#1609 Comprehensive Multi-Omic Analysis of Circulating Tumor Cells Isolated from a Metastatic Triple-Negative Breast Cancer Patient to Identify Pathogenic Genomic Aberrations

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Abstract

Increasing evidence confirms the prognostic relevance of Circulating Tumor Cells (CTCs) in a variety of cancers including advanced breast cancer. Recent data also suggests that CTCs are a useful tool for monitoring treatment and identifying potential targets for therapeutic intervention. The objective of this study was to investigate technologies for defining the genomic landscape of CTCs in order to compare genomic information (1) between CTCs to assess genetic heterogeneity, and (2) between CTCs and bone-marrow metastasis tissue biopsies (BMB) and cell-free DNA plasma to assess how reflective the molecular profile of CTCs is to cancer tissue and plasma samples. To evaluate these potential applications, positive CTCs were identified using the AccuCyte® CytocyteFinder system (RareCyte, Inc., Seattle WA) from the blood of a patient with triple negative breast cancer (TNBC). Twenty CTCs and twenty white blood cells (WBCs) were individually retrieved from CyteSpreader™ prepared slides using the ACDFC system. We used whole genome amplification (WGA) followed by next-generation sequencing to perform a comprehensive analysis using WBCs as genome controls. Whole genome, white exome and targeted sequencing of known cancer-associated genes using Illumina® and Life Technology panels identified mutations in TP53, PTEN, STK11, AB1L, HRAS, MPL1L and INPP2 which were present in CTCs but not in WBCs. Results from the whole genome sequencing analysis comparing variants identified between CTCs revealed that the vast majority (≥85%) of variants were specific to individual CTCs revealing a high degree of noise and sequence variation. Variants among the CTCs identified by WGA included mutations in ATM, ALK, BRAF, NOTCH1, ATR and XPC. 80% of the CTCs contained a novel variant in LPP which was not present in the WBCs. Mutations in this gene have recently been associated with aggressive solid tumors. Sequencing variation was also observed in the WBC population, enabling the calculation and subsequent subtraction of background noise associated with WGA of single cells. Molecular information derived from the CTCs was compared to multiple BMIM samples and cfDNA from the same patient. Additional analyses of copy number and structural variations and transcriptomic analysis are being performed in order to gain further insights into the genomic heterogeneity of CTCs and identify genomic markers to establish the utility of CTCs as a non-invasive real-time liquid biopsy for breast cancer.

Study Rationale

During the study period the patient underwent weekly chemotherapy treatments and her CTC counts were routinely assessed.

Single CTCs were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle WA (see poster 1081)

Compared to typical CTC counts this patient had extremely high CTC counts.

Plasma was still available for each of these blood draws to isolate CTCs from the same patient.

CTCs were regularly enumerated over the study period. CTCs were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA on one of the last blood draws before the patient's death.

Figure 1. Clinical status, bone marrow biopsies and mutation profiles. A Patient with extensive tumor involvement in a bone marrow biopsy (A) and a progressive decline in platelet counts (B) received treatment and was enrolled in the TOMIC trial (C). Patient Cyto provides a schematic depiction of free cancer cells (circles) from the left (L) and right (R) posterior iliac crest. Filled circles represent single cells that were analyzed via Whole Exome Sequencing (WES). Filled squares indicate normal hematopoietic cell (green) versus tumor cell (red) in a bone marrow sample.

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Figure 2. Dynamic changes in CTCs. CTCs were regularly enumerated over the study period. CTCs were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA.

Figure 3. Genomic analysis of isolated CTCs. 20 CTCs and 20 WBCs were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA. Shown is one of the last blood draws before the patient's death. Cell-free DNA (cfDNA) was extracted by LeuCo. Numerous follow-up generations of CTCs were performed to identify variants specific to the patient’s CTCs and cell-free tumor DNA (cfDNA) using WBCs as non-mutator controls.

Figure 4. Variant detection in CTCs. 20 CTCs and 20 WBCs were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA. Shown is one of the last blood draws before the patient's death. Cell-free DNA (cfDNA) was extracted by LeuCo. Numerous follow-up generations of CTCs were performed to identify variants specific to the patient’s CTCs and cell-free tumor DNA (cfDNA) using WBCs as non-mutator controls.

Figure 5. CTCs vs. cfDNA. CTCs were isolated from 8 different time points throughout the patient's treatment period. Coupled with CTC characterization, genomic analysis of cfDNA can provide complementary information to detect mutations, which is especially valuable if CTC counts are lowered from a positive treatment response.

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Figure 7. Variant detection in cfDNA. cfDNA extracted from plasma samples from 8 time points throughout the study period were analyzed via WGS and the results were compared with results from the WES data of the patients three metastatic bone marrow biopsies (B1-B3) and the CTCs isolated after leukapheresis. 200 variants were identified as present in the bone marrow samples and the mix CTCs.

Acknowledgements

The patients and their families

Elisabeth Mahen at the University of Washington for help with acquiring the plasma for cfDNA extraction

The AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA allows the identification and isolation of single CTCs that can be used for downstream multi-omic analyses

Analysis of CTCs via WGS showed high sequence variability such that ≥85% of the identified variants were present in a single CTC

WBCs can be used to filter out non-tumor-specific variants

The majority of variants found in the cfDNA were also present in the WBC controls

A number of variants in known cancer-associated genes were identified in the CTCs and cfDNA that were also shared with variants identified in the metastatic biopsy samples and bulk CTC population

Variant information derived from purified CTCs can be combined with primary or metastatic tumor data to give a more comprehensive picture to evaluate metastases or a patient’s response to therapy

Additional analysis of WES data, network analysis to assign biological context to the identified variants, analysis of methylated DNA fragments, targeted mutational analysis and total cfDNA quantification will be performed to continue to understand the clinical utility of CTCs and cfDNA

Summary

The AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA allows the identification and isolation of single CTCs that can be used for downstream multi-omic analyses

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Patient History

The patient was a 56-year-old woman with metastatic triple negative breast cancer (TNBC)

Patient consented to enrollment in the Intensive Trial of OMCs in Cancer clinical trial (TOMIC-001, ClinicalTrials.gov ID NCT01957514)

The TOMIC design characterizes the molecular features of a cancer, deploys a distributed network to analyze results and predict drug susceptibilities, allows for treatment in accordance with these predictions, and aims to learn from individual patient experiences to iterate and improve over time (Trends Genet. 2013; 29: 6-10)

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