Simultaneous Multi-parametric Profiling of Cell-free RNA (cfRNA) and Cell-free Tumor DNA (ctDNA) in Advanced-stage Colorectal Cancer

Abstract No. A22

Zheng Feng [1], Danyi Wang [1], Dennis Merkle[2], Jianjun Yu[3], Shidong Jia[3], Juergen Scheuenpflug[1#]

[1]Global Clinical Biomarkers and Companion Diagnostics, Translational Medicine, Global Development, EMD Serono Research and Development Institute, Inc. a business of Merck KGaA, Darmstadt, Germany
2Clinical Biomarkers & Companion Diagnostics, Merck KGaA, Darmstadt, Germany 3Predicine, Inc, Hayward, CA, USA

Corresponding author

INTRODUCTION

It has been recognized that intra-tumoral heterogeneity together with therapeutic intervention associated multiple genomic alterations added complexity for precision therapy in late stage colorectal cancer (CRC). The clinical utility/validity of circulating tumor-derived DNA (ctDNA) assays have been systematically analyzed and proved under clinical settings in select cancer types with demonstrated specificity and sensitivity. Notably, circulating cell-free RNA (cfRNA) sequencing confirmed and enhanced detection sensitivity for variants with low allele frequency.

In this study, plasma-based ctDNA mutation and cfRNA gene expression in patients with advanced CRC were simultaneously tested using NGS liquid biopsy panels.

METHODS

Sample collection: Plasma samples were collection from 25 treatment-naive patients with advanced CRC (stage III/IV), circulating cell-free DNA (cfDNA) and cfRNA are extracted from the same tube of plasma.15~30ng purified cfDNA and >5ng cfRNA were recommended for cfDNA and cfRNA assay, respectively.

NGS assay: UMI(unique molecular identifier)-tagged libraries are enriched by 600-gene PredicineATLAS™ ctDNA panel and 234-gene PredicinePLUS™ cfRNA panel using hybrid capture method and deep sequenced by Illumina pair-end sequencing.

Bioinformatics pipeline: Raw sequencing data (BCL files) are fed through DeepSEA™, Predicine's proprietary NGS analysis pipeline. Variants with mutation allele frequency (MAF) >=0.25% and hotspot variants with MAF down to 0.1% are reported. Benign and likely benign variants (clinical significance defined based on in-house and public databases) are excluded. Tumor mutation burdens (TMB) was calculated based on 600 genes covered in the PredicineATLAS™ panel and germline variants were further removed according to sequencing data derived from both plasma and tumor tissue. cfRNA-based variants and gene expression were also reported using the DeepSea™ pipeline.

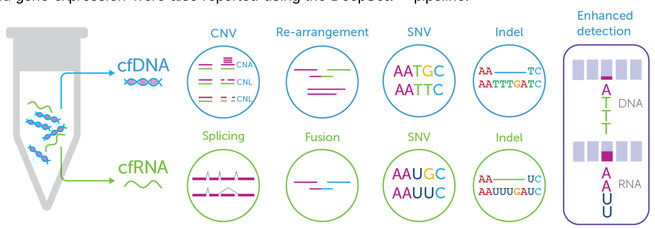

Figure 1. Predicine ctDNA + cfRNA combo liquid biopsy solution to detect genomic alterations occured at DNA or RNA transcripts.

Table 1. Specifications of PredicineATLAS™ ctDNA and PredicinePLUS™ cfRNA panel

Parameter	PredicineATLAS™ ctDNA Panel	PredicinePLUS™ cfRNA Panel
Regions Analyzed	600 genes	234 genes
Panel Size	2.4Mb	163Kb
Assay Sensitivity	0.25%, report down to 0.1%	≥3 unique reads
Sequencing Depth	>20,000X	>20,000X
Turnaround Time	2-4 weeks	2-4 weeks
Input Sample Type	Plasma 2-5ml Whole-blood (5-10ml)	Plasma 2-5ml Whole-blood (5-10ml)

