Introduction

One of the major challenges to cancer treatment is acquired resistance to targeted therapies. 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.

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.

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.

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 were 16 males and 13 females, and the average age of the patients was 45.8.

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