#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 (BMM) and cell-free plasma DNA 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® -CyteFinder® (AC/CF) system (RareCyte®, 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 AC/CF system. We used whole genome amplification (WGA) followed by next-generation sequencing to perform a comprehensive analysis using WBCs as genome controls. Whole genome, whole exome and targeted sequencing of known cancer-associated genes using Illumina® and Life Technology panels identified mutations in TP53, PTEN, STK11, ABL1, HRAS, MLL2 and INPPL1 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 WGS 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. Sequence 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 BMM 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 genetic heterogeneity of CTCs and identify genomic markers to establish the utility of CTCs as a non-invasive real-time liquid biopsy for breast cancer.

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 OMics in Cancer clinical Trial (ITOMIC-001; ClinicalTrials.gov ID NCT01957514)
- The ITOMIC 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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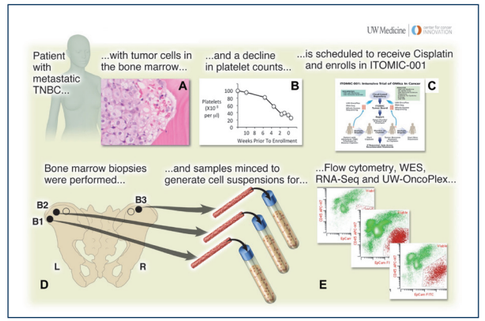
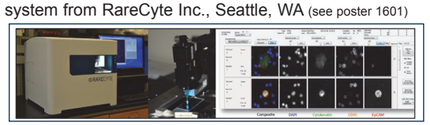


Figure 1. Clinical status, bone marrow biopsies and mutation profiles.
A Patient with extensive tumor involvement in a bone marrow biopsy (A) and a progressive decline in platelet counts (B) received treatment and was enrolled in the ITOMIC clinical Trial (C). Panel D provides a schematic depiction of five core biopsy sites (circles) from the left (L) and right (R) posterior iliac crest. Filled circles (designated B1, B2, and B3) indicate samples that were analyzed via Whole Exome Sequencing (WES), RNA-Seq, UW-OncoPlex and Flow cytometry at the University of Washington. Panel E shows a representative flow cytometry profile of hematopoietic cells (green) versus tumor cells (red) in a baseline bone marrow sample.

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 1601)



- Compared to typical CTC counts this patient had extremely high CTC counts
- Plasma was still available for each of these blood draws to isolate cell-free DNA
- The presence of such high numbers of CTCs, the accessibility of matched plasma samples and the availability of mutation data from Whole Exome Sequencing (WES) of the metastatic biopsy sites and bulk CTC analysis following leukapheresis allowed for a rare opportunity to define the genomic landscape of CTCs in a TNBC patient

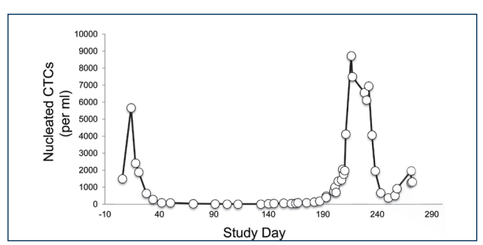


Figure 2. Dynamic changes in CTCs.
CTCs were regularly enumerated over the study period. CTCs were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA.

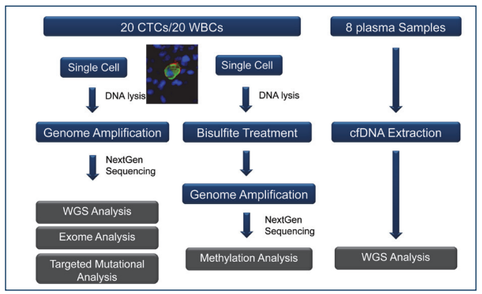


Figure 3. Genomic analysis of isolated CTCs.
20 CTCs and 20 WBCs were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA at one of the last blood draws before the patient's death. Cell-free DNA (cfDNA) was extracted by LabCorp. Numerous Next Generation Sequencing tools were performed to identify variants specific to the patient's CTCs and cell-free tumor DNA (ctDNA) using WBCs as non-tumor controls.

