A comprehensive genomic profiling approach to interrogate hematologic malignancies using a novel multimodal next generation sequencing assay in a single-tube

#5639

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Background

Many new guidelines require a comprehensive genomic profiling approach for diagnosis, risk stratification and therapy decisions. Limitations in sample quantity and throughput may limit the number of single biomarker tests (i.e. FISH, karyotyping, sequencing, qRT-PCR) that can be performed for a patient. There currently are multiple commercial NGS assay options for total nucleic acids; however, they require independent parallel workflows, and twice the amount of sample and effort. Here we developed a novel single-tube consolidated workflow of DNA/RNA utilizing custom QIAseq multimodal chemistry (Figure 1). This simplified workflow enables a discovery approach of all critical DNA/RNA abnormalities in hematologic malignancies, extending our NGS capabilities to large structural changes, RNA fusions and gene expression.

Methods

The 297 genes and 14 chromosomes specific to hematological cancers were targeted in the genome, along with over 200 RNA genes in the transcriptome using a custom QIAseq workflow. Captured DNA/RNA targets from 135 patients were sequenced with unique dual indices on an Illumina NovaSeq 6000. Coverage and variant allele frequency from all gene and chromosomal targets in 25 disease free patients, denoted as a panel of normal (PoN), was compared to the same genomic targets in 76 patients that were referred for a suspected or diagnosed hematological malignancy (i.e. MDS, CML, AML, ALL); furthermore, the analysis was extended to additional 546 patients. We compared results from our custom algorithm to karyotyping and FISH, and assessed the relationship between structural changes and tumor mutation burden (TMB). Gene fusions were confirmed by qRT-PCR or Sanger sequencing. Moreover, 69 fusion positive samples reported by ArcherDX containing 20 independent fusions were used for orthogonal validation.

Results

- Cytogenetic abnormalities in 30/32 patients were confirmed by karyotyping and FISH data; two cases with abnormalities were missed by NGS.
- The extended analysis on 546 patients comparing to karyotyping shows 92.5% accuracy (data not shown).
- NGS detected additional abnormalities not detected by cytogenetics, including a case with loss of chr17 (Figures 2 and 3).
- TMB was calculated for each tumor type, but no direct correlation with the structural changes was observed (Figure 4).
- RNA fusions were confirmed with 100% of BCR-ABL fusions (p210, p190) in all patients (59/59) reported by qRT-PCR with International Scale (IS) percentages ranging from 2.4-100%. Notably, we confirmed two clinically significant fusions, SET-NUP214 and RUNX1-RUNX1T1, in two patients not previously interrogated by qRT-PCR. The SET-NUP214 fusion, normally associated with ALL, was identified in a patient with suspected CML. A ROS1-GOPC gene fusion was detected in a commercial universal human RNA reference material, commonly used in expression profiling studies (Figure 5). In addition, orthogonal validation was performed on 69 fusion positive samples reported by ArcherDX resulting 92.5% (64/69) concordance (Table 1).

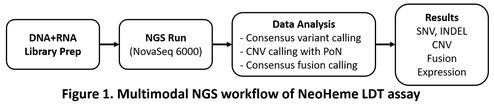

Figure 1. Multimodal NGS workflow of NeoHeme LDT assay

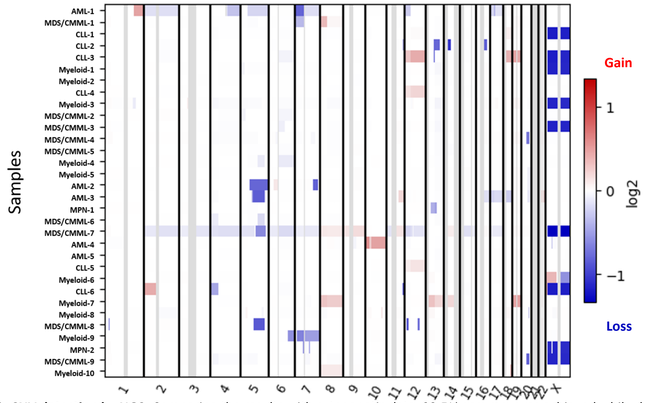

Figure 2. CNV detection by NGS. Comparing the results with cytogenetic data, 93.5% accuracy was achieved while detecting additional abnormalities in other chromosomes.

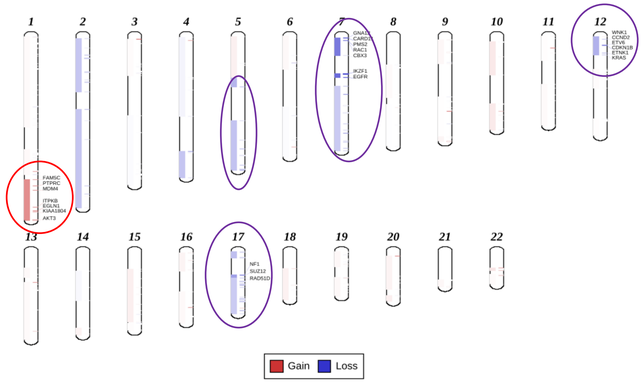

Figure 3. Additional abnormality in AML-1 detected by NGS. While confirming the gain and loss in chr1, 5, 7, and 12, loss in 17q was detected including deletion of driver genes-NF1 and SUZ12.

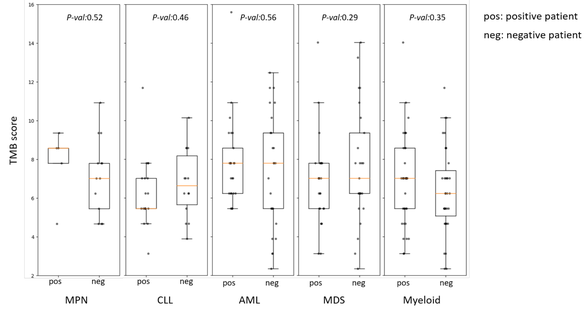

Figure 4. TMB distribution. TMB scores were calculated and compared between positive and negative patients by unpaired two-tail t-test

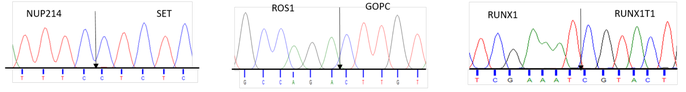

Figure 5. Additional fusions detected by NGS. Three clinically significant fusion genes discovered by NGS were confirmed by Sanger-sequencing. Black arrow lines indicate the fusion breakpoint between the two genes.

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.

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

Conclusion

- CNV and fusion detection using a novel multimodal NGS can offer a better diagnostic and prognostic testing service for patients with hematologic disease
- CNV and TMB are not directly correlated for hematological cancers
- The study confirms the validity and utility of simple but efficient comprehensive genomic profiling for use in hematologic malignancies