Analysis and single-cell retrieval of circulating tumor cells to monitor treatment response and assess genotype in triple-negative breast cancer

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Abstract

We used a high recovery real-time analysis and single-cell picking system to enrich, visualize, and isolate circulating tumor cells (CTCs) for genomic analysis from the blood of patients with metastatic triple-negative breast cancer (TNBC) undergoing treatment with cisplatin as part of a study to intensively characterize TNBC. CTCs were evaluated and treated during treatment to monitor CTC burden and characteristics that could be associated with treatment response or disease progression, and perform single-cell mutational analysis to inform clinical decision making.

Methods: Patients were enrolled in the study at the University of Washington Center for Cancer Innovation after informed consent for participation in monitoring of their disease, including molecular analysis of multiple lines of approachable CTCs. CTCs were evaluated prior to treatment and tracked longitudinally using the AccuCyte – CyteFinder system. Circulating blood cell-based enrichment of blood cells and processing to microscopic slides was performed using the AccuCyte kit. Fluorescently labeled antibodies to cytokeratin (CK), CD45, and a nuclear dye were applied to samples using an automated slide stainer. Slides were scanned on the CyteFinder digital microscope and candidate CTCs identified using image analysis software. CTCs were verified by appropriate morphology and expression of epithelial and nuclear stains without CD45 expression. Other antibodies used to characterize cells included Her2, M30, and Ki-67. A mutation hypothesis to lead to the activation of ROS1 was identified in the cancer cells isolated from the bone marrow of one patient. CTCs were retrieved from slides using the integrated semi-automated CyteFinder and evaluated for the ROS1 variant using whole genome amplification followed by nested PCR and Sanger sequencing. Activating mutations in FGFR2 were identified in tumor isolated from lymph node of a second patient. CTCs were picked and evaluated as above for these mutations.

Results: Nine patients have been enrolled to date. At least 1 CTC/7 mL has been found in all patients. Pre-treatment CTC levels in the patient with the ROS1 mutation were extremely high (1500/mL). One week after treatment, CTC levels spiked to over 5000/mL. CTC counts then dropped exponentially to 50/mL after 4 months. CTC clusters and Ki-67 positive cells also decreased during treatment. Treatment with cisplatin was discontinued in this patient due to toxicity and progression, and CTC levels increased to nearly 9000/mL over 6 months. The ROS1 mutation was found in 50% of individual picked CTCs before treatment with cisplatin, a ROS1 inhibitor. After cisplatin treatment, the ROS1 mutation was only detected in 30% of CTCs. A second patient was found to have somatic loss of BRCA1, and was therefore treated with the PARP inhibitor, veliparib. CTC levels decreased during therapy, followed by a significant decrease in numbers which correlated very well with CA 15-3, CA 125, and Ki-67.

Conclusions: Analysis of CTCs may provide a non-invasive measure of cancer progression/response and the molecular evolution of tumor cells in patients with TNBC. Single-cell CTC retrieval after slide-based immunofluorescence visualization is compatible with whole genome amplification and sequencing methods.

Methods and Technology

Figure 1. CTC collection by AccuCyte System buffy coat isolation

Figure 2. Slide based blood cell analysis

Figure 3. Imaging of CTCs in TNBC

Figure 4. Sensitivity of RareCyte platform and monitoring of TNBC patients over time

Figure 5. Molecular profiling of CTCs