Assessment of UltraSEEK Colon Cancer Panel for Detection of Low Frequency Somatic Mutations in Blood

•[1]Rajeswari Avula, [1]W. Edward Highsmith, Jr, [1,2]Minetta C. Liu, [1]Jesse S. Voss, [3]Darryl Irwin, [3]Ryan T. Birse, [1]Benjamin R. Kipp.

•[1]Department of Lab Medicine and Pathology, [2]Department of Oncology, Mayo Clinic, Rochester, MN. [3]Agena Bioscience, San Diego, CA

Introduction

Current guidelines suggest that all patients with metastatic colorectal cancer (mCRC) have their tumors tested for *KRAS, NRAS and BRAF* mutations to predict for response to EGFR inhibitors. Testing for these alterations has traditionally been performed on tissue biopsies which are invasive and sometimes not feasible. Blood-based analyses ("liquid biopsy") may overcome these limitations by evaluating circulating cell-free DNA (cfDNA) from peripheral blood. This study evaluates the performance characteristics of the Agena Bioscience UltraSEEK™ chemistry on the MassARRAY® System to detect low frequency mutations in cfDNA.

Method

The UltraSEEK™ Colon Panel assesses 107 mutations in 5 genes (*KRAS, NRAS, BRAF, PIK3CA* and *EGFR).* The method uses as little as 10ng of total DNA and involves a multiplex PCR followed by a mutation-specific single base extension reaction. The extension reaction utilizes multiple, mutation-specific chain terminators labeled with a moiety for solid phase capture. After capture, the extension products (analyte) are desalted, transferred to an Agena Bioscience (San Diego, CA) SpectroCHIP® Array and loaded into the MassARRAY® System. Data acquired by the MassARRAY® System is processed by the MassARRAY® Typer software and reports are generated.

UltraSEEK Colon Panel

Gene	# of Mutations	Coverage
BRAF	5	Codon 469 of Exon 11; Codon 594, 600 of Exon 15
EGFR	10	Extracellular domain mutations across Exon 12
KRAS	48	Codons 12, 13 of Exon 2; Codons 59, 61 of Exon 3; Codons 117, 146 of Exon 4
NRAS	40	Codons 12, 13 of Exon 2; Codons 59, 61 of Exon 3; Codons 117, 146 of Exon 4
PIK3CA	4	Codons 542, 545 of Exon 9; Codon 1047 of Exon 20

Table 1: Genes and mutations covered in the Agena Colon Panel.

Mutation detection on MassARRAY

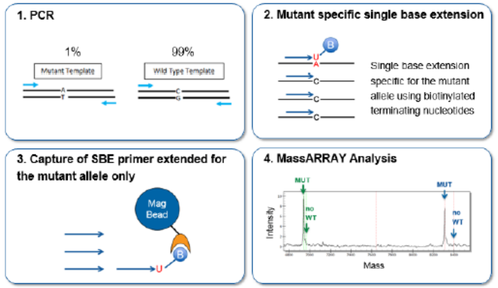

Figure 1: UltraSEEK mutation detection process on the MassARRAY System
Figure Courtesy of Agena Biosciences

Mass ARRAY Spectra

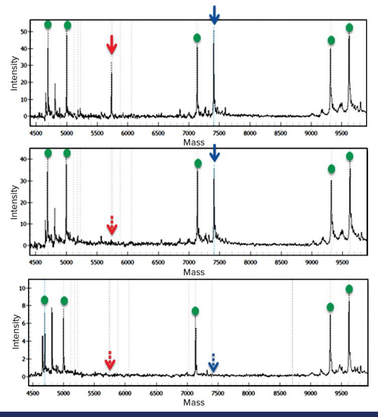

Figure 2: Colon panel spectra. Green - capture controls, blue - process control, red - mutation specific assay. Top panel - 0.5% mutant sample, middle - wild type DNA, bottom - NTC.

Figure Courtesy of Agena Biosciences.

Results

•We analyzed 55 samples including DNA controls from Horizon Discovery, Saint Louis, MO (HD), FFPE tumor DNA, cfDNA samples, and cell line DNA.

•Twenty Horizon Discovery DNA samples evaluated gave accurate mutation results. Mutations detected in 10 FFPE tumor DNA samples matched results from previously run NGS (Illumina, San Diego, CA) data.

