Droplet digital PCR monitoring of BRAF and NRAS plasma DNA as biomarkers of treatment response in stage IV melanoma

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Introduction

Currently, there is no sensitive and specific blood-based biomarker in use for the management of patients with metastatic melanoma. Our group has been developing methods to quantitatively detect circulating cell-free DNA (ctDNA) in the plasma of metastatic melanoma patients, with the aim of developing new biomarker assays. In this study, we evaluated the potential of a probe-based, droplet digital PCR (ddPCR) assay to quantitively measure copies of DNA encoding the mutant BRAFV600E and NRASQ61L mutations and their respective wild-type alleles in the plasma of patients undergoing treatment with targeted therapy or immune checkpoint blockers.

Methods

We studied patients with unresectable stage IVc, metastatic melanoma undergoing treatment with either a BRAF/MEK inhibitor or Ipilimumab who had at least 3 sequentially collected plasma samples available, including 1 drawn prior to treatment or biopsy and 2 or 3 following cycles of therapy. Patient samples were collected at the time of the first scans to evaluate the specificity of these assays, we analyzed 12 replicate wells for each plasma sample according to the manufacturer’s protocol. DNA was extracted from plasma samples (Qiagen QIAamp DSP Circulating NA Kit) and concentrations were measured using a Qubit fluorometer. Mutant ctDNA was detected in 5/5 melanoma patients, and 13/30 BRAF wild-type patients. All plasma samples amplified their respective wild-type and mutant BRAF or NRAS were tested for mutations.

Results and Conclusions

We found that ctDNA assays may become useful predictive markers of response to both MAPK-reinforced therapies and immune-based therapies.

Conclusions

- Droplet digital PCR assays are able to measure clinically meaningful changes in the plasma levels of BRAF and NRAS mutant DNA.
- ctDNA assays have a greater dynamic range than LDH levels and respond more quickly to changes in disease status.
- ctDNA assays may become useful predictive markers of response to both MAPK-reinforced therapies and immune-based therapies.