Quantitative assessment of circulating BRAF DNA in stage IV melanoma patients undergoing BRAF inhibitor treatment

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Introduction
Our group has been developing methods to quantitatively detect mutant BRAF DNA 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, digital PCR (dPCR) assay to quantitatively measure copies of DNA encoding the mutant BRAF V600E and wild-type alleles in the plasma of patients undergoing treatment with a BRAF inhibitor. We previously showed this assay to be 100% specific with a sensitivity to detect 1 mutant copy among 10,000 wild type copies when sufficient input DNA was used.

Methods
Ten patients with BRAF-mutant stage IV melanomas were prospectively studied. At least 3 serially-collected plasma samples were available for each patient, corresponding to pre-treatment, best treatment response, and progression of disease as evidenced by PET/CT scan. DNA was extracted from plasma samples (Qiagen QIAamp DSP Circulating NA Kit) and concentrations were measured using a Qubit fluorometer. The relevant BRAF region was assayed using 24 to a maximum of 337 ng of DNA from each sample, using multiple replicate wells for dilute samples to maximize the probability of detecting BRAF mutant copies. Specific TaqMan S5 probes for wild-type and mutant BRAF were employed along with common primers to measure the number of copies of each. The amount of amplifiable DNA was measured using RPP30 as a control gene.

Results
Histogram of amount of DNA analyzed for samples

Representative Raw Data (copies/u): Plasma sample 2 and NTC

Discussion
All 10 patients had partial responses. We analyzed 36 plasma samples, of which 30 had a minimum of 4ml of plasma. Qubit-based DNA concentrations ranged from 6.0 to 1158 ng/ml. All samples yielded positive amplification of the BRAF and RPP30 amplicons. Total BRAF DNA concentrations ranged from 719 to 156,920 copies/ml plasma. Mutant BRAF DNA concentrations ranged from 0 to 15,028 copies/ml. The fraction of BRAF V600E amplicons ranged from 0 to 45%, with a minimal detected mutant fraction of 0.01%. In 8/10 patients, levels of mutant BRAF fell with clinical response and/or with disease regression. In 3 of these 8 patients, samples drawn 10 days to 2 months prior to imaging studies showed increased levels of BRAF mutant DNA prior to evidence of disease progression. In 1 of the remaining 2 patients levels of total BRAF DNA tracked with clinical outcome better than levels of mutant BRAF DNA. In 1 patient neither levels of mutant or total BRAF DNA tracked with clinical responses.

Conclusions
These results demonstrate that a dPCR assay is able to quantitatively measure total and mutant BRAF DNA in the plasma of patients with stage IV melanoma. Levels of mutant and total BRAF DNA tracked with disease progression showing promise for this assay as a potential biomarker that may predict disease progression. Analyses of additional tumor-derived mutations in plasma DNA, and more precise assessments of disease response, are underway to determine optimal biomarkers to incorporate into melanoma clinical trial and patient care protocols.

References

Number.

Johannes W. Tonti, V; Shao, Y; Polito, A; Lau, D; Maragakis, L; Reaman, G; Fleming, N H; Shao, Y; Darvishian, F; Pavlick, A C; Polsky, D; Yancovitz, M; Polsky, D; Yoo, J; Yoo, J; Mikhail, M; Gai, W; Shapiro, R; Berman, R; Pavlick, A; Chapman, P; Osman, I. A single-center phase II trial of vemurafenib in patients with intermediate-stage or metastatic melanoma. J Clin Oncol 2013 31(28):3493–9.


Table: Quantitative BRAF DNA in Plasma

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Total BRAF</th>
<th>Mutant BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12000</td>
<td>1200</td>
</tr>
<tr>
<td>2</td>
<td>24000</td>
<td>2400</td>
</tr>
<tr>
<td>3</td>
<td>36000</td>
<td>3600</td>
</tr>
<tr>
<td>4</td>
<td>48000</td>
<td>4800</td>
</tr>
</tbody>
</table>

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