Serial monitoring of circulating tumor cells and circulating tumor DNA in metastatic lobular breast cancer identifies intra-tumor heterogeneity and precision and immuno-oncology biomarkers.

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ABSTRACT

Background: Clinical decisions on precision and immunotherapy oncology are based on predictive biomarkers commonly obtained from a single metastatic biopsy or archived primary tumor tissue. Circulating genomic biomarkers offer a minimally invasive approach to monitor intra-patient tumor heterogeneity and detect in real-time the clinically-relevant evolving clinical architecture. Although currently understudied, we hypothesize that single-cell Next generation sequencing (scNGS) of circulating tumor cells (CTC) is a particularly well-suited method to complement biomarker information obtained from tissue and cell-free circulating tumor DNA (ctDNA).

Methods: We analyzed 113 individual CTC, 21 ctDNA, and 15 white blood cells (WBC) samples, from 15 CTC-positive lobular breast cancer patients, four of whom had CTC available at both metastatic baseline and after progression on a variety of therapies chosen at their physician’s discretion. Clinical NGS of 15 tumor tissues obtained using a ~1700-gene DNA panel and whole transcriptome sequencing were available for comparison. CTC were enriched with the CellSearch® system and isolated as single cells with the DEPArray™ system. Whole genome amplified CTC and WBC, as well as ctDNA underwent scNGS with the Oncomine Comprehensive Assay covering ~500 genes and 1.1Mb of genomic information. Targetable mutations, copy number alterations, tumor mutation burden (TMB) and microsatellite instability (MSI).

Results: 99.1% of single cells and 95.2% of ctDNA samples were informative, with a mean sequencing depth of 686x. Using our previously developed, CTC-based precision medicine reporting platform, Mi-CTCseq, CTC in 15 of 15 patients (60%) had mutations that were actionable by FDA-approved targeted therapies including the oncogenes PIK3CA and FGFR2 and HER2. 3 of these 9 patients (33%) harbored actionable alterations not shared between all 3 analyte types (tissue, CTC, and ctDNA). These included 3 actionable mutations found in CTC and ctDNA only, 1 in tissue and ctDNA, and 1 in tissue only. However, 2 of those ctDNA mutations were identified near the limit of detection and with a priori knowledge of their presence from tissue or CTC. Further, 1 patient with plentiful CTC had no detectable ctDNA and one patient’s tissue biopsy was inadequate for sequencing while both liquid biopsy analytes were abundant. 13 patients (87%) displayed intra-patient, inter-CTC genomic heterogeneity of putative driver alterations. 1 of 4 (25%) patients with CTC available in >1 timepoint displayed fluctuations in their ctDNA mutations. 1 of 4 (25%) patients with CTC available in >1 timepoint displayed fluctuations in their mutations.

Conclusions: These data suggest the feasibility of the non-invasive interrogation of the circulating tumor cell genome for the detection of actionable and immunogenic biomarkers with biological and clinical implications. The data suggest that single-CTC interrogation is particularly suited to lobular breast cancer, an especially high CT-producing cancer. In a novel application, we show the ability to perform measurement of single-cell tumor mutation burden, reported to be enriched in lobular breast cancer), and, therefore, an important target for instability detection. Remarkably, we document the presence of TMB heterogeneity and evolution over a sequence of endocrine, chemotherapeutic and immunotherapies.

Our data support continued investigation of the potential utility of Circulating Tumor Cell genomic profiling to complement DNA as a targetable alteration platform suited to the detection and monitoring of Targetable Alterations, Tumor Mutation Burden and Microsatellite instability in lobular breast cancer.

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