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

Yao Sun1, Lance U'Ren1, Daniel Campton1, Arturo Ramirez1, Nolan Ericson1, C. Anthony Blau1, VK Gadi2, Alisa Clein2, Celestia Higano2, Stephen Plymate3, Tad George1, Pei-Ching (Tessa) Tsai1, Daniel Sabath2, Eric Kaldjian1

1RareCyte, Inc., Seattle, WA; 2University of Washington, Seattle, WA; 3University of Washington, Seattle, WA

Background

Circulating tumor cells (CTCs) can reliably be identified in cancer patients and are associated with clinical outcomes. We have developed a next-generation "liquid biopsy" platform to expand CTC investigation beyond identification to multi-parameter phenotyping and mechanistic characterization. The RareCyte CTC platform has four components in an integrated workflow: 1) CTC retrieval with classification as CTCs in cancer patient samples. We demonstrate by single-cell isolation and molecular analysis that CTCs can reliably be identified in cancer patients and are associated with clinical outcomes.

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 2 additional channels for custom investigational biomarkers. Using spike-in and model cancer patient samples, we present here 5- and 6-channel assays that use additional channels to investigate biomarkers for prostate cancer (androgen receptor – AR-V7) and breast cancer (Ki-67/ER/HER2) and for immune checkpoint inhibition (PD-L1 / IRF1) beyond identification to multi-parameter phenotyping.

Conclusions

Table 1. CTC assays built on the RareCyte CTC-4 and CTC-3 panel framework. The CTC-4 panel identifies epithelial CTCs using four fluorescence channels.

Sample preparation and analysis workflow

Figure 1. 6-parameter assay for CTC investigation of AR / AVT1 applied to prostate tumors. Note that DAPI/PI double stains both AR and AVT1. EGFR expresses AR but not AR-V7. PC3 expresses neither AR nor AVT1.

Table 2. Investigational Biomarkers

Figure 2. 6-parameter assay for CTC investigation of AR / AVT1.

Figure 3. Immunofluorescence detection of AR and AVT by DAPI/PI/Ab staining of CTCs. Expression of AR is present in less than 1% of the total CTCs and the vast majority of the total CTCs express AR-V7.

Figure 4. Immunofluorescence detection of AR by DAPI/PI/Ab staining of CTCs. Expression of AR is present in less than 1% of the total CTCs and the vast majority of the total CTCs express AR-V7.

Figure 5. Cell separation by immunofluorescence of CTCs. Blood samples from a prostate cancer patient with known TP53 mutations (G244S) were analyzed with the CTC-4 and CTC-3 EMT assay. Individual CTCs were retrieved by CytePicker® and single-cell molecular analysis. The platform has four components in an integrated workflow: 1) CTC retrieval with classification as CTCs in cancer patient samples. We demonstrate by single-cell isolation and molecular analysis that CTCs can reliably be identified in cancer patients and are associated with clinical outcomes.

Figure 6. CTC assay framework

Figure 7. Table 1. CTC assays built on the RareCyte CTC-4 and CTC-3 panel framework. The CTC-4 panel identifies epithelial CTCs using four fluorescence channels.

Figure 8. Figure 2. 6-parameter assay for CTC investigation of AR / AVT1 applied to prostate tumors. Note that DAPI/PI double stains both AR and AVT1. EGFR expresses AR but not AR-V7. PC3 expresses neither AR nor AVT1.

Figure 9. Figure 3. Immunofluorescence detection of AR and AVT by DAPI/PI/Ab staining of CTCs. Expression of AR is present in less than 1% of the total CTCs and the vast majority of the total CTCs express AR-V7.

Figure 10. Figure 4. Immunofluorescence detection of AR by DAPI/PI/Ab staining of CTCs. Expression of AR is present in less than 1% of the total CTCs and the vast majority of the total CTCs express AR-V7.

Figure 11. Figure 5. Cell separation by immunofluorescence of CTCs. Blood samples from a prostate cancer patient with known TP53 mutations (G244S) were analyzed with the CTC-4 and CTC-3 EMT assay. Individual CTCs were retrieved by CytePicker® and single-cell molecular analysis. The platform has four components in an integrated workflow: 1) CTC retrieval with classification as CTCs in cancer patient samples. We demonstrate by single-cell isolation and molecular analysis that CTCs can reliably be identified in cancer patients and are associated with clinical outcomes.

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Figure 17. Figure 11. Figure 5. Cell separation by immunofluorescence of CTCs. Blood samples from a prostate cancer patient with known TP53 mutations (G244S) were analyzed with the CTC-4 and CTC-3 EMT assay. Individual CTCs were retrieved by CytePicker® and single-cell molecular analysis. The platform has four components in an integrated workflow: 1) CTC retrieval with classification as CTCs in cancer patient samples. We demonstrate by single-cell isolation and molecular analysis that CTCs can reliably be identified in cancer patients and are associated with clinical outcomes.

Figure 18. Figure 12. Figure 6. CTC assay framework

Figure 19. Figure 13. Table 1. CTC assays built on the RareCyte CTC-4 and CTC-3 panel framework. The CTC-4 panel identifies epithelial CTCs using four fluorescence channels.

Figure 20. Figure 14. Figure 2. 6-parameter assay for CTC investigation of AR / AVT1 applied to prostate tumors. Note that DAPI/PI double stains both AR and AVT1. EGFR expresses AR but not AR-V7. PC3 expresses neither AR nor AVT1.

Figure 21. Figure 15. Figure 3. Immunofluorescence detection of AR and AVT by DAPI/PI/Ab staining of CTCs. Expression of AR is present in less than 1% of the total CTCs and the vast majority of the total CTCs express AR-V7.

Figure 22. Figure 16. Figure 4. Immunofluorescence detection of AR by DAPI/PI/Ab staining of CTCs. Expression of AR is present in less than 1% of the total CTCs and the vast majority of the total CTCs express AR-V7.

Figure 23. Figure 17. Figure 5. Cell separation by immunofluorescence of CTCs. Blood samples from a prostate cancer patient with known TP53 mutations (G244S) were analyzed with the CTC-4 and CTC-3 EMT assay. Individual CTCs were retrieved by CytePicker® and single-cell molecular analysis. The platform has four components in an integrated workflow: 1) CTC retrieval with classification as CTCs in cancer patient samples. We demonstrate by single-cell isolation and molecular analysis that CTCs can reliably be identified in cancer patients and are associated with clinical outcomes.