Evaluation of OGT's SureSeq™ Myeloid Fusion Complete NGS Workflow Solution V2 for partner-agnostic fusion gene detection in acute leukemias

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Recurrent gain-of-function translocations often result in gene fusion events, which are a hallmark of acute leukemia, and many of these fusion events can significantly impact disease classification, prognosis and treatment approaches. Traditionally, fusion events are detected using fluorescence *in situ* hybridization (FISH) and/or polymerase chain reaction (PCR)-based techniques, but these methods can be limited by their reliance on prior knowledge of what partner genes or gene breakpoints are involved and cannot be used to detect multiple and/or novel fusions. Conversely, next-generation sequencing (NGS) assays allow simultaneous detection of multiple fusion genes in a single assay, including multiple partners for the same genes and novel fusions.

Here we demonstrate the utility of a partner-agnostic targeted RNA sequencing workflow using OGT's SureSeq™ Myeloid Fusion Complete NGS Workflow Solution V2 (890001-24) for functional detection of both canonical and rare fusions.

MATERIALS AND METHODS

Patients

Bone marrow from 15 acute leukemia patients (9 AML and 6 ALL), who had agreed to genetic testing, was collected for routine molecular and cytogenetic tests.

Sample preparation and next-generation sequencing

NGS fusion testing was performed using OGT's SureSeq™ Myeloid Fusion Complete NGS Workflow Solution V2 as per manufacturers guidelines. All samples used in this workflow had undergone prior cytogenetic analysis using either qPCR and tagmentation-based sequencing, GTG and/or FISH. For OGT's workflow, we used Trizol™ extraction to obtain 300ng total RNA per sample and were able to detect 14 previously characterized rearrangements withpartner-agnostic fusion detection method by baiting for 18 clinically relevant target genes.

Quality of sequencing data

The median total read count across all samples was 5.9 M (2.5 – 9.3 M) with 67.4% median aligned reads (49.2-82.6%). Duplicate rates ranged from 7.2 to 14%. The fusion-contributing read counts ranged from 10 to 4776.

Orthogonal methods

Bone marrow from the patients was cultured for GTG-banding and FISH testing using routine diagnostic procedures. FISH analysis was performer using Metasystems™ probes. qPCR was used to confirm the expression of canonical fusions using standardized EAC methodology [1] or Zheng et al. protocol [2]. After qPCR, products of amplification were processed using Illumina DNA prep™ and sequenced to confirm the exonic coordinates of breakpoints.

RESULTS

Detected fusions

The SureSeq™ Myeloid Fusion Complete NGS Workflow Solution V2 allowed 100% concordant detection of clonal reciprocal translocations in all 14 samples, compared with either FISH or GTG-banding.

We successfully detected canonical gene fusions (*RUNX1::RUNX1T1, CBFB::MYH11, PML::RARA* and *BCR::ABL1*), known *KMT2A* rearrangements (*KMT2A::ELL* and *KMT2A::ENL*) and *MECOM* locus rearrangements (*RUNX1::MECOM* and *ETV6::MECOM*). In 3 cases, non-canonical translocations were detected: *KMT2A::TET1* (known) [3], *MECOM::CBFA2T3* and *NUP98::HOXD8* (novel) [4,5]. No fusions were detected in negative samples or samples bearing non-targeted fusions.

