ASSAYS BY AGENA OncoFOCUS™ PANEL v3

EVALUATING EXPANDED *RAS* MUTATIONS IN COLORECTAL ADENOCARCINOMA USING A TARGETED, HIGH THROUGHPUT, COST-EFFECTIVE SOLUTION

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INTRODUCTION

It has been known for several years that patients with metastatic colorectal adenocarcinoma (mCRC) who harbor an activating mutation in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) exon 2 will not benefit from monoclonal antibodies (mAbs) targeting the epidermal growth factor receptor (*EGFR*). Recent research evaluating anti-*EGFR* monoclonal antibodies has indicated that up to 20% of the *KRAS* exon 2 wild type tumors may contain other *RAS* mutations that are similarly predictive of drug resistance.[1,2] Currently, common *KRAS* testing methodologies only assay codons 12/13 in exon 2 and sometimes codon 61 in exon 3. This application note describes the development of the Assays by Agena (AbA) OncoFOCUS Panel v3 for the retrospective testing of archived CRC samples. The panel, for use on the MassARRAY® System, was developed to include expanded coverage of *KRAS* and *NRAS* mutations.

ASSAY DESIGN

Agena Bioscience's OncoFOCUS Panel v1 is a set of pre-validated assays covering 200+ somatic mutations in four key oncogenes observed in lung, colorectal, and metastatic melanoma tumors. The panel was designed to detect significant driver mutations in *EGFR, KRAS, NRAS*, and *BRAF* with extensive coverage of *EGFR* exon 19 and 20 insertions and deletions. The AbA OncoFOCUS+KIT Panel v2 was developed to include mutations in the c-KIT oncogene for additional coverage of melanomas. Table 1 summarizes the mutations covered by these two panels.

The flexibility of the MassARRAY System allows for easy customization of an existing panel. For this study, additional mutations in *KRAS* and *NRAS* exons 2 and 3, together with mutations in exon 4, were added to the existing multiplexed wells of the AbA OncoFOCUS+KIT Panel v2. Table 2 details the additional *RAS* mutations detected by the AbA OncoFOCUS Panel v3.

Table 1

OncoFOCUS™ Panel v1 and AbA OncoFOCUS+KIT Panel v2 Contents		
Genes	**Coverage**	**# of Mutations**
BRAF	Codon 469 of exon 11; codons 594, 597 and 600 of exon 15. **The rare V600E (c.1799_1800TG>AA), substitutions and deletions in codon 601 of exon 15.**	20 **(5)**
EGFR	Substitutions, insertions and deletions across exons 18, 19, 20 and 21. Includes key mutations such as L858R, L861Q and T790M.	125
KRAS	Codons 12, 13 of exon 2; codon 61 of exon 3; codon 146 of exon 4.	37
NRAS	Codons 12, 13 of exon 2 and codon 61 of exon 3.	20
KIT	**Substitution mutations in exons 11, 13 and 17.**	**6**
The additional contents of the AbA OncoFOCUS+KIT Panel v2 are indicated in **green**.		

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Table 2

RAS Mutation Coverage in OncoFOCUS™ Panels				
	KRAS Mutations Detected		*NRAS* Mutations Detected	
	Amino Acid Change	Nucleotide Change	Amino Acid Change	Nucleotide Change
EXON 2, CODON 12	p.G12S	c.34G>A	p.G12S/N	c.34G>A/c.34-35GG>AA
	p.G12R	c.34-36GGT>AGA	p.G12R/P	c.34G>C/c.34-35GG>CC
	p.G12T	c.34-35GG>AC	p.G12C/Y	c.34G>T/c.34-35GG>TA
	p.G12V	c.35G>T	p.G12D/E	c.35G>A/c.35-36GT>AG
	p.G12F	c.34-35GG>TT	p.G12A	c.35G>C
	p.G12P	c.34-35GG>CC	p.G12V	c.35G>T
	p.G12R	c.34G>C		
	p.G12A	c.35G>C		
	p.G12C	c.34G>T		
	p.G12C	c.34-36GGT>TGC		
	p.G12W	c.34-36GGT>TGG		
	p.G12D	c.35G>A		
	p.G12N	c.34-35GG>AA		
	p.G12I	c.34-35GG>AT		
	p.G12L	c.34-35GG>CT		
	p.G12Y	c.34-35GG>TA		
	p.G12E	c.35-36GT>AA		
	p.G12E	c.35-36GT>AG		
	p.G12D/V	c.35-36GT>AC/ c.35-36GT>TC		
EXON 2, CODON 13	p.G13C	c.37G>T	p.G13S/N	c.37G>A/c.37-38GG>AA
	p.G13S	c.37G>A	p.G13R/P	c.37G>C
	p.G13A	c.38G>C	p.G13C/Y	c.37G>T/c.37-38GG>TA
	p.G13V/I	c.38G>T/c.37-38GG>AT	p.G13D/E	c.38G>A
	p.G13D/N	c.38G>A/c.37-38GG>AA	p.G13A	c.38G>C
	p.G13R	c.37G>C	p.G13V	c.38G>T/c.38-39GT>TC
EXON 3, CODON 59	p.A59T	c.175G>A	**p.A59T**	**c.175G>A**
	p.A59G	**c.176C>G**	**p.A59G**	**c.176C>G**
	p.A59E	**c.176C>A**		

