Comprehensive molecular analysis of tissue samples from cancer patients: Evaluation of a novel all-in-one NGS strategy for diagnosis and theranostic

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Background

DNAseq, RNAseq, and determination of microsatellite instability (MSI) are now routine analyses in precision medicine for the management of patients with cancer. Analyzing DNA/RNA requires reliable assays often performed in two separate runs. This can be challenging considering the limited quantity of total nucleic acids (DNA) extracted from some FFPE samples. Sequential approaches (i.e. DNAseq followed by RNAseq) increase the turnaround time and costs, delaying treatment. Here, we evaluated a novel NGS approach developed by Invitae (San Francisco, USA) which allows the detection of single nucleotide variants (SNVs), indels, MSI and structural rearrangements in one single NGS run using DNA extracted from FFPE tissue.

Methods

A total of 24 FFPE samples from patients with various cancers previously characterized using conventional methods: DNAseq, RNAseq, PCR, IHC or FISH were included. Selected samples were qualified, and the tumour cell content evaluated by a senior pathologist. After macrodissection, TNAs were extracted with ReliaPrep™ FFPE gDNA Miniprep System from Promega (Madison, USA) and libraries were prepared using in parallel VariantPlex (DNA) and FusionPlex (RNA) kits from Invitae. One single NGS run was performed with NextSeq 500/550 High Output v2 kit from Illumina (San Diego, USA) (Figure 1). Kits were designed to detect fusions without a priori knowledge of the gene fusion partner, MSI, and variants using a panel of 156 cancer-relevant target genes (71 DNA, 136 RNA).

Results

The single extraction of TNA allowed sufficient quality and quantity of RNA and DNA to perform the all-in-one NGS. Among 9 previous SNVs/indels and 11 MSI samples identified by orthogonal methods, all-in-one NGS approach confirmed all (100%) (Table 1). Among 12 fusions detected by the reference methods, 11 were detected by FusionPlex (91.7%) (Table 2). The ROS1 fusion missed by NGS was scored low (1+) by IHC which was at the limit of sensitivity of the IHC method. Further analyses are conducted to determine whether the ROS1 fusion is a false positive result obtained by IHC, or a false negative result by all-in-one NGS.

Conclusion

Novel, all-in-one NGS approach from Invitae shows promising results by comprehensively detecting SNVs, indels, MSI and structural variants. Analyses of DNA and RNA using the novel NGS strategy improves the overall turn-around-time and cost-effectiveness. This can potentially aid clinicians and patients to take timely decisions regarding treatment modalities.