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.

Andi K. Cani[1,2,#], Emily M. Dolce[1,2], Elizabeth P. Darga[1,2], Kevin Hu[3,4], Chia-Jen Liu[3,4], Dan Robinson[3,4], Yi-Mi Wu[3,4], Dafydd G. Thomas[3], Costanza Paoletti[1,2], Scott A. Tomlins[3,4], James M. Rae[5], Aaron M. Udager[2,3,4], Arul M. Chinnaiyan[3,4], Erin F. Cobain[1,2], Daniel F. Hayes[1,2].

1 Division of Hematology and Oncology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI. 2 Rogel Cancer Center, Michigan Medicine, University of Michigan, Ann Arbor, MI. 3 Department of Pathology, Michigan Medicine, University of Michigan, Ann Arbor, MI. 4 Michigan Center for Translational Pathology, Michigan Medicine, University of Michigan, Ann Arbor, MI. 5 Department of Pharmacology, Michigan Medicine, University of Michigan, Ann Arbor, MI. # Corresponding.

ABSTRACT

Background:

Clinical decisions on precision and immuno-oncology therapies 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 clonal architecture. Although currently underutilized, we hypothesize that single-cell DNA 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:

In this study 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 data from 15 tumor tissue biopsies 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 space to detect mutations, copy number alterations, tumor mutation burden (TMB) and micro-satellite instability (MSI).

Results:

99.1% of single cells and 95.2% of ctDNA samples were informative, with a mean sequencing depth of 664x. Using our previously developed, CTC-based precision medicine reporting platform, MI-CTCSeq, CTC in 9 of 15 patients (60%) had mutations that were actionable by FDA-approved targeted therapies including in 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 only, and 1 in ctDNA 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 mutations. 1 of 4 (25%) patients with CTC available in >1 timepoint displayed fluctuations in their CTC subclonal makeup between timepoints. Data from this patient's 2 tissue biopsies, 5 ctDNA samples, and 30 individual CTC over 6 timepoints combined to reveal in unprecedented detail inter-metastatic lesion, and inter-CTC heterogeneity and tumor evolution in response to endocrine and immunotherapy selective pressures. ScNGS of CTC helped provide an additional level of detail not appreciated by sequencing of the other two analyte types. In another patient, CTC were composed of 2 subclones which were indistinguishable by ctDNA, 1 of which appears to have not been sampled by the tissue biopsy.
Using a novel method, we enabled detection of single-cell CTC TMB and MSI. CTC TMB scores were highly concordant with their matched tissue samples (R=0.81). CTC MSI was also accurately detectable compared to tissue at the bulk and single cell level. Furthermore, in a novel observation, we detected intra patient, inter-CTC heterogeneity and evolution of TMB and MSI, which has potential implications for immunotherapy response and development of resistance.

Conclusions:

Taken together, these data support the non-invasive biomarker interrogation and monitoring by liquid biopsy that incorporates CTC scNGS and complements tissue in informing precision and immuno-oncology approaches. This may have important implications for appropriate treatment selection and identification of therapeutic resistance mechanisms.

Figure 1

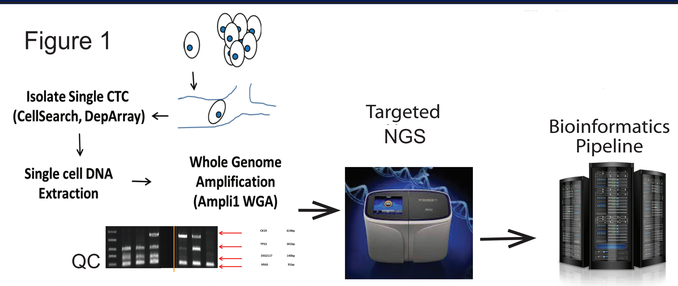

Figure 1. Workflow of single Circulating Tumor Cell NGS. CTC enrichment and staining (Cytokeratin, CD45, DAPI) was carried out with CellSearch®. Enriched CellSearch® cartridge contents were subjected to single cell isolation with DEPArray™ using dielectrophoretic cell sorting. Individuallly isolated Circulating Tumor Cells and control white blood cells underwent whole genome amplification (WGA, Ampli1) and quality control (QC, Ampli1 QC). Amplified DNA of high quality underwent tageted next generation sequencing (NGS) using the (Oncomine Comprehensive Assay Plus, Ion Torentand, ThermoFisher) and data analysis with Ion Reporter.

Figure 2

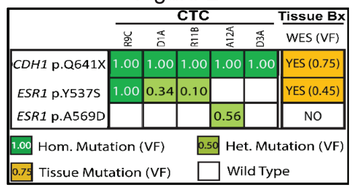

Figure 2. DNA scNGS of matched tumor tissue and 5 CTC (columns) shows hetero-geneity not discernible in bulk tissue sequencing. VF=variant fraction. Bx=biopsy.

Figure 3

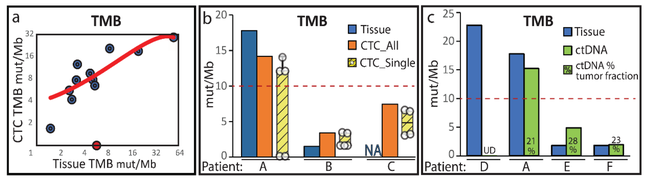

Figure 3. a) Matched tissue and CTC (overall) TMB in lobular mBC patients. (Tissue TMB from clinical sequencing. Low CTC TMB sample (red) mutations not covered by CTC panel) b) Matched tissue (blue bars) and CTC TMB (overall: orange bars, single-cell: yellow boxplots, white balls) in lobular mBC patients. Dashed red line=tissue TMB cutoff. c) Matched tissue, ctDNA TMB. % ctDNA tumor fraction is shown.