Table 2 (continued)

	KRAS Mutations Detected		*NRAS* Mutations Detected	
	Amino Acid Change	Nucleotide Change	Amino Acid Change	Nucleotide Change
EXON 3, CODON 61	p.Q61K	c.181C>A	p.Q61H	c.183A>C
	p.Q61E	c.181C>G	p.Q61H	c.183A>T
	p.Q61L	c.182A>T	p.Q61L	c.182A>T
	p.Q61R	c.182A>G	p.Q61L	c.182-183AA>TG
	p.Q61P	c.182A>C	p.Q61R	c.182A>G
	p.Q61H	c.183A>C	p.Q61R	c.182-183AA>GG
	p.Q61H	c.183A>T	p.Q61P	c.182A>C
			p.Q61K	c.181C>A
			p.Q61E	c.181C>G
			p.Q61Q/K	c.183A>G/c.181-183CAA>AAG
EXON 4, CODON 117	**p.K117E**	**c.349A>G**	**p.K117E**	**c.349A>G**
	p.K117N	**c.351A>T**	**p.K117N**	**c.351G>C**
	p.K117R	**c.350A>G**	**p.K117N**	**c.351G>T**
	p.K117R	**c.351A>C**	**p.K117R**	**c.350A>G**
EXON 4, COLON 146	p.A146T	c.436G>A	**p.A146T**	**c.436G>A**
	p.A146P	c.436G>C	**p.A146P**	**c.436G>C**
	p.A146G	c.437C>G	**p.A146S**	**c.436G>T**
	p.A146V	c.437C>T	**p.A146V**	**c.437C>T**
			p.A146G	**c.437C>G**

Additional *RAS* mutations added to the AbA OncoFOCUS Panel v3 are indicated in **Green**.
The rest of the mutations are covered in both the OncoFOCUS Panel and the AbA OncoFOCUS+KIT Panel v2.

MATERIALS AND METHODS

DNA from 266 archived formalin-fixed, paraffin-embedded (FFPE) tissue samples that were histologically confirmed CRC cases from a Midwestern US population were supplied by the South Bend Medical Foundation. DNA from these samples was previously tested for *RAS* mutations using either the AbA OncoFOCUS+KIT v2 Panel from Agena Bioscience on the MassARRAY System or the Asuragen® Signature® *KRAS* Mutations Kit on the Luminex® 100™ System. DNA was extracted using the QIAamp® DNA FFPE Tissue Kit and tested for integrity using the iPLEX® Pro Sample ID Panel from Agena Bioscience. Only samples that had a sufficient number of amplifiable copies of DNA were then used for testing with the expanded *RAS* panel, AbA OncoFOCUS Panel v3. The EntroGen *RAS* Mutation Analysis Kit was used on the Roche Diagnostics LightCycler® 480 Platform to confirm selected mutations that were identified exclusively by the AbA OncoFOCUS Panel v3.

The AbA OncoFOCUS Panel v3 contains two multiplexed PCR reactions, and requires only 20ng of input DNA per sample. The *KRAS* and *NRAS* assays are separated into two wells to avoid the amplification of pseudogenes. Amplification of the target region is followed by single base extension into the mutation site using the iPLEX Pro biochemistry. The extension products are desalted and dispensed onto a SpectroCHIP® Array and detected via MassARRAY MALDI-TOF mass spectrometry.

