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2757. Using Liquid Biopsies and NGS as Tools to Analyze Mutation Burden and Copy Number Variation in the Blood of a Patient with Triple Negative Breast Cancer to Better Inform Therapeutic Targets

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Abstract
The ability to characterize molecular features of cancer from liquid biopsies is resulting in the development of innovative health care for patients. Longitudinal changes in the mutational profiles of DNA isolated from liquid biopsies are being used to better understand and monitor the development, progression, and evolution of therapy resistance in cancer patients. To define changes in the mutational landscape and predict drug susceptibilities in Triple Negative Breast Cancer (TNBC) patients, we used whole exome analysis to profile circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) from selected time points of a patient enrolled in the Intensive Trial of Omics in Cancer clinical Trial (ITOMIC). The patient initially received weekly cisplatin infusions followed by additional targeted therapy. Peripheral blood samples were collected at specific time points over a period of 272 days following enrollment in the clinical trial. Our data indicates that the identified mutations in genomic DNA isolated from CTCs and ctDNA can be used to understand and mitigate the impact of tumor heterogeneity in addition to identifying clinically relevant mutations at those selected time points. To further increase the resolution of our analysis, we profiled ctDNA from these samples to a higher depth targeting only clinically relevant genes. These analyses increased the sensitivity of detection and identified additional targets that could have been used for therapeutic intervention. In addition to sequence variants, copy number variations (CNVs) have also been significantly associated with the development of metastasis and changes in CNVs have been used to monitor disease progression. We performed a bioinformatics analysis of genomic instability and CNVs across 32 different time points from ctDNA from the same patient throughout the treatment period. The genomic instability number (GIN) calculated for each of the 32 time points seems to mirror the overall CTC burden in the patient at each time point tested. CNV analysis is ongoing and these data sets are being further analyzed in combination with TCGA data to define possible cancer driver genes for the functional prediction of significant TNBC candidate alterations and the results of these analyses will be presented.

Patient History
The patient was a 56-year-old woman with metastatic triple negative breast cancer (TNBC).1
- 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 treatment and her CTC/cfDNA were collected.

Methods

Figure 1. Genomic Analysis of CTCs and cfDNA from Different Time Points. CTCs and cfDNA were isolated using the AccuCyte–CyteFinder system from RareCyte Inc., Seattle, WA. ctDNA was extracted from 32 serum samples from various time points over the study period. In the above figure, solid arrows indicate weekly cisplatin treatment. Open arrows indicate cisplatin resistance. After 3 weeks of cisplatin treatment, the patient was treated with an open biopsy and Enbrel treatment are identified with check marks.

Figure 2. Genomic tools used to better understand the etiology of TNBC.

Figure 3. Representative Network from one of the CTCs at a selected time point. Mutated genes were identified and network analysis was performed using String that looks for various interactions shown above.

Figure 4. The Genome Instability Number (GIN) was calculated for each of the 31 ctDNA were extracted from the study period where enough mass was available for genome wide sequencing. GIN is a metric designed to quantify the cumulative number and magnitude of the Copy Number changes within a given sample. The results show that although there are changes over time with respect to the GIN which coincide with treatment, overall the genomic profile is not consistent with what would be seen for a normal healthy individual.

Figure 5. Copy number analysis of individual ctDNA samples. The Copy Number Variation (CNV) was analyzed for each of the 31 ctDNA samples. The three profiles shown above are representatives from three different time points (indicated above each figure). Multiple CNV changes can be identified in every sample, the magnitude of which tracks with the tumor burden seen in the plasma over time (one time point above to pre-treatment and the other two were during treatment with cisplatin).

Conclusions and Future Directions
- Each CTC examined contains different affected pathways suggesting a highly heterogeneous CTC population
- The GIN calculated for the ctDNA follows the CTC profile as expected due to genomic instability
- Numerous genes show CNV based on the ctDNA analysis
- A targeted mutation panel was used on the ctDNA and no actionable mutations were identified
- Future work includes:
  - Examining the CNV profiles for all ctDNA samples analyzed to perform network analysis
  - Determine whether there are pathways in common between CTCs and other ctDNA timepoints

2. ITOMIC-001. ClinicalTrials.gov ID NCT01985704.