### ABSTRACT

Small fragments of cell-free DNA (cfDNA) that are shed by normal and tumor cells can be detected in the plasma of patients with advanced melanoma. Quantitative measurement of BRAF V600 mutant DNA within the cfDNA holds promise as a tumor specific biomarker for diagnosis and therapeutic monitoring in patients with BRAF V600 mutant melanoma.

### OBJECTIVES

To assess the value of BRAF V600 mutant DNA from plasma as a diagnostic test and as a monitoring tool in BRAF V600 mutant melanoma.

### METHODS

- Prospective blood sample collection in 10 ml EDTA tube
- Immediate centrifugation and separation of plasma
- Immediate storage of plasma at -80°C
- Extraction of ctDNA from 1 ml plasma (Idylla™, Biocartis)
- Allele-specific qPCR for BRAF V600E/E2/D/K/R/M (Biocartis, blinded for clinical information)

Analysis of BRAF mutant ctDNA from plasma allows for a rapid diagnosis of the BRAF status in pts with advanced melanoma. BRAF mutant ctDNA likely reflects the BRAF mutant proliferative tumor burden and holds promise as a therapeutic monitoring tool for pts with advanced BRAF V600 mutant melanoma.

### RESULTS diagnostic test

<table>
<thead>
<tr>
<th>n=13</th>
<th>Tissue test</th>
<th>Tissue test</th>
<th>Tissue test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF V600 mut</td>
<td>36 patients, 229 plasma samples</td>
<td>13 patients with stage III/IV melanoma</td>
<td>Sample before initiation of first-line treatment</td>
</tr>
<tr>
<td>BRAF V600 wild-type</td>
<td>36 patients, 229 plasma samples</td>
<td>13 patients with stage III/IV melanoma</td>
<td>Sample before initiation of first-line treatment</td>
</tr>
</tbody>
</table>

Kappa 0.68 [95% CI 0.3-1]

Plasma cDNA

| BRAF V600 mut | 4 |
| BRAF V600 wild-type | 0 |

Plasma cDNA of BRAF V600 mut: 2 | 7 |

ctDNA as a diagnostic test compared to tissue test:
- Accuracy = 85%
- Specificity = 100%

Evolution of the BRAF mutant ctDNA fraction upon initiation of BRAF/MEK inhibitor

- At treatment initiation (+/- 2 weeks)
  - Mutant fraction detected in 60% (12/20)
  - After median of 13 days (range 6-40) of treatment
  - Mutant fraction undetectable in 67% (8/12)
  - Absence of CR in 160 % (12/12)

Rationale for cDNA to detect PD according to RECIST 1.1:
- Accuracy = 83%
- Specificity = 100%

### RESULTS monitoring

<table>
<thead>
<tr>
<th>n=36</th>
<th>Progressive disease (RECISTv1.1)</th>
<th>No progressive disease (RECISTv1.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increases in BRAF V600 mutant ctDNA fraction</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>No increase in BRAF V600 mutant ctDNA fraction</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

Hypothesis: BRAF V600 mutant fraction rather represents proliferative tumor burden, than tumor mass

Evolution of the BRAF mutant ctDNA fraction compared to PD (RECIST 1.1)

- At treatment initiation (+/- 2 weeks)
  - Mutant fraction detected in 60% (12/20)
  - After median of 13 days (range 6-40) of treatment
  - Mutant fraction undetectable in 67% (8/12)
  - Absence of CR in 160 % (12/12)

Concurrent PD and increase mutant fraction (n=23)

- No detectable BRAF mutant fraction in 160 samples
  - No disease progression in following month in 88% (p<0.001)
  - No disease progression in following 2 months in 78% (p<0.001)

Evolution of the BRAF mutant ctDNA fraction compared to PD (RECIST 1.1)

- Accuracy = 83%
- Specificity = 100%

- Increase mutant fraction prior to PD (12/23)
- PD prior to increase mutant fraction (6/23)
- Concomitant PD and increase mutant fraction (5/23)

Analysis of BRAF mutant ctDNA from plasma allows for a rapid diagnosis of the BRAF status in pts with advanced melanoma. BRAF mutant ctDNA likely reflects the BRAF mutant proliferative tumor burden and holds promise as a therapeutic monitoring tool for pts with advanced BRAF V600 mutant melanoma.

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