RESULTS

Of the 266 samples that were tested, 58% were wild type, 38% carried a *KRAS* mutation, and 4% had an *NRAS* mutation. Figure 1 shows the distribution of the 115 *RAS* mutations detected across the various exons. These mutations were detected in 110 samples, five of which contained two separate mutations. Three of the samples showed a mutation in *KRAS* exon 2 codon 12, plus a second *KRAS* mutation: A59T, G13D/N, or K117N. Two samples carried a mutation in *BRAF* (D594G, c.1781A>G), plus a second *RAS* mutation, one in *KRAS* (A146T) and one in *NRAS* (G12C/Y).

A subset of 80 samples had been previously tested with only the Signature *KRAS* kit, assessing mutations in codons 12 and 13 of *KRAS*. Additional *RAS* mutations were detected in 15% of samples by the AbA OncoFOCUS Panel v3 (Figure 2) with these additional mutations being present in *KRAS* exons 3 and 4, and *NRAS* exons 2 and 3 (Figure 3).

SUMMARY

The AbA OncoFOCUS Panel v3 was developed for the identification of extended *RAS* mutations that may be present in mCRC samples. From this study, it was found that 42% of the FFPE samples harbored extended *RAS* mutations. Mutations in *KRAS* exon 4, codon 146 and *NRAS* exon 2, codon 12 were most frequently represented in addition to the common mutations in *KRAS* exon 2, codons 12 and 13. Based on these results, testing for only *KRAS* exon 2 could miss up to 15% of *RAS* mutations that are now considered significant for metastatic CRC. New research has shown that the presence of these expanded *RAS* mutations indicates resistance to anti-*EGFR* monoclonal antibody therapy and can be used for improved treatment stratification. The AbA OncoFOCUS Panel v3 provides a rapid, cost-effective, and comprehensive method for the identification of *RAF, EGFR,* and an expanded set of *RAS* mutations.

Figure 1

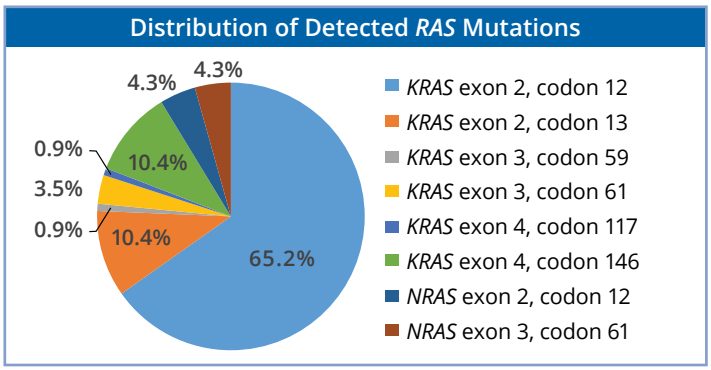


Figure 2

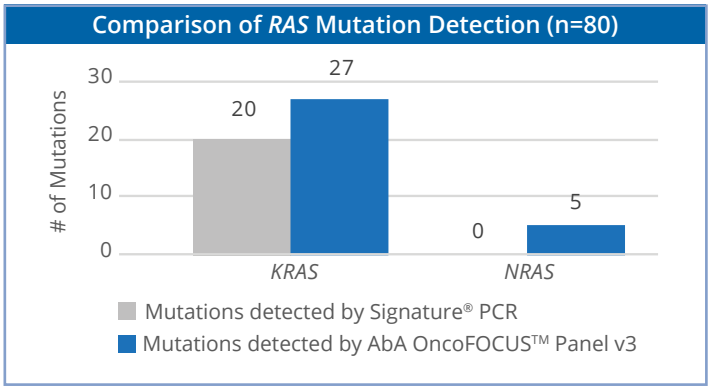
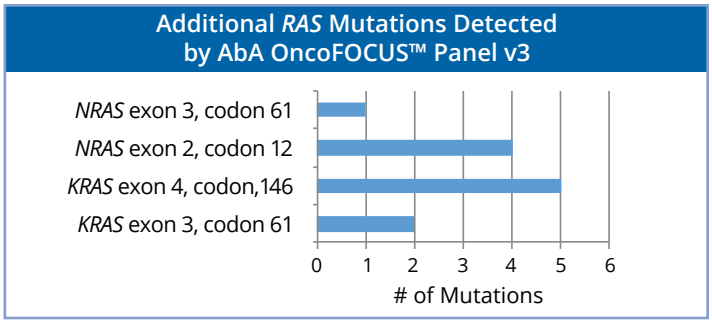


Figure 3



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2. Atreya CE, Corcoran, RB, Kopetz, S. J *Clin Oncol* 33. 2015. 1-4.

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