnostic and prognostic recommendations.
- The single-tube assay for detection of both RNA fusions and DNA variants using the same sample could offer comprehensive and cost-effective solution for clinical laboratory test for ALL patient care.
- This is a promising approach that might be used as a dual DNA/RNA alterations detection on other hematological neoplasia.