Detecting and Intervening on Rare Pre-Existing Resistant Subclones to BRAF/MEK Inhibitors in Melanoma

Maria Bao-Loc-Trung[1], Michael Nakazawa, M.D., Ph.D.,[2] Lawrence Kwong, Ph.D.[2]

[1]Louisiana State University School of Medicine, New Orleans, LA

[2]Department of Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, TX

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Introduction

One of the major challenges to cancer treatment is **acquired resistance to targeted therapies.**[3] However, little is known about how drug resistance arises, including if they occur *de* novo or how to best counteract them in the clinic.

For melanoma, patients with BRAF activating mutations are commonly treated with the **BRAF plus MEK inhibitor (BRAFi+MEKi)**. Although the majority of these patients respond to therapy, **over 80% of patients have tumor progression within one year.**[3]

At progression, a vast majority of resistant mutations found are in the **MAPK or PI3K pathways** and are postulated to be the mechanism of resistance to these therapies. Furthermore, targeting resistant mechanisms after patients have relapsed have also **not been effective**, and thus, more research needs to be done in order to increase the long-term outcome of patients with pre-existing low-VAF resistance subclones.[2]

Goals/Objective

We integrated multiple blocker displacement amplification (mBDA), patient samples with clinical data, and mouse models in order to investigate the following questions:

1. What is the percentage of melanoma patients that have **actionable, low-VAF, pre-existing resistant subclones to BRAFi/MEKi**?
2. What is the difference in **progression free survival (PFS)** of patients with and without these mutations on BRAFi + MEKi therapy?

Methods

DNA Extraction:
We identified 147 patients with metastatic BRAF-mutated cutaneous melanoma from The University of Texas MD Anderson Cancer Center. 79 FFPE patient tumor samples from 29 patients DNA samples were extracted using a COVARIS truXTRAC Automated FFPE Kitkit according to manufactured protocol with the addition of UNG treatment at 50C for 1 hour after reverse crosslinking step.

A NanoDrop Spectrophotometer was used to measure the concentration of DNA. If above 200 ul/ng, a Zymo Quick-DNA Microprep kit was used to remove excess melanin from the sample to optimize readings.

mBDA:
mBDA technology enriches several different groups of genetic loci via different BDA primer/blocker sets, so that the mutations with originally low VAFs will have over 1% VAF and can then be profiled using next-generation sequencing.[1]

Sample	BRAF		ddPCR	NRAS		ddPCR	MAP2K1		MAP2K2		AKT3		AKT1	KRAS	PIK3CA
FFPE2	V600E	34.41%	30.90%	Q61K*	0.27%	0.03%	0%		0%		0%		0%	0%	0%
FFPE4	V600E	37.92%		0%			0%		0%		C17K*	3.03%	0%	0%	0%
FFPE6	V600E	23.13%		0%			P124L	0.33%	S127L	0.17%	0%		0%	0%	0%
FFPE11	A598_i	30.23%		0%			0%		0%		0%		0%	0%	0%
FFPE3	0%			Q61K	3.86%		R201H	0.28%	P128L	0.11%	0%		0%	0%	0%
FFPE7	0%			Q61R	7.87%		0%		0%		0%		0%	0%	0%
FFPE9	0%			Q61K	16.93%	31.00%	0%		0%		0%		0%	0%	0%
FFPE13	0%			Q61R	2.74%		0%		0%		0%		0%	0%	0%
FFPE1	0%			0%			R201H	0.18%	S127L	0.18%	0%		0%	0%	0%
	0%			0%			P124L	0.20%	0%		0%		0%	0%	0%
FFPE12	0%	0%		0%		0.00%	P124L	0.12%	0%		0%		0%	0%	0%
FFPE15	0%			0%			P124L	0.21%	S127L	0.21%	0%		0%	0%	0%
	0%			0%			R201H	0.14%	0%		0%		0%	0%	0%
FFPE8	0%			0%			0%		S127L	0.18%	0%		0%	0%	0%
FFPE5	0%			0%			0%		0%		0%		0%	0%	0%
FFPE10	0%	0%		0%		0%	0%		0%		0%		0%	0%	0%
FFPE14	0%	0%		0%		0%	0%		0%		0%		0%	0%	0%

Table 1. VAFs for 17 FFPE melanoma samples. *likely resistant subclone.