Figure 4

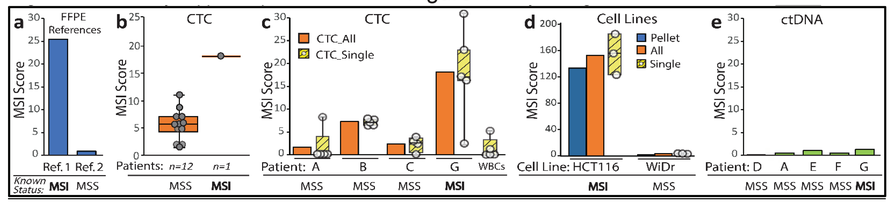

Figure 4. a) FFPE, b) c) CTC, d) cell line and e) ctDNA MSI determination by NGS. MS references from Horizon Diagnostics. Tissue MS status from clinical lab shown in bottom. MSS =microsatellite stable. CTC-all MSI score in orange bars=mean of single CTC scores. Single CTC scores shown as yellow hatched boxplots and dots. WBCs=white blood cells.

Figure 5

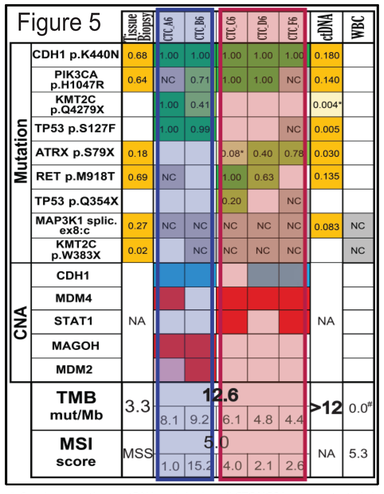

Figure 5. Synchronous tissue, ctDNA and single-cell CTC NGS in one metastatic lobular breast cancer patient. Tis-sue (from clinical sequencing), and ctDNA driver mutations are shown in orange (variant frequency shown inside box). CTC mutations and their variant frequencies are shown as homozygous (dark green) and heterozygous (light green, including one below pre-specified detection cutoff *). Select copy number alt-erations (CNAs) are shown as blue for 1-copy loss and red for copy gains. TMB and MSI (whole sample, and single-cell for CTC) are shown. Blue and red boxes mark the two subclones present based on mutations and TMB. NC= no sequenc-ing coverage, NA=not available. # TMB of white bloodcell (WBC) is defaulted to 0.

Figure 6

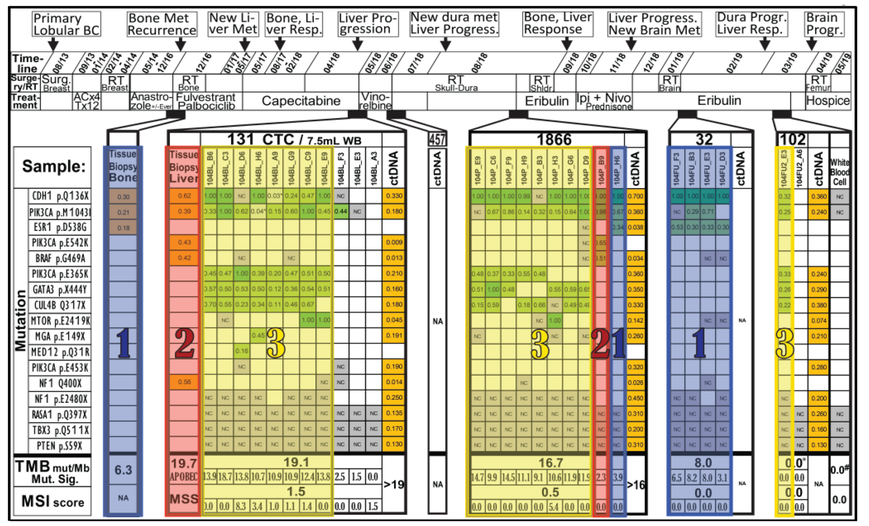

Figure 6. Tissue, ctDNA and single-cell CTC serial NGS in one lobular breast cancer patient undergoing a seq-uence of therapies from primary cancer to metastatic progres-sion and hospice. Synchronous samples are grouped in boxes and time of collection is marked in the timeline by black horizontal bars. Tissue (from clinical sequencing), and ctDNA driver mutations are shown in orange boxes (variant frequency shown inside box). CTC count per 7.5 mL whole blood is shown above. CTC mut-ations and their variant frequencies are shown as homo-zygous (dark green) and heterozygous (light green, including two below prespecified detection cutoff). TMB and MSI (whole sample, and single-cell for CTC) are shown. Blue, red and purple boxes denote the various subclones present based on mutations and TMB. RT= radiotherapy, AC=doxorubicin, cyclo-phosphamide, T= paclitaxel, Ipi=ipilimumab, Nivo=Nivolumab, NC=no sequencing coverage, NA=not available. *TMB calcul-ation for 1 lone CTC in a sample is defaulted to 0, #TMB of a white blood cell is defaulted to 0.

CONCLUSIONS

These data suggest the feasibility of the non-invasive interrogation of the circulating tumor cell genome for the detection and monitoring of precision and immuno-oncology biomarkers with biological and clinical implications.

The data suggest that single-CTC interrogation is particularly suited to lobular breast cancer, an especially high CTC-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 micro-satellite instability detection. Remarkably, we document the presence of TMB hetero-geneity and evolution over a sequence of endocrine, chemo- and immuno-therapies.

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

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