Fusion	Adjacent exons (MANE select)	ISCN description	Total supporting reads	Validation
RUNX1::MECOM	RUNX1ex7, MECOM ex2	t(3;21)(q26.2;q22.12)	1242	qPCR, tagment.*, FISH, GTG
CBFB::MYH11	CBFB ex5, MYH11 ex33	t(16;16)(q22.1;p13.11)	64	qPCR, tagment.*, FISH, GTG
KMT2A::TET1	KMT2A ex8, TET1 ex11	t(10;11)(q21.3;q23.3)	10	FISH, GTG
MECOM::CBFA2T3	MECOM ex2, CBFA2T3 ex1	t(3;16)(q26.2;q24.3)	116	FISH
BCR::ABL1	BCR ex14, ABL1 ex2	t(9;22)(q34.12;q11.23)	306	FISH, GTG, qPCR*
RUNX1::MECOM and BCR::ABL1	RUNX1ex7, MECOM ex2 and BCR ex13, ABL1 ex2	t(3;21)(q26.2;q22.12) and t(9;22)(q34.12;q11.23)	1260 and 1541	FISH, GTG, qPCR*
KMT2A::MLLT1	KMT2A ex10, MLLT1 ex2	t(11;19)(q23.3;p13.3)	286	qPCR, tagment.*, FISH, GTG
MECOM::ETV6	MECOM ex2, ETV6 ex2	t(3;12)(q26.2;p13.2)	971	FISH, GTG
KMT2A::ELL	KMT2A ex8, ELL ex2	t(11;19)(q23.3;p13.11)	149	qPCR, tagment.*, FISH, GTG
RUNX1::RUNX1T1	RUNX1 ex5, RUNX1T1 ex3	t(8;21)(q21.3;q22.12)	3718	qPCR*, FISH, GTG
BCR::ABL1	BCR ex1, ABL1 ex2	t(9;22)(q34.12;q11.23)	162	FISH, GTG, qPCR*
PML::RARA	PML ex4, RARA ex2	t(15;17)(q24.1;q21.2)	1598	qPCR*, FISH, GTG
NUP98::HOXD8	NUP98 ex12, HOXD8 ex2	t(2;11)(q31.1;p15.4)	49	FISH

Table 1: Fusions detected by SureSeq Myeloid Fusion Complete NGS Workflow Solution V2.

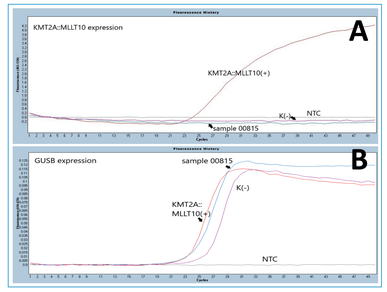

Figure 1: *KMT2A::MLLT10* rearrangement excluded by qPCR (**A** and **B**), detected by OGT SureSeq™ Myeloid Fusion panel (**C**).

Fusion verification

All fusion events identified with the SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 were in concordance with GTG and/or FISH results. Fusion partners orientation were compatible, and exons involved coherent with functional studies. qPCR reactions yielded positive results for 10 canonical fusions. Tagmentation-based sequencing or qPCR showed the involvement of the same exons described in the NGS panel (see table 1, labeled*). In two negative samples there were suspected non-canonical fusions of *ETV6* and *ABL1* genes identified by FISH, which were not targeted by the OGT workflow and therefore are considered outside of the test's scope. The third negative sample was extracted from a healthy donor's bone marrow and had no expected fusion events.

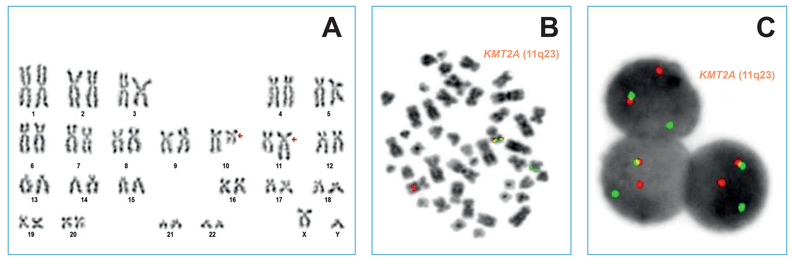

Figure 2: Unknown *KMT2A* rearrangement, described initially as 46,XY,t(10;11)(q22;q23) with *KMT2A::MLLT10* rearrangement GTG (**A**), ISH (**B**) and nucISH(**C**).

CONCLUSIONS

In this study we demonstrated the capability of OGT's SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 to achieve 100% accurate detection for novel and canonical fusion events. By allowing concurrent detection of multiple known and novel rearrangements, NGS assays offer an economical and efficient alternative and addendum to routine cytogenetic approaches.

Disclosure

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Literature

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