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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LAY ABSTRACT

Metastatic lobular breast cancer is incurable due to the tumor molecular evolution that leads to therapy failure. However, monitoring of tumor molecular evolution is not performed in the clinic since tissue biopsies are invasive and costly whereas free-floating tumor DNA in blood is in limited amounts. Given that circulating tumor cells (cell that break off from a tumor and end up in blood) are plentiful in metastatic lobular breast cancer, and that tumor cells in a patient are not all identical, isolation and analysis of a number of individual circulating tumor cells represents a unique opportunity to monitor tumor evolution for treatment purposes.

In this study we analyzed over 180 samples from 15 metastatic lobular patients, including 120 circulating tumor cells and several tissue and free-floating DNA samples to detect cancer driving mutations and cancer immunotherapy biomarkers using a new method developed by us. This analysis revealed extensive heterogeneity of cancer driving mutations (presence of cells with or without the mutation). Mutations were often not present in all three matched samples from individual patients (circulating tumor cells, tissue, and free-floating tumor DNA). We also show that monitoring by circulating tumor cells reveals tumor evolution in response to endocrine and immunotherapy in unprecedented detail. Further, our new method to detect immunotherapy biomarkers in individual circulating tumor cells reveals good, but imperfect concordance with tissue biomarkers, indicating further heterogeneity among single tumor cells within individual patients. Altogether, our data suggest that single-cell profiling in metastatic lobular breast cancer represents a unique opportunity to monitor tumor heterogeneity and evolution with the potential to inform therapy and extend survival.

SCIENTIFIC ABSTRACT

Background:
Despite treatment advances, metastatic lobular breast cancer (mLBC) remains incurable mainly due to tumor heterogeneity and evolution leading to exhaustion of treatment options. However, longitudinal molecular monitoring of the evolving disease is not routinely performed and is unfeasible or ambiguous with tissue biopsies or ctDNA. Circulating tumor cells (CTCs) are common in LBC, and we hypothesized that single-cell CTC genomic profiling allows detection and monitoring of the clinically-relevant biomarkers, their heterogeneity and evolution.

Methods:
We analyzed 121 individual CTC, 26 ctDNA, 15 white blood cells and 24 tissue samples, from 15 CTC-positive mLBC patients. CTC were enriched with CellSearch® and isolated as single cells with the DEPArray™ system. CTC, WBC, and ctDNA underwent targeted scNGS at 733x depth covering ~500 genes and 1.1Mb of genomic space to detect mutations, copy number alterations, tumor mutation burden (TMB) and microsatellite instability (MSI) with 99.1% of single cells and 95.2% of ctDNA informative samples.

Results:
Our CTC-based precision medicine reporting platform, MI-CTCSeq, detected targetable CTC alterations in 10 of 15 patients (67%). 14 of 22 alterations (64%) in 8 of 11 patients (73%) harbored actionable alterations not shared between all three analyte types (tissue, CTC and ctDNA), including undetectable mutations in each of the three. 13 patients (87%) displayed inter-CTC genomic heterogeneity of 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 6 tissue, 5 ctDNA samples, and 28 individual CTC over 7 timepoints revealed in unprecedented detail heterogeneity and evolution in response to endocrine, chemo and immunotherapy pressures. Our novel detection of single-cell TMB and MSI showed highly concordant (R 0.81) CTC and tissue TMB and intra patient, inter-CTC TMB and MSI heterogeneity. These data support the non-invasive biomarker detection and monitoring by liquid biopsy in mLBC.

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 chemo-, endocrine, precision and immuno-oncology approaches in lobular breast cancer. This may have important implications for appropriate treatment selection and identification of therapeutic resistance mechanisms.

Figure 1

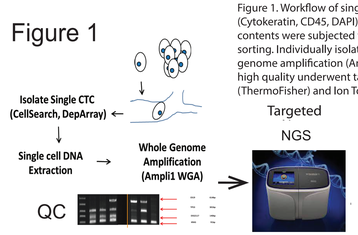


Figure 1. Workflow of single Circulating Tumor Cell NGS. CTC enrichment and staining (Cytokeratin, CD45, DAPI) was carried out with CellSearch. Enriched CellSearch cartridge contents were subjected to single cell isolation with DEPArray by dielectrophoretic cell sorting. Individually isolated CTCs and control white blood cells underwent whole genome amplification (Ampli1 kit) and quality control (Ampli1 QC kit). Amplified DNA of high quality underwent targeted NGS using the Oncomine Comprehensive Assay Plus (ThermoFisher) and Ion Torrent sequencing. Data were analyzed with Ion Reporter and an in-house pipeline.

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 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

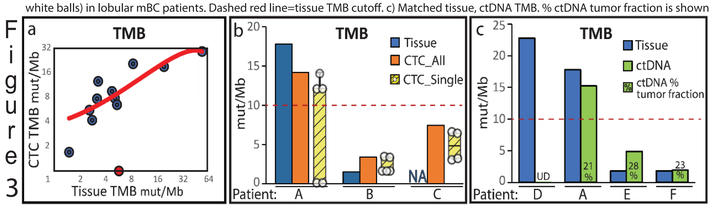


Figure 2. DNA scNGS of matched tumor tissue and 5 CTC (columns) shows heterogeneity not discernible in bulk tissue sequencing. VF=variant fraction. Bx= biopsy.

	CTC					Tissue Bx
	R9C	D1A	R11B	A12A	D3A	WES (VF)
CDH1 p.Q641X	1.00	1.00	1.00	1.00	1.00	YES (0.75)
ESR1 p.Y537S	1.00	0.34	0.10			YES (0.45)
ESR1 p.A569D				0.56		NO

1.00 Hom. Mutation (VF) · 0.50 Het. Mutation (VF) · 0.75 Tissue Mutation (VF) · Wild Type

Figure 5

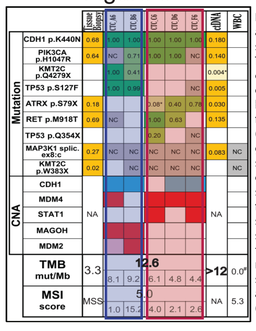


Figure 5. Synchronous tissue, ctDNA and single-cell CTC NGS in one metastatic lobular breast cancer patient. Tissue (from clinical sequencing), and ctDNA driver mutations are shown in orange (variant frequency shown inside box). CTC mutations and their variant frequencies are shown as homozygous (dark green) and heterozygous (light green, including one below pre-specified detection cutoff*). Select copