Analysis of plasma-based BRAF and NRAS mutation detection in patients with stage III and IV melanoma

Jyothirmayee S. Tadepalli[1] *MS*, Shria Hafner[2] *BS*, Gregory Chang[1] *MBA*, Nathaniel H. Fleming[1] *BA*, Yongzhao Shao[1] *PhD*, Farbod Darvishian[1] *MD*, Anna Pavlick[1] *DO*, Russell Berman[1] *MD*, Richard Shapiro[1] *MD*, Iman Osman[1] *MD*, Cindy Spittle[2] *PhD*, and David Polsky[1] *MD, PhD*

[1]New York University Langone Medical Center, New York, New York & [2]Molecular MD Corporation, Portland, Oregon

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Introduction

Patients with metastatic melanoma are eligible for BRAF inhibitor therapy if the *BRAF V600E* mutation can be identified in their tumor specimen. Patients lacking an available specimen for genotyping are unable to receive inhibitor therapy. We developed 2 mutation-specific genotyping platforms and tested their ability to detect *BRAF* and *NRAS* mutations in archived plasma and tumor samples to determine the potential utility of blood-based tumor genotyping in melanoma.

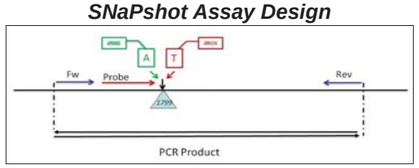
BRAF mutation table

	Nucleotide Position		
	1798	1799	1800
BRAF WT	G	T	G
BRAF V600E	G	A	G
BRAF V600K	A	A	G
BRAF V600R	A	G	G
BRAF V600D	G	A	T

NRAS mutation table

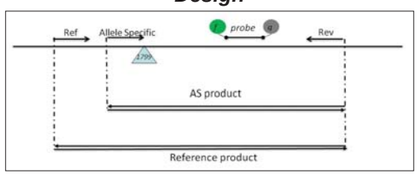
	Nucleotide Position		
	181	182	183
NRAS WT	C	A	A
NRAS Q61K	A	A	A
NRAS Q61L	C	T	A
NRAS Q61R	C	G	A

SNaPshot Assay Design



Note: BRAF V600E shown

TaqMan Allele Specific PCR Assay Design



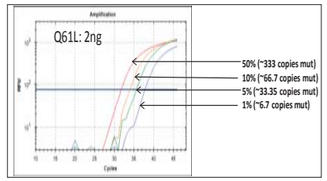
Methods

Allele-specific PCR (TaqMan™) and SNaPshot™ assays were designed and evaluated to determine their sensitivities to detect *BRAF V600* and *NRAS Q61* mutations in tissue and plasma samples. Platform comparisons were conducted to validate assay performance using cell line DNA diluted into normal lymphocyte DNA. Concordance between both mutation detection methods was evaluated using patient FFPE tumor tissue and plasma samples.

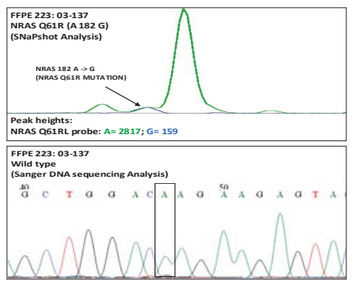
Cell Lines with Mutations and Limits of Detection

Cell line	Mutation	Limit of detection (Number of copies)	
		TaqMan Assay	SNaPshot Assay
SK-Mel 29	BRAF V600E	Not done	7.5
Colo 201	BRAF V600E	20	Not done
M6	BRAF V600K	20	6.7
CHP212	NRAS Q61K	6.7	6.7
C3A	NRAS Q61L	6.7	6.7
HT1197	NRAS Q61R	6.7	6.7

TaqMan Assay Amplification Plot- C3A cell line dilution series



SNaPShot Analysis detects NRAS Q61R mutation missed by Sanger Sequencing

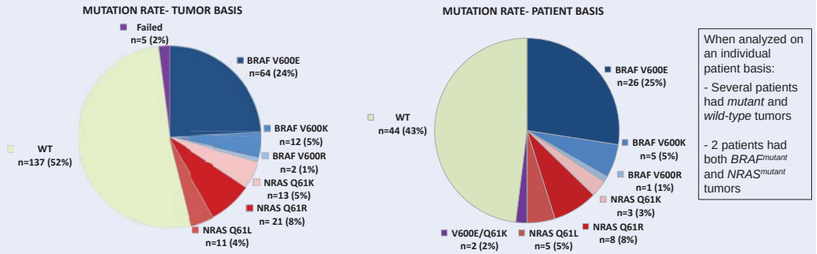


Results and Conclusions

Study Design

- Retrospective analysis
- 94 patients with Stage III/IV melanoma enrolled in the NYU Melanoma Biorepository
  - Median Age: 59; Gender: 56 Males, 38 Females
- 1 or more plasma samples (2ml) collected and frozen at time of enrollment or as available (n=98)
- 2 or more tumor specimens (n=265)
  - 65 patients with primary and metastatic tumor(s)
  - 29 patients with multiple metastatic tumors only
- Complete clinical information

