Longitudinal Analysis of Circulating Tumor Cells and Cell Free Tumor DNA by Next Generation Sequencing in Triple Negative Breast Cancer

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Abstract
As the practice of genetically profiling patient tumors is considered for making clinical treatment decisions, recent methodologies for screening of genomic aberrations in circulating tumor cells (CTCs) and cell-free plasma DNA (cfDNA) may provide non-invasive tools for such applications. Genomic analysis of DNA from CTCs and plasma can also provide useful insight into tumor heterogeneity and thus disease progression by revealing sub-populations of tumor cells that evolve during treatment, have novel drug-resistant genotypes, or carry alternate cancer driver mutations not identified by the sequencing of primary tumors.

Comprehensive evaluation of DNA isolated from CTCs and cfDNA from a breast cancer patient by whole exome sequencing was performed to better understand the role of liquid biopsies in investigating the etiology of tumor progression. The patient was diagnosed with metastatic triple negative breast cancer (TNBC) six years after remission from estrogen receptor (ER3+), progesterone receptor (PR+/-), human epidermal growth factor 2 negative (HER2-), grade 3 intraductal carcinoma of the right breast. Metastatic lesions were found in the spine, pelvis and sacrum and bone-marrow. The patient was enrolled in the Intensive Trial of Omics in Cancer clinical trial (ITOMIC-001, ClinicalTrials.gov ID NCT01957514) and initially received weekly cetaplatin infusions followed by additional targeted therapy. Peripheral blood was obtained during regular clinic visits over the 272 days the patient was enrolled in the clinical trial. CTCs were identified and enumerated from each blood draw using the AccuCyte-CyteFinder® (ACCF) system (RareCyte, Seattle WA).

Multiple CTCs along with whole blood (WBCs) were picked from various time points throughout the treatment regimen. The selected CTCs and WBCs were whole genome amplified and whole exome sequencing was performed to identify tumor-specific variants. A comparative analysis with variants present in genomic DNA isolated from the bone-marrow metastasis tissue biopsy samples and cfDNA revealed the evolution of tumor-specific variants during therapy. Each CTC had somatic alterations in genes associated with therapies in current use or those in the clinical trials setting. Sequencing analysis of cfDNA provided similar information on potential therapeutic approaches. The monitoring of disease over time through genomic analysis of CTCs and cfDNA can identify novel sub-populations related to disease progression for the tailored cancer treatment regimens. Further analysis is being performed to better understand the evolution of the genomic heterogeneity among CTCs at the same time point and across different time points and therefore better understand the etiology of progression of metastatic breast cancer in this patient.

Patient Clinical History
The patient was a 56-year-old woman with metastatic triple negative breast cancer (TNBC). She consented to enrollment in the Intensive Trial of Omics in Cancer (ITOMIC) clinical trial.1

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.1

Isolation of Circulating Tumor Cells from Blood
During the study period the patient underwent weekly chemotherapy treatments and her CTC counts were routinely assessed.1

CTCs were isolated using the AccuCyte-CyteFinder system from RareCyte Inc., Seattle, WA. Compared to typical CTC counts, in general this patient had extremely high CTC counts.2

CTCs were regularly enumerated over the study period. CTCs were isolated using the AccuCyte-CyteFinder system from RareCyte Inc., Seattle, WA and sequencing was performed after Whole Genome Amplification (WGA). CTCs with available sequence data were regularly enumerated over the study period. CTCs were isolated using the AccuCyte-CyteFinder® (ACCF) system (RareCyte, Seattle WA).

For all 5 CTCs, CTN308, ITOMIC-001, ClinicalTrials.gov ID NCT01957514, the deleted region included the promoter region of lncRNA308 on chromosome 21. This deletion was identified in all 5 CTCs analyzed.

Figure 6. Detection of variants across various time points from individual CTCs. All variants (rows) were identified in the cell-free DNA (cfDNA) across 6 different time points (columns). For each column, the variants identified across all 5 CTCs are shown (as described in the Key above). The colors show the number of CTCs with that variant, ranging from 1 to 5.

Figure 4. CNV Analysis of the CTCs identified deletions in the promoter region of lncRNA308 on chromosome 21. A portion of chromosome 21 was found to be deleted in the CNV analysis performed on CTCs. The deletion included the promoter region of lncRNA308.

Figure 3. CNV analysis of CTCs vs WBCs. Copy Number Variation (CNV) analysis was performed using whole genome sequencing data from five CTCs and WBCs at the same time point. Coverage data from the WBCs was used to detect CNVs in the CTCs. Shown is a CNV plot for 5 CTCs vs 5 WBCs.

Figure 2. Genetic analysis of CTCs and cfDNA from different time points. CTCs were regularly enumerated over the study period. CTCs were isolated using the AccuCyte-CyteFinder system from RareCyte Inc., Seattle, WA. Sequencing was performed after Whole Genome Amplification (WGA). CTCs with available sequence data were regularly enumerated over the study period. CTCs were isolated using the AccuCyte-CyteFinder® (ACCF) system (RareCyte, Seattle WA).

Figure 7. Detection of variants across various tissue points from cfDNA. All variants (rows) were identified in the cell-free DNA (cfDNA) across 6 different time points (columns) of cfDNA. The colors are described in the Key above.

Figure 5. Using genomic tools for informed therapies.

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.

CNV analysis of CTCs identified a deletion in chromosome 21 that includes ITOM308, which has been shown to be positively associated with breast cancer survival.

Conserved, dynamic and novel variants were identified in the CTCs and cfDNA across six time points.

Using network analysis of the variants identified in the CTCs, four compounds currently in clinical trials were identified as potential therapies for the patient (mirtinib, Rituximab, Bortezomib and Dasabulin). Variant information derived from purified CTCs can be combined with cfDNA and primary or metastatic tumor data to give a more complete picture to evaluate metastases or an inform targeted therapy.

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References