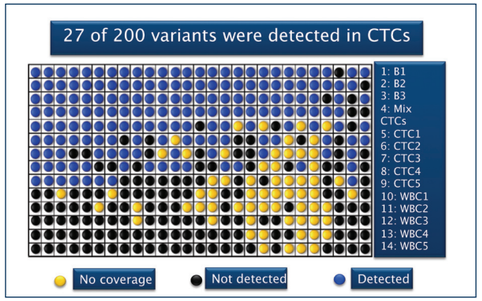


Figure 4. Variant detection in CTCs.
5 CTCs and 5 WBCs were analyzed via WGS. Variant analysis was performed and compared with results from the WES data of the patients three metastatic bone marrow biopsies (B1-B3) and mix CTCs isolated after leukapheresis. 200 variants were identified as present in the bone marrow samples and the mix CTCs.

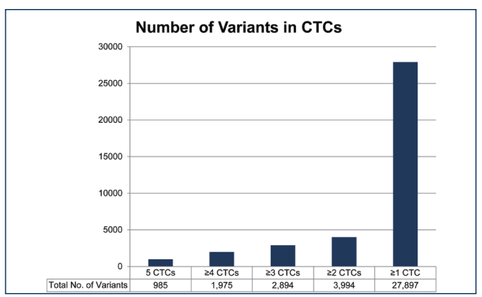


Figure 5. Assay characterization.
A total of 27,897 variants were detected in the CTCs as a whole. Further analysis of different subsets of the CTCs revealed that 85-99% of these variants were identified in a single CTC suggesting a high degree of sequence variability.

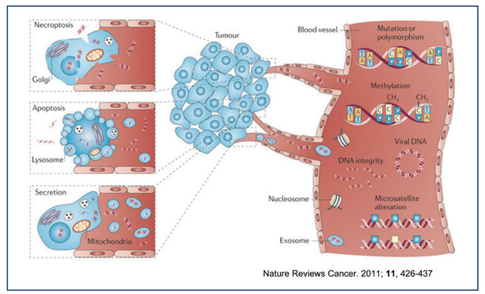


Figure 6. CTCs vs. cfDNA.
Cell-free DNA (cfDNA) was isolated from 8 different time points throughout the patient's treatment period. Coupled with CTC characterization, genomic analysis of cfDNA can provide complementary information to detect mutations, which is especially valuable if CTC counts are lowered from a positive treatment response.

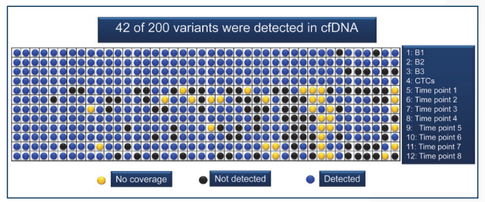


Figure 7. Variant detection in cfDNA.
cfDNA extracted from plasma samples from 8 time points throughout the study period were analyzed via WGS and the variants were compared with the results from the WES data of the patients three metastatic bone marrow biopsies (B1-B3) and mix CTCs isolated after leukapheresis.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

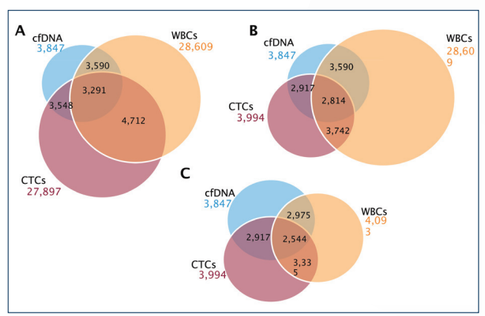


Figure 8. Common variants identified in CTCs, cfDNA and WBCs.
Variants present in ≥ 1 CTC, WBC and cfDNA sample are shown in (A); variants present in ≥ 2 CTCs, ≥ 1 WBC and ≥ 1 cfDNA sample are shown in (B) and variants present in ≥ 2 CTCs, ≥ 2 WBCs and ≥ 1 cfDNA sample are shown in (C).

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
- 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 complete 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

Acknowledgements

- The patients and their families
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