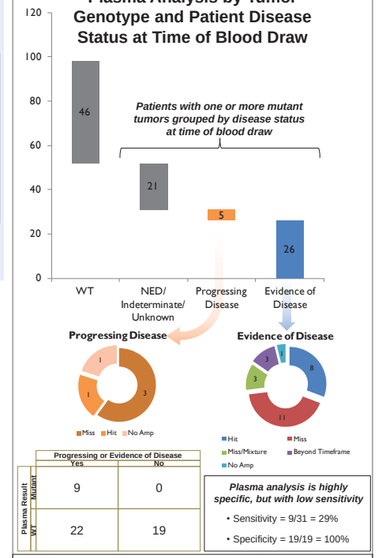
Overall mutation rates reveal tumor heterogeneity within individual patients



When analyzed on an individual patient basis:

- Several patients had *mutant* and *wild-type* tumors
- 2 patients had both $BRAF^{mutant}$ and $NRAS^{mutant}$ tumors

Plasma Analysis by Tumor Genotype and Patient Disease Status at Time of Blood Draw



Plasma Result	Progressing or Evidence of Disease: Yes	No
Mutant	9	0
WT	22	19

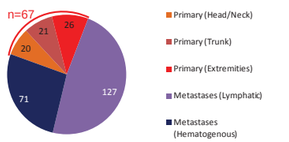
Plasma analysis is highly specific, but with low sensitivity

- Sensitivity = 9/31 = 29%
- Specificity = 19/19 = 100%

Of the 98 plasma samples analyzed, 46 were samples from patients who had *wild-type* tumors only. The remaining samples were drawn from patients with one or more *mutant* tumors: 21 samples drawn when the patient disease status was NED, indeterminate, or unknown; 5 samples drawn when the patient was NED but progressed within three months; and 26 had evidence of disease at the time of blood draw. Miss/Mixture cases are defined as WT plasmas within one year of an analyzed tumor in a patient that had heterogeneous tumors.

Tumor Samples Analyzed

Primary/Metastases Breakdown

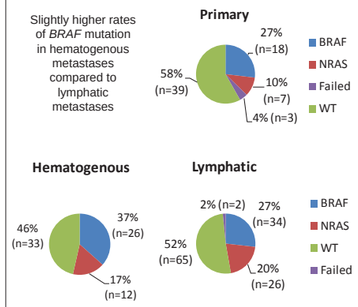


Greater sensitivity of SNaPshot compared to Sanger sequencing for mutations under study

SNaPshot \ Sanger	Mutant	Wild-type
Mutant	95	15 (-10 BRAF - 5 NRAS)
Wild-type	0	125

Mutation Rates in Primary and Metastatic Tumors

Slightly higher rates of *BRAF* mutation in hematogenous metastases compared to lymphatic metastases



15/94 patients with heterogeneous tumors

Patient ID	Primary	Met 1	Met 2	Met 3	Met 4
09-036	BRAF V600E	BRAF V600E	BRAF V600E	WT	BRAF V600E
06-002	BRAF V600E	BRAF V600E	WT	BRAF V600E	
06-001	BRAF V600E	WT	NRAS Q61K	NRAS Q61K	
06-040	WT	WT	BRAF V600E	BRAF V600E	
06-004	BRAF V600E	WT	WT		
09-203	WT	BRAF V600E	BRAF V600E		
08-051	WT	NRAS Q61R	NRAS Q61R		
03-092	WT	WT*	NRAS Q61R		
10-142	BRAF V600E	WT*			
06-075		BRAF V600E	NRAS Q61K	WT	
07-014		NRAS Q61R	NRAS Q61R	WT	
07-080		NRAS Q61L	WT	WT	
03-075		BRAF V600E	BRAF V600E		
08-090		WT	BRAF V600E		
04-100		WT	BRAF V600E		

* <= 5%

Case 06-075

Three mutational genotypes in single patient

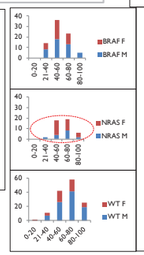
6/27/2006 Left Axilla *BRAF V600E*

6/21/2007 Left Flank *NRAS Q61K*

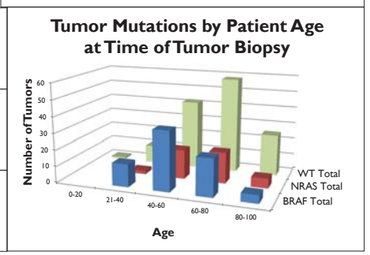
8/7/2007 Left Flank *WT*

Gender Bias in Tumor Mutations

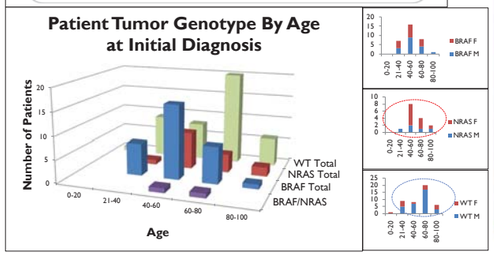
- Among patients aged 40-60 years, *NRAS* mutations are more common among women
- Among patients aged 60 – 80 years *wild-type* tumors are more common among men



Tumor Mutations by Patient Age at Time of Tumor Biopsy



Patient Tumor Genotype By Age at Initial Diagnosis



Conclusions

- Tumor-associated $BRAF^{V600}$ and $NRAS^{Q61}$ mutations can be detected with high specificity in plasma of patients with evidence of disease at the time of blood draw
- Increasing the amount of plasma analyzed may improve sensitivity and the detection of disease activity in patients without evidence of disease
- Tumor heterogeneity may contribute to "false negative" plasma results