Detection and Monitoring of the \textit{BRAF} Mutation in Circulating Tumor Cells and Circulating Tumor DNA in \textit{BRAF}-Mutated Lung Adenocarcinoma

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Introduction
The B-Raf proto-oncogene, serine/threonine kinase gene (\textit{BRAF}) V600E mutation occurs in less than 2% of cases of non–small cell lung carcinoma (NSCLC); however, it has been associated with interesting response rates to B-Raf proto-oncogene, serine/threonine kinase (BRAF) (Minhibitors either alone or associated with mitogen-activated protein kinase kinase (MEK) inhibitors.\textsuperscript{1} Cell-free circulating tumor DNA (cfDNA) and circulating tumor cells (CTCs) have been described as noninvasive tools to detect and monitor epidermal growth factor gene (\textit{EGFR}) mutations in NSCLC\textsuperscript{2,3} during cancer treatments but never for a \textit{BRAF} mutation. Moreover, no study has yet compared CTCs and cfDNA for this purpose.

Case Reports
cfDNA and DNA extracted from CTCs obtained by isolation according to size of epithelial tumor cells from six patients treated for metastatic \textit{BRAF} V600E NSCLC were tested for the \textit{BRAF} V600E mutation using digital droplet polymerase chain reaction (PCR). This mutation was detected in the cfDNA of all six patients but in the CTCs of only one patient (Table 1).

In the first of the six cases, the initial sample was obtained at the time of resistance to the BRAF inhibitor. Despite the addition of a MEK inhibitor, the patient suffered disease progression. A dissociated plasma response was then observed, with a decrease in the \textit{BRAF} mutant and an increase in \textit{BRAF} wild type (WT) in cfDNA (Table 1 and Fig. 1). Targeted next-generation sequencing of the biopsy specimen identified, besides the known \textit{BRAF} mutation, a p.Arg132Cys-\textit{IDH1} mutation. For patients 2 through 4 (Fig. 2), for whom first blood samples were obtained after failed chemotherapy and before initiation of the BRAF inhibitor, we observed a good correlation between variations in plasma \textit{BRAF} mutants in cfDNA and a response to BRAF inhibitors (Response Evaluation Criteria in Solid Tumors 1.1 criteria).

Discussion
Rapid, noninvasive, and repeatable access to the molecular profile of NSCLC is challenging. We herein demonstrated the feasibility of detecting and monitoring \textit{BRAF} mutations in blood samples using digital droplet PCR on a small number of patients. Of particular interest, apart from in the intriguing first case, the kinetics of mutant cfDNA correlated well with changes in tumor burden. These results are in agreement with another report on \textit{BRAF}-mutated melanoma.\textsuperscript{4} \textit{BRAF} mutants in cfDNA were often detected in small amounts, but no positive droplets were detected in the WT samples, indicating good specificity (see Table 1).

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<table>
<thead>
<tr>
<th>Patient</th>
<th>Last Treatment Received (at Time of Blood Collection)</th>
<th>BRAF Mutated and Wild-Type DNA in Plasma and CTCs during Treatment with BRAF Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patient of Blood Collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTCs/2 Spots Mutant Copies/mL in CTCs Negative Control (WT Patients) Mutant Copies/mL in ctDNA WT Copies/mL in ctDNA Mutant/Total cfDNA RECIST Evaluation</td>
</tr>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>53 0 00 00 145.9 161.7 154.6 DP</td>
</tr>
<tr>
<td>2</td>
<td>Bevacizumab</td>
<td>0 0 274 1167 27120 50.4% DP</td>
</tr>
<tr>
<td>3</td>
<td>Pemetrexed</td>
<td>0 0 600 290100 590 1250 970 5.8 350 27120 2.3% DP</td>
</tr>
<tr>
<td>4</td>
<td>Pemetrexed</td>
<td>0 0 1.6 0 152.8 146 124 1.6 0 5.8 350 1250 0.2 0.4% DP</td>
</tr>
<tr>
<td>5</td>
<td>Cisplatin + pemetrexid</td>
<td>1 NA 0.04 0 141.1 149.8 146 1.4 5.2 350 27120 0.04% DP</td>
</tr>
<tr>
<td>6</td>
<td>Pemetrexed</td>
<td>0 NA 0.12 0 144.2 149.8 146 1.4 5.2 350 27120 0.04% DP</td>
</tr>
</tbody>
</table>

Note: Positive control: 10 ng DNA extracted from A375 cell line (BRAF V600E-mutated and BRAF WT lung adenocarcinomas); Negative control: cfDNA extracted from plasma of patients with Kirsten rat viral sarcoma oncogene (KRAS)-mutated and BRAF WT lung adenocarcinomas. BRAF, B-Raf proto-oncogene, serine/threonine kinase; CTCs, circulating tumor cells; WT, wild-type; ctDNA, circulating tumor DNA; cfDNA, circulating free DNA; RECIST, Response Evaluation Criteria in Solid Tumors; DP, disease progression; PR, partial response; NA, not applicable.
In patient 1, the decreased BRAF-mutated DNA indicated that the BRAF inhibitor was still active on the BRAF clone. However, the concomitant increase in BRAF WT in cfDNA and tumor progression suggests that this clone was no longer predominant. The isocitrate dehydrogenase (NADP(+)) 1 sytoic gene (IDH1) mutation confers in vivo growth of the BRAF-mutated melanoma cell line and was probably the mechanism of resistance in this case. No archival tissue was available to confirm that this alteration was not initially present.

Our observations suggest that plasma has better sensitivity compared with CTCs. However, CTCs have several advantages (prognostic value, fluorescence in situ hybridization, immunocytochemistry) but are probably not as effective at detecting and monitoring mutations.

In conclusion, analyses of BRAF mutants using digital droplet PCR on cfDNA is feasible and appears to be more sensitive than CTCs.

In patient 2, the increase in B-Raf proto-oncogene, serine/threonine kinase gene (BRAF) V600E-mutated circulating tumor-specific DNA in patient 2 during treatment with vemurafenib concomitant with dramatic disease progression, as seen on a computed tomography scan. The BRAF V600E probe plot is shown. The pink line is the threshold for positive versus negative droplets.
sensitive than analyzing CTCs. This test could be useful when following up $BRAF$-mutated lung adenocarcinoma.

References


