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 (TNA) 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 TNA 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 Outpout 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).

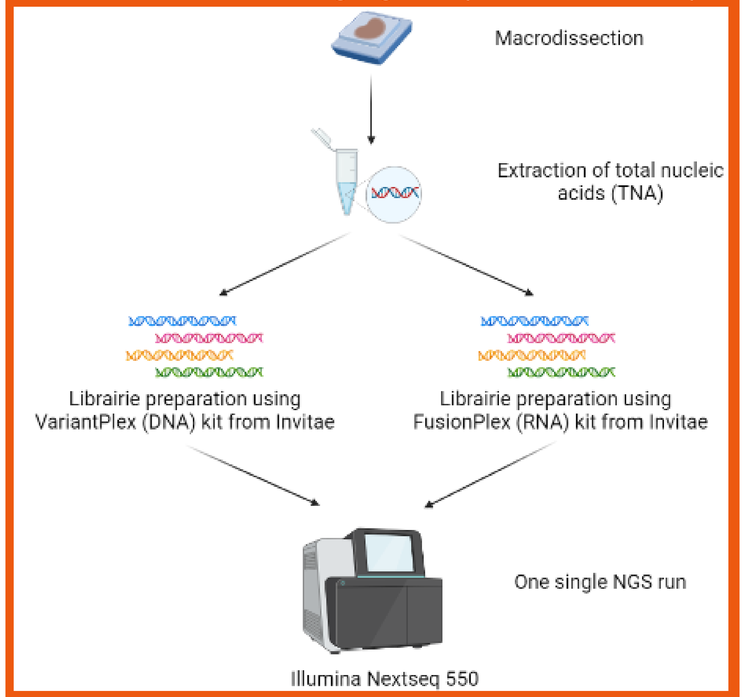

Figure 1: Protocol steps

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.

Variants detection with VariantPlex

Table 1: VariantPlex analyses

Gene	Variant	Expected VAF % (NGS)	Observed VAF % (VariantPlex)
BRAF	V600E	28,2	29,32
SMAD4	R497H	25	38,6
CTNNB1	T41I	25	40,41
ESR1	S450A	12,7	11,11
KRAS	G12A	20,7	21,14
AKT1	E17K	47	55,56
KRAS	G13D	17,5	22,01
TP53	Q192R	31,8	21,58
PIK3CA	Q546R	22	7,46

Variants were previously detected in DNASeq with NGS using SOPHiA Solid Tumor Solution (STS). Variant allele frequency (VAF) obtained with NGS is compared with VAF obtained with VariantPlex kit.

Methods comparison

		Conventional methods	Conventional methods concordance	Sensibility	Specificity
VariantPlex	MSI Status	PCR, NGS, IHC	100%	100%	100%
	Variants	NGS	100%	100%	100%
FusionPlex	Fusions	RNAseq	91,7%	91,7%	92,3%

Fusions detection with FusionPlex

Table 2: FusionPlex analyses

Conventional method	Expected fusions	Observed fusions	Number of cases
RNAseq	BRAF exon 11 - KIAA1549 exon 16	BRAF exon 11 - KIAA1549 exon 16	1
	BRAF exon 9 - KIAA1549 exon 15	BRAF exon 9 - KIAA1549 exon 15	1
	EML4 exon 2 - ALK exon 20	EML4 exon 2 - ALK exon 20	1
	BRAF exon 11 - KIAA1549 exon13	BRAF exon 11 - KIAA1549 exon13	1
	BRAF exon 9 - KIAA1549 exon 16	BRAF exon 9 - KIAA1549 exon 16	2
IHC	ALK	EML4 exon 13 - ALK exon 20	3
	ROS1	CD74 exon 6 - ROS1 exon 34	1
	ROS1	-	1
	ALK	EML4 exon 6 - ALK exon 20	1

Fusions were previously detected in RNASeq with NGS Archer FusionPlex Lung and in IHC

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.

Disclosure: All kits and reagents were provided free of charge by Invitae