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Combined cell-free DNA and cell-free RNA NGS testing for cancer molecular profiling

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INTRODUCTION

NGS-based liquid biopsy has its unique advantage in cancer patient care and monitoring as it allows repeated testing of a spectrum of target sites across multiple time points. In order to monitor dynamic fusion and alternative splicing variants, Predicine has developed PredicineRNA™, a proprietary NGS assay coupling cell-free RNA (cfRNA) with cell-free DNA (cfDNA) to get comprehensive snapshots of genomic landscape in cancer patients. Case studies are reported in this study.

METHODS

A single tube of blood is taken from cancer patient, which is used to extract both cfDNA and cfRNA. Predicine's proprietary cfDNA and cfRNA NGS assays and analysis pipelines are used to detect single nucleotide variations (SNVs), insertions and deletions (indels), copy number variations (CNVs) as well as fusions and alternative splicing events from cancer samples that were collected at multiple time points from patients during or after treatment.

Figure 1. PredicineRNA™ NGS assay workflow

Table 1. Assay performance of PredicineRNA™ NGS assay

Variant type	Report range	Variant read copies	Sensitivity (%)	PPV (%)
Fusion and splice variants	≥2 copies	≤10 copies	92.5	100
		10-30 copies	97.6	100
		≥30 copies	100	100

Table 2. Representative cfRNA events detected by PredicineRNA™ NGS assay

Validated fusions in cfRNA (selected)	PAX8—PPARG1, EML4—ALK, CD74—ROS1, FGFR3—TACC3, NCOA4—RET, EGFR—SEPT14, SLC45A3—BRAF, ETV6—NTRK3, FGFR3—BAIAP2L1, LMNA—NTRK1, TPM3—NTRK1, KIF4B—RET, TMPRSS2—ERG
Validated splicing variant in cfRNA (selected)	MET14 skipping, EGFR Variant III, AR-V3, AR-V7, AR-V9

RESULTS

Case 1. Confirming KIF5B-RET (K15;R12) DNA fusion event in pleural effusion supernatant using PredicineRNA™ cfRNA NGS assay.

A lung cancer patient had only pleural effusion available for testing. A testing of the cfDNA in the supernatant of the pleural effusion identified an actionable KIF5B-RET fusion (K15;R12, which meant the breakpoint was in intron 15 of the upstream gene KIF5B and intron 11 of the downstream gene RET). However, the molecular fraction of the KIF5B-RET fusion was only 0.75% from cfDNA. Although the molecular fraction was above the LOD of our cfDNA assay, considering the sample type, we further carried out the PredicineRNA™ assay to confirm the fusion event using the pleural effusion supernatant. In the cfRNA assay, 81 supporting reads (including 34 Junction Reads and 47 Span Reads) were identified for the fusion event (fusion between exon 15 of KIF5B and exon 12 or RET), which was 16.9% in molecular fraction at the RET breakpoint (**Figure 2**). This result offered a confirmation from the cfRNA level for the fusion event detect in cfDNA from pleural effusion supernatant.

Case 2. Monitoring EML4-ALK(E13;A20) fusion events in cfDNA and cfRNA at multiple timepoints and in different sample types for a NSCLC patient.

Plasma samples from multiple time points of a NSCLC patient were tested for both cfDNA and cfRNA. In addition, cfDNA from pericardial effusion taken at one time point was also tested. The patient had been treated with ALK inhibitors (Alectinib and Lorlatinib) and progressed (the patient also has a TP53 truncating mutation). The samples were taken at time points after the progression. The testings carried out at each time point are listed in **Table 3**. As the patient has been treated with ALK inhibitors and progressed before time point 1, we observed that at the DNA level, the molecular fractions of the EML4-ALK fusion were close between time point 1 and 2 in plasma, as well as in pericardial effusion. However, the fusion fraction in cfRNA differed greatly between time points, with the fusion not detected at time point 1 and 21.5% at time point 2 (two months later). These results reflected a relapse in EML4-ALK RNA expression after ALK targeted treatment was stopped for a period of time, even though the DNA fusion fraction remained unexpanded.

Case 3. Using cfRNA to monitor the incidence of splicing events of AR-V3, AR-V7, AR-V9 and fusion events of TMPRSS-ERG in plasma of mCRPC patients

Splicing events of AR-V3, AR-V7, AR-V9, and fusion events of TMPRSS2-ERG are known characteristics of prostate cancer. **Table 4** shows the incidence rates of these events in mCRPC plasma samples by PredicineRNA™ assay. The presence of AR splicing variants and TMPRSS2-ERG fusion events have been reported to influence treatment effects. The capability of detecting splicing and fusion events from plasma cfRNA, complementary to cfDNA, may greatly facilitate personalized cancer care in a large fraction of mCRPC patients.

Figure 2. Reads covering the fusion junction between KIF5B exon 15 and RET exon 12 from the cfRNA assay

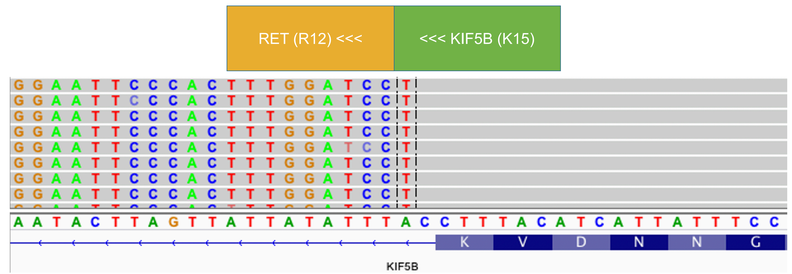

Table 3. Molecular fractions of EML4-ALK fusion at different time points for Case 2

Time point	Sample/Test	EML4-ALK fusion molecular fraction (%)
1	Plasma/cfDNA	1.33
1	Plasma/cfRNA	Not detected
2 (two months later)	Plasma/cfDNA	1.11
2 (two months later)	Pericardial effusion/cfDNA	1.89
2 (two months later)	Plasma/cfRNA	21.5 (26 supporting unique reads)

Table 4. Incidence of AR-V3, AR-V7, AR-V9 and TMPRSS2-ERG in mCRPC patients

Event	Incidence
AR-V3	14%
AR-V7	14%
AR-V9	6%
Any AR-V*	26%
TMPRSS2-ERG (T-E)	37%
Any AR-V* and T-E	51%

CONCLUSIONS

The cfDNA+cfRNA combined approach allows cross-validation of cancer variants using both methods, which greatly elevates the confidence level of certain clinical testing results in a variety types of samples, such as pleural effusion and pericardial effusion in clinical testing. Further studies are warranted to investigate its clinical applications.