Clinical Data:

Patient data was obtained from EPIC, including progression-free survival, (PFS), best response (RECIST), and overall survival (OS) while the patients were on the BRAFi/MEKi therapy.

PFS and OS Kaplan-Meir curves were generated through Graph Pad Prism 9. Statistical significance was determined through Log-rank (Mantel-Cox) test using Prism software. Values were determined to be statistically significant if $P < 0.05$.

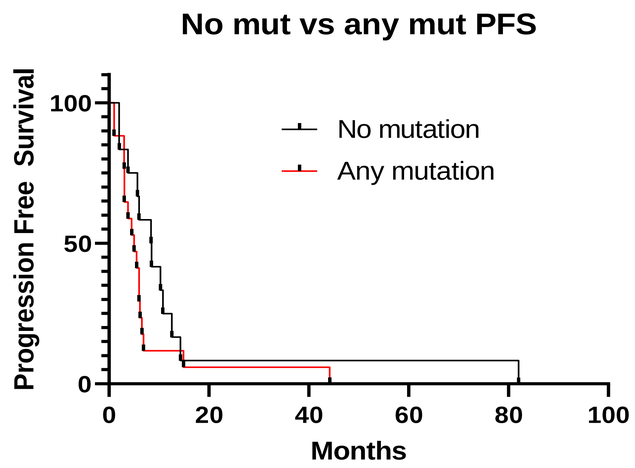
Results

Out of the 29 patients, 12 (41.4%) patients were determined to have no mutation, while 17 (58.6%) patients had detectable mutations. Most patients (22) utilized Dabrafenib and Trametinib for the BRAFi+MEKi therapy. There was 16 males and 13 females, and the average age of the patients was 45.8.

DRUG	# OF PATIENTS
DABRAFENIB + TRAMETINIB	22
VEMURAFENIB	6
ENCORAFENIB + BINIMETINIB	2
NIVOLUMAB + DABRAFENIB	1
TEMOZOLOMIDE (NO BRAFI/MEKI)	1

MRN	Age	Sex	Cancer Stage
683562	63	M	IVM1c
726482	55	M	IVM1d
740894	42	F	IVM1b
761609	21	F	IVM1b
779963	34	F	IVM1c
794947	49	F	IVM1a
797326	53	M	IVM1a
889445	30	M	IVM1c
902928	58	F	IVM1d
1072277	26	F	IVM1d
1082540	45	F	IVM1d
1084002	36	M	IVM1a
2193823	18	M	IVM1c
255644	32	M	IVM1d

MRN	Age	Sex	Cancer Stage
665405	23	M	IVM1c
712459	43	F	IVM1d
715122	61	M	IVM1d
911056	66	M	IVM1a
926828	32	M	IVM1c
1003885	53	F	IIIc
1018953	57	F	IVM1c
1024139	68	F	IVM1b
1025884	43	F	IVM1d
1029229	57	M	IVM1d
1043020	75	M	IVM1c
1072277	26	F	IVM1d
715122	61	M	IVM1d
724886	57	M	IVM1c
733774	43	M	IVM1b



The median PFS of patients without any mutation was 8.45, while with any mutation, the median survival was 5.00. The p-value for the data was .137, indicating insignificant data.

Conclusions

In this interim analysis, patients that did not have a detectable resistance mutation using qBDA in the MAPK/PI3K pathways had a trend towards **overall higher PFS** compared to the group with any mutation. This preliminary data supports the hypothesis that pre-existing mutations may infer resistance to BRAFi/MEKi therapy that leads to tumor progression.

The next steps are to obtain more patient samples to demonstrate how personalized counter-resistance therapies can increase the long-term outcome of patients harboring pre-existing low-VAF resistance subclones.

References

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