COLORECTAL CANCER PATIENTS COMPREHENSIVE GENOMIC PROFILING USING TRUSIGHT ONCOLOGY 500

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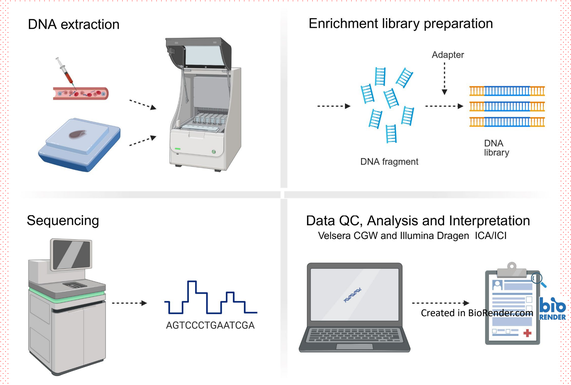
Introduction

Comprehensive genomic profiling (CGP) provides testing of hundreds of pan-cancer biomarkers with tumor markers specific for individual tumor types. This approach brought the possibility of single nucleotide variants (SNV), insertions and deletions (INDELS), copy number variations (CNV), fusions and splice variants detection in DNA and RNA at one time, including biomarkers tumor mutational burden (TMB) and microsatellite instability (MSI) additionally. The aim of this study was to validate TruSight Oncology 500 (TSO500) NGS assay using different types of DNA input samples from solid tumor and different types of bioinformatician analysis.

Material and method

In this study, we included in total of 513 samples obtained from colorectal cancer patients and commercial reference standard Mimix™ OncoSpan Formalin-fixed paraffin-embedded (FFPE) cell line-derived Reference Standard containing over 380 variants across 152 key cancer genes. Part of the samples, 295, came from Masaryk Memorial Cancer Institute Brno, other 218 come from Olomouc University Hospital, Pathology department. All participants consented to the processing of their data and samples, which was documented by signing an informed consent form. The input amount of DNA, isolated from FFPE and RNA-later tissue samples, was 40 ng. All FFPE samples were mechanically fragmented to the 90-250 bp dsDNA fragments using Covaris focused ultrasonicator prior to library preparation. TruSight Oncology 500 High Throughput kit combined with IDT for Illumina – UMI DNA/RNA UD Indexes set A/B were used for library preparation. The quality control (QC) of all completed libraries was performed prior to sequencing using a Qubit® 2.0 Fluorometer with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and 2100 Bioanalyzer using High Sensitivity DNA kit (Agilent Technologies). Sequencing was performed on an Illumina NovaSeq 6000 with a read length of 100 bp PE (Fig.1).

Fig. 1: TSO500 HT workflow



Results and discussion

The results showed that the TSO500 HT kit provides high sensitivity and specificity in detecting tumor markers in patients with colorectal cancer. Sequencing quality parameters have shown satisfactory quality of sequencing data (Fig.2). The average total number of reads obtained was 118 thousand with an average coverage per sample of 271, with a minimum coverage of 124, and a maximum coverage of 1127, whereas the %>Q30 parameter reached the value of 93.28. It was found that biomarkers were significantly elevated in samples with higher amounts of cancer cells. 241 patients carried a variant in at least one of the genes *KRAS*, *NRAS*, *ERBB2*, which are typical for colorectal cancer, which corresponds to a frequency of 68% (Tab. 1), with *KRAS* p.G12D and p.G12V being the most common variants. The variant frequencies in pan-cancer genes as *BRAF*, *NTRK1*, *NTRK2* and *NTRK3*, and other cancer associated variants are shown in the Fig.3. For reliable data, the median insert size at the DNA level should be >70 bp and the median target coverage >150x, which was the case for 98.73% and 95.14% of the validation samples, respectively. As the last set of samples is still in the process of analysis using CGW software (Velsera), a final evaluation of all patient data will be available soon.

Fig. 2: Per Read Metrics

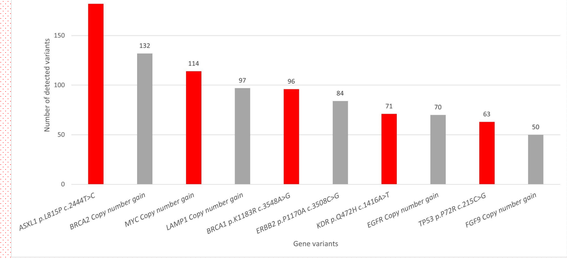
Per Read Metrics

READ	CYCLES	YIELD	PROJECTED YIELD	ALIGNED (%)	ERROR RATE (%)	INTENSITY CYCLE 1	%>Q30
Read 1	101	1.32 Tbp	1.32 Tbp	1.35	0.09	1329.91	93.75
Read 2 (I)	10	119.04 Gbp	119.04 Gbp	0.00	0.00	1493.18	92.93
Read 3 (I)	10	119.04 Gbp	119.04 Gbp	0.00	0.00	1559.93	90.05
Read 4	101	1.32 Tbp	1.32 Tbp	1.31	0.31	1103.59	93.13
Non-index Reads Total	202	2.65 Tbp	2.65 Tbp	1.33	0.20	1216.75	93.44
Total	**222**	**2.88 Tbp**	**2.88 Tbp**	**1.33**	**0.20**	**1371.65**	**93.28**

Tab. 1: The most frequent colorectal cancer associated variants

Gene	Number of gene variants	Number of mutation carriers
KRAS	26	172
ERBB2	20	166
BRAF	13	51
NRAS	8	18
RET	4	10
NTRK1,2,3	0	0

Fig. 3: The most frequent variants



Conclusion

It may be concluded that targeted known cancer variants detection using NGS technology is a cost-effective solution for analysing low frequency SNV´s, CNVs, INDELS, gene fusions, splice variants, somatic variants, structural and transcript variants, TMB and MSI are also included in this pan-cancer cancer enrichment panel. 523 cancer-related genes in TSO500 kit were chosen based on alignment to guidelines, drug labels and clinical trials across multiple tumor types. The concurrently delivered Illumina Connected Analytics/Illumina Connected Insights (ICI) bioinformatics data analysis solution complements the laboratory portion of the testing, providing a comprehensive solution even for high-volume sites. Compared to Velsera CGW, Illumina provides a more automated analytical workflow, where raw data from the sequencer are sent directly to the Dragen (Dynamic Read Analysis for GENomics) for secondary analysis and then automatically processed in the ICI for tertiary analysis. This significantly speeds up the data processing while avoiding the generation of errors in the analysis workflow. The high reproducibility and robustness of the TSO500 kit in clinical settings indicate that this tool can be valuable for personalized treatment and monitoring of oncology patients. Furthermore, the results of this research can contribute to the further development of diagnostic methods for colorectal cancer and similar oncological diseases.

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