Precise and Accurate Detection of Mutations and Copy Number Alterations in CLL Using Enrichment-Based Targeted NGS

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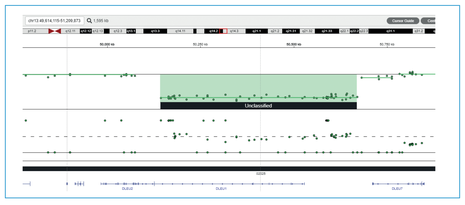
The need for better and faster molecular risk stratification has continued to emerge alongside the development of novel treatment regimens for chronic lymphocytic leukemia (CLL). Traditionally, comprehensive genetic testing in CLL requires multiple laborious and costly diagnostic strategies. Traditionally, actionable copy number alterations (CNAs) are tested by cytogenetic methods. Typically, these CNAs are heterogeneous in size and the detection of CNAs smaller than 1Mbp by FISH is often challenging for the cytogeneticist. The application of next-generation sequencing may serve to help reduce the burden of issues associated with CLL testing.

In this study we analyze the concordance of CNA size and architecture as well as the sensitivity of small variant detection using OGT's SureSeq™ CLL+CNV V3 Panel, FISH and Nextera® sequencing.

MATERIALS AND METHODS

- Genomic DNA was extracted from peripheral blood of 28 progressive or recurrent CLL patients.
- FISH analysis of deletions in 11q, 13q, 17p and trisomy 12 was performed.
- NGS testing was carried out using the SureSeq CLL + CNV V3 Panel, covering mutations in 16 genes and CNAs in chromosomes 6,11,12,13, and 17.
- The validation of small variants in *TP53, SF3B1, BTK, BRAF* and *KRAS* genes was carried out with Nextera sequencing using Illumina DNA prep (M) [1-2].

RESULTS: CNV CALLING



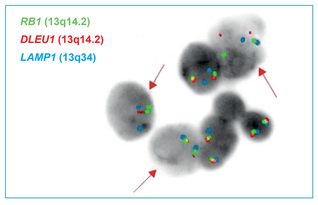
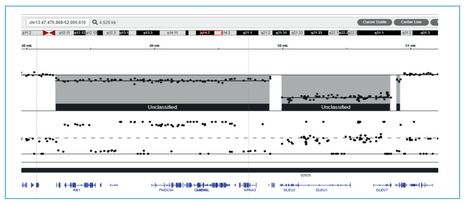


Figure 1: OGT's Interpret NGS Analysis Software visualization and FISH result of a small (450kbp) deleletion in 13q (sampleCG136), resulting in a partial deletion of *DLEU1* locus.



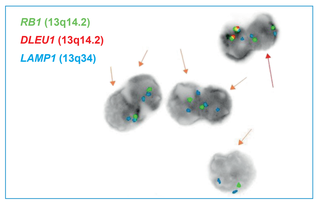


Figure 1: OGT's Interpret NGS Analysis Software visualization and FISH result of a compound rearrangement resulting from biclonal 13q deletion (sample 1262). The major clone (83% of cells) harboured sole *DLEU1* deletion, the minor clone (12% of cells) both – *DLEU1* and *RB1* deletions.