RESULTS

PredicineALTAS™ ctDNA-based molecular profiling

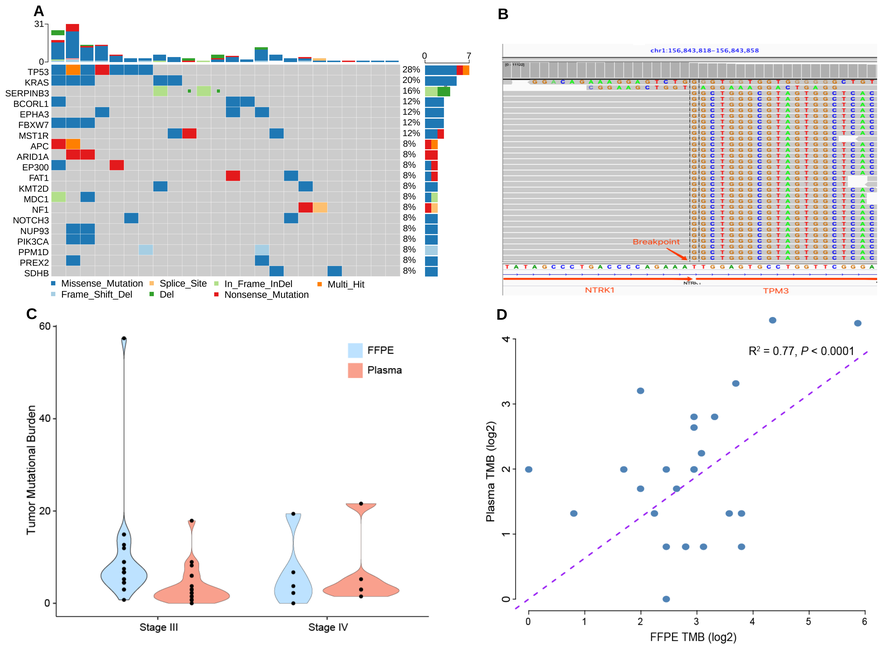

Figure 2. Genomic alterations and TMB detections using PredicineATLAS™ ctDNA assay.
A: Heatmap of genomic alterations in the top 20 genes. **B.** TPM3-NTRK1 fusion detection was confidently identified in a patient sample, which was confirmed by the matched tissue sample. **C.** Distribution of TMB scores in different tumor stages. **D.** TMB agreement between tissue and plasma samples. Samples with ≥ 95% probes having >1500x coverage pass NGS QC and all samples passed NGS QC.

PredicineATLAS™ ctDNA™ assay detects total of 169 genomic alterations including SNV, Indel and CNV in 25 advanced stage CRC samples.

Table 2. Performance metrics of PredicineATLAS™ ctDNA panel

Alterations	Report Range	Sensitivity	PPV*
SNV	>0.25% AF	94.4%	99.7%
SNV	0.1%-0.25% AF	78.6%	94.3%
CNV	2.3 copies	95.0%	100%

Based on cfDNA input of ≥15ng in cell lines/clinical samples.
*PPV: Positive Predict Value; AF: Allele frequency; SNV: single nucleotide variant; CNV: copy number variation

PredicinePLUS™ cfRNA-based molecular profiling

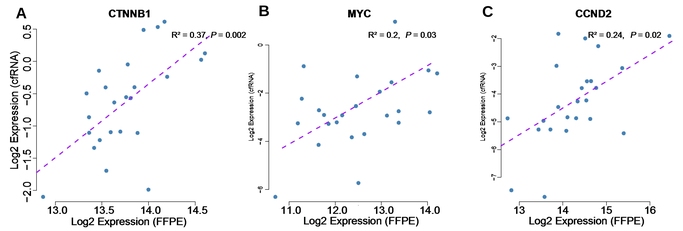

Figure 3. Agreement of gene expression for CTNNB1 (A), MYC (B) and CCND2 (C) genes between tissue and plasma.

Table 3. RNA-based NGS confirms the variants detected at DNA level

Sample ID	Diagnosis	Stage	Gene	HGVSp	AF (cfDNA, %)	AF (cfRNA, %)
P00022	Adenocarcinoma	IIIB	CHEK2	p.Leu348Val	1.43	1.136
P00043	Rectal Cancer	IIIC	SMAD4	p.Asp142His	0.295	0.212
P00050	Colon Cancer	IIIB	MYD88	p.Leu273Pro	0.274	0.989
P00050	Colon Cancer	IIIB	JAK2	p.Val617Phe	0.491	1.002
P00053	Rectal Cancer	IIIC	PTPN11	p.Thr543Ile	0.33	0.172
P00059	Colon Cancer	IVA	RB1	p.Glu440Lys	0.43	0.182
P00059	Colon Cancer	IVA	KRAS	p.Ala146Thr	42.954	0.188
P00059	Colon Cancer	IVA	FBXW7	p.Tyr427Cys	24.7	4.724
P00059	Colon Cancer	IVA	TP53	p.Arg248Trp	0.325	6.78
P00060	Colon Cancer	IVA	DNMT3A	p.Cys562Tyr	0.994	3.887
P00061	Colon Cancer	IVA	KRAS	p.Gly12Asp	27.936	0.026
P00061	Colon Cancer	IVA	PIK3CA	p.Cys420Arg	29.925	0.164
P00061	Colon Cancer	IVA	TP53	p.Arg273Cys	52.365	2.639
P00062	Colon Cancer	IVA	KRAS	p.Gly12Asp	3.453	0.088

Minimal 100x unique coverage is required for more than 60% regions to pass NGS QC. Among the 25 samples, 24 samples passed NGS QC.

CONCLUSIONS

- Our results demonstrated the potential clinical utility/feasibility of simultaneous multi-parametric profiling of ctDNA and cfRNA for comprehensive molecular insight/characterization in advanced stage cancer. cfRNA-derived variant detection confirms the variants originally detected in ctDNA test and further validates the high specificity results for report interpretation.
- The ctDNA and cfRNA-based combined liquid biopsy solution provides holistic molecular overview for detecting driver mutations, monitoring efficacy / disease burden, and identifying sub-clonal alterations that may related to drug resistance and tumor heterogeneity.

REFERENCES

1. Merker JD, et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol. 2018;36(16):1631-1641
2. Davis AA, et al. Association of a novel circulating tumor DNA next-generating sequencing platform with circulating tumor cells (CTCs) and CTC clusters in metastatic breast cancer. Breast Cancer Res. 2019 Dec 4;21(1):137.

ACKNOWLEDGEMENTS AND DISCLOSURES

This study was jointly sponsored by EMD Serono R&D, Inc, a business of Merck KGaA, Darmstadt, Germany, and Predicine Inc in California, US.