#498 Meta-Analysis of Genetic Aberrations Identified in CTCs and ctDNA in Triple Negative Breast Cancer

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Abstract

Technological innovation and scientific advances in understanding cancer at the molecular level have accelerated the discovery and development of both diagnostics and therapeutics. Circulating tumor cells (CTCs) and plasma circulating tumor DNA (ctDNA) are non-invasive prognostic markers that have been associated with metastatic and aggressive disease. Both CTCs and ctDNA allow molecular characterization of a tumor that is inaccessible or too risky to biopsy. The analysis of genomic aberrations in both sample types provides insights into drug resistance and can help determine appropriate, targeted cancer treatments. Mutations found in the primary or metastatic tumor can be identified in both CTCs and ctDNA as well as novel mutations that may reflect intra-tumoral and inter-metastatic heterogeneity. When collected and evaluated over an extended period of time, changes in the CTC and/or ctDNA mutational profile can offer guidance in the effective treatment of a disease, indicate disease progression, and detect recurrence of disease earlier.

We have performed whole genome sequencing of CTCs and ctDNA from a metastatic triple negative breast cancer (TNBC) patient to better understand the evolution of tumor heterogeneity during therapy. The patient was enrolled in the Intensive Trial of Omics in Cancer clinical trial (ITOMIC)—001 and initially received weekly capcitabine infusions followed by additional targeted therapy. Longitudinal peripheral blood samples were collected over a period of 372 days following enrollment in the clinical trial. CTCs were identified using the AccuCyte–CyteFinder® system from RareCyte Inc., Seattle, WA and Whole Exome Sequencing (WES) was performed after Whole Genome Amplification (WGA). CTCs with available sequence data are indicated with arrows (h4). Whole Genome Sequencing was also performed on ctDNA isolated from the plasma at the same time points.

Patient History

- The patient was a 56-year-old woman with metastatic triple negative breast cancer (TNBC).
- In October 2013, she consented to enrollment in the Intensive Trial of Omics in Cancer clinical trial (ITOMIC).²
- During the study period the patient underwent weekly chemotherapy treatments and her CTC/ctDNA were collected.

Figure 1. Genomic analysis of CTCs and ctDNA 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 and Whole Exome Sequencing (WES) was performed after Whole Genome Amplification (WGA). CTCs with available sequence data are indicated with arrows (h4). Whole Genome Sequencing was also performed on ctDNA isolated from the plasma at the same time points.

Figure 2. Using genomic tools for a better understanding of TNBC etiology.

Table 1. Top Three Ranked Common Pathways Associated with Cancer Driver Variants Identified in Individual CTCs.

Table 2. Top Three Ranked Common Pathways Associated with Cancer Driver Variants Identified in Individual CTCs.

Figure 3. Number of variants identified in various CTCs at various time points using bio-informatic filtering. Sequencing data was aligned against the hg19 reference sequence using bwa. Samtools were used to call variants.

Figure 4. Detection of variants across various time points from individual CTCs. Each column indicates a different CTC. The larger columns indicate time points for which there are multiple CTCs. Each row represents a mutation identified in the WES data. Those variants that are detected in CTCs are represented by red (same time point) and blue (different time point). Variants that are shared across all time points are at the top, variants that evolve over time are at the bottom of the figure.

Figure 5. Identified variants occupy key nodal points of cancer associated pathways. Various cancer driver variants were mapped across known pathways using Ingenuity. The colors are described in the key to the left.

Figure 6. Summary of the study period. CTCs were regularly enumerated over the study period. CTCs were isolated using the AccuCyte–CyteFinder® system from RareCyte Inc., Seattle, WA and Whole Exome Sequencing (WES) was performed after Whole Genome Amplification (WGA). CTCs with available sequence data are indicated with arrows (h4). Whole Genome Sequencing was also performed on ctDNA isolated from the plasma at the same time points.