RESULTS: SNV & INDEL CALLING

Sample ID	TP53 variants	TP53 VAF	BTK variants	Other variants	17p [Mbp]	11q [Mbp]	12p [Mbp]	13q [Mbp]	FISH cel %
01008	c.818G>C	3%	no	BIRC, XPO1, MYB	no	del11q	no	del13q [0,8M]	77% / 48%
01055	c.626_627del	12%	no	no	no	no	no	del13q [1,48M]	33%
01262	c.707A>G, 45%	45%	no	no	del17p	no	no	del13q [2,5M]	8% / 95%
01358	c.721T>G	26%	no	no	no	no	no	del13q [3,5M]	40%
01550	c.743G>A	91%	no	no	del17	no	no	no	97%
01968	c.375G>A	45%	no	no	no	no	no	del13q [0,9M]	48%
01973	c.578A>G	73%	no	SF3B1	del17	no	no	del13q [1,4M]	80% / 90%
02477	c.183dup, c.159G>A, c.818G>C , c.273G>A c.602T>A	4%, 4%, 1%, 1%, 1%	no	SF3B1, SAMHD1, MYB	del17p [8,9M]	no	no	no	63%
B1077	no	No	c.1442G>C	BIRC3	del17p [18,1M]	del11q [59,1M]	no	no	na
00366	c.536A>T, c.77C>A	41%, 1%	no	no	del17	del11q	no	no	35% / 21%
00481	no	no	no	no	no	no	trisomy 12	no	26%
01450	no	no	no	BIRC3, ATM	no	del11q [61,7M]	no	del13q [1,5M]	92% / 94%
CG162	c.473G>T	34%	c.1442G>C	no	del17p	no	trisomy 12	del13q [9.9M]	54% / 58% / 54%
01507	no	no	no	no	trisomy 17	no	no	del13q	11% / 10%
2423	no	no	no	no	del17p	del11q	no	no	80 / 21%
B1027	no	no	no	SF3B1	no	no	no	no	na
B0904	no	no	no	ATM	no	no	no	no	na
B0883	no	no	no	XPO1	trisomy 17 [0,3M]	no	no	del13q [85,8M]	40% / 48%
CG136	no	no	no	no	no	del11q [4,6M]	no	no	80%
CG270	no	no	no	no	Del17p [2,1M]	no	no	del13q	21% / 20%
1107	c.524G>A	3%	no	no	del17p	del11q	no	no	50% / 48%
1953	c.448_460del	78%	no	SF3B1	no	no	no	no	na
2025	c.1015G>T	5%	no	no	no	no	no	del13q	8%
B0368	no	no	no	BRAF	no	no	dup 12q [81,8M]	no	35%
1509	c.638G>T	16%	no	no	no	no	no	del13q [6,1M]	38%
2465	no	no	no	no	no	del11q [22,9M]	no	no	58%
2870	c.747G>C, c:743G>A, c.527G>T, c.524G>A	4%, 3%, 4%, 4%	no	KRAS	no	no	no	del13q [35,9M]	21%
3181	no	no	no	SF3B1 (3x)	no	del11q [34,8M]	no	no	64%

Table 1: CNVs and small pathogenic variants detected by OGT's SureSeq™ CLL+CNV V3 Panel. The variants in green were confirmed by orthogonal methods: Nextera sequencing (SNV/indel) or FISH (CNVs). In orange are variants that were detected by orthogonal method but were not confirmed by OGT's SureSeq CLL + CNV V3 Panel as they were below its detection limit. In red are variants detected by FISH, but not detected by OGT's SureSeq CLL + CNV V3 Panel as they were below its detection limit.

Figure 2: Examples of small pathogenic variants detected by OGT's SureSeq™ CLL+CNV V3 Panel. *BTK* resistance variant (**A**), multiple *TP53* variants (**B**).
Green arrow indicates a benign variant, red arrows- pathogenic.

23 pathogenic variants in *TP53* were detected with allele burden (VAF) of 1-81%. 7 variants in *SF3B1* were detected (VAF ranged 1-48%). All were confirmed by the orthogonal method. Two resistance mutations in *BTK* were also detected by OGT's SureSe CLL+CNV V3 Panel in patients resistant to the BTK inhibitors. Moreover, *BRAF* and *KRAS* pathogenic variants were also detected and confirmed by Nextera sequencing [2].

Within the declared detection limit of the NGS panel, there were 9 deletions in 11q, 10 losses of 17p, two trisomies and one partial duplication of 12q and 11 deletion of 13q. The size of aberrations ranged from 298kbp to full chromosomes. All aberrations were confirmed by FISH. Deletions smaller than 1Mbp were described in FISH as "dim", indicating correct hybridization pattern was present with different signal intensities (example shown in Fig.1.)

In case of 13q deletions both FISH and NGS concordantly described partial losses and compound deletions of *DLEU and/or RB1* loci. In 4 instances 13q deletion was further characterized as clonally heterogeneous, manifesting in two distinct hybridization patterns in FISH and in differences of logR in NGS (example shown in Fig. 2.).

Samples harboring CNAs with less than 20% aberration burden in FISH were not detected by the SureSeq™ CLL+CNV V3 Panel, as they were below its detection limit.

CONCLUSIONS

We demonstrate that hybrid-capture next generation sequencing is an efficient tool combining small variant and CNA detection in clinical CLL samples. The actionable mutations of at least 2,5% VAF were called, as well as all of disease associated clonal copy number alterations within the methods detection limit (20%). Our findings confirm that applicability of NGS panels in CLL diagnostics [3]. The benefit of NGS testing is its higher resolution, enabling reliable detection of < 1Mbp alterations which may be overlooked by the human eye during FISH analysis.

Disclosure

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Statements of fact and opinions expressed are those of the presenters individually and, unless expressly stated to the contrary, are not the opinion or position of the Oxford Gene Technology Group (OGT).

Literature

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