•A total of 18 blood samples were assessed (Table 2); cfDNA from 9 out of 10 mCRC patients gave concordant results with matched tumor tissue DNA. 8 cfDNA samples from normal controls showed no mutations.

•The cfDNA results from the 10 CRC patients were confirmed by Digital Droplet PCR (both RainDance, Boston, MA and BioRad, Hercules, CA).

•A subset (n = 3) of the positive cfDNA samples were run in triplicate with concordant results. DNA samples from 3 HD controls were also run in triplicate with concordant results.

•Horizon Multiplex cfDNA reference standard DNA containing *KRAS* G12D, *NRAS* Q61K, *NRAS* A59T, *PIK3CA* E545K and *BRAF* V600E mutations were detected at 0.5 to 1% allelic frequency. In addition, in normal plasma spiked with mix of mutant cell line DNA mutations were detected at 5-10 copies.

1% Multiplex 1 cfDNA Reference Standard

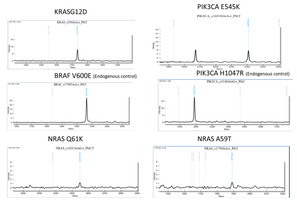

Figure 3: Multiplex 1 cfDNA reference standard (Horizon) had KRAS G12D, PIK3CA E545K, NRAS A59T and NRAS Q61K mutations at 1%. The standard also had BRAF V600E and PIK3CA H1047R, as an endogenous control.

Blood cfDNA Results

Sample ID	DNA Source	cfDNA Results	KRAS Mutation Tumor tissue Results
T1	cfDNA_CRC	KRAS G12R	kRAS Exon 2 Mutant
T2	cfDNA_CRC	Wild-type	KRAS Exon 2 Wild-type
T3	cfDNA_CRC	KRAS G13D	KRAS Exon 2 Mutant
T4	cfDNA_CRC	WT	KRAS G12V
T5	cfDNA_CRC	KRAS G12S	KRAS G12S
T6	cfDNA_CRC	Wild-type	KRAS Exon 2 Wild-type
T7	cfDNA_CRC	KRAS G12D	kRAS Exon 2 Mutant
T8	cfDNA_CRC	Wild-type	KRAS Exon 2 Wild-type
T9	cfDNA_CRC	Wild-type	KRAS Exon 2 Wild-type
T10	cfDNA_CRC	Wild-type	KRAS Exon 2 Wild-type
23N	cfDNA Normal	Wild-type	
1N	cfDNA Normal	Wild-type	
2N	cfDNA Normal	Wild-type	
11N	cfDNA Normal	Wild-type	
15N	cfDNA Normal	Wild-type	
16N	cfDNA Normal	Wild-type	
17N	cfDNA Normal	Wild-type	
18N	cfDNA Normal	Wild-type	

TABLE 2: Comparison of protein changes in cfDNA and primary tumor tissue DNA. cfDNA results from normal donors. The results for samples T1 – T10 were confirmed by ddPCR and NGS (See Results)

UltraSEEK Report

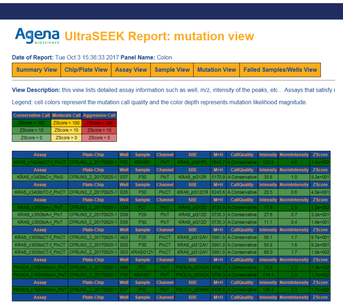

Figure 4: UltraSEEK report showing list of mutations detected in the samples

Conclusions

- **The UltraSEEK™ Colon Cancer Panel on the MassARRAY® System has excellent accuracy and is able to detect mutations in *KRAS, BRAF, NRAS, PIK3CA and EGFR* at low mutation frequencies.**
- **The turnaround time of 2 days is an advantage over NGS based methods.**
- **Validation with additional blood samples collected from patients with mCRC and known tissue mutation status is underway.**

References

- Kuo YB, Chen JS, Fan CW, Li YS, Chan EC. Comparison of KRAS mutation analysis of primary tumors and matched circulating cell-free DNA in plasmas of patients with colorectal cancer. Clin Chim Acta.;Vol 433:284-9. (June 2014)
- Graham Brock et al: Liquid biopsy for cancer screening, patient stratification and monitoring, Translational Cancer research, Vol. 4, No3. (June 2015)