Novel multi-parameter assays for investigational phenotyping of circulating tumor cells

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Background
Circulating tumor cells (CTCs) can reliably be identified in cancer patients and are associated with clinical outcome. We have developed a new-generation "liquid biopsy" platform to expand CTC investigation beyond identification to multi-parameter phenotyping with classification as CTCs in cancer patient samples.

Methods and Results

Sample preparation and analysis workflow

Methods and Results

To identify epithelial CTCs, we employ a canonical 4-color immunofluorescence staining panel that includes a nuclear stain, antibodies to exclude white blood cells (CD45) and to identify epithelial cells (EpCAM and cytokeratin -- allowing up to 6 additional channels for custom investigational biomarkers.

Conclusions

We have designed novel multi-parameter CTC assay frameworks that incorporate 6 fluorescence channels and 3 additional channels to investigate biomarkers for prostate cancer (androgen receptor, AR and AR-V7), breast cancer (epithelial marker EpCAM, vimentin and PD-L1), and melanoma (EpCAM and vimentin).

Single cell molecular analysis

Prostate cancer

CTC Identification Markers

CTC-4 + AR / AR-V7

Breast cancer

CTC Identification Markers

CTC-4 + EGR7 / HER2

Epithelial-mesenchymal transition

Table 1. CTC assays built on the RareCyte CTC-4 and CTC-3 panel framework.

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<thead>
<tr>
<th>CTC-4 core epithelial identification panel</th>
<th>Marker 1</th>
<th>Marker 2</th>
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<tr>
<td>CTC-4 core epithelial identification panel</td>
<td>AR</td>
<td>AR-V7</td>
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<td>CTC-4 core epithelial identification panel</td>
<td>Vimentin</td>
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<td>CTC-4 core epithelial identification panel</td>
<td>EpCAM</td>
<td>CK</td>
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Figure 1: 6-parameter assay for CTC investigation of AR / AR-V7 applied to prostate tissue.

Figure 2: 6-parameter assay for CTC investigation of PD-L1 / IRF1.

Figure 3: Mesenchymal differentiation assay applied to prostate and breast cancer patient samples.

Figure 4: Epithelial differentiation assay applied to prostate and breast cancer patient samples.

Panels show individual channel images of CTCs identified from prostate and breast cancer patients.

Figure 5: Single-cell sequencing of CTCs. Blood samples from a prostate cancer patient with breast TRIM51 (G563D) were analyzed with the CTC-4 and CTC-3 BRT assay. Individual CTCs were identified by CyteMapper. Four distinct single gene amplification peaks (G443A, G443B, G443C, G443D) and four distinct single gene deletions (D581A, D581B, D581C, D581D) were observed in CTCs with (a) negative hbb-G/D/EpCAM phenotypes. These results support the hypothesis that CTCs expressing both markers have a higher likelihood of being of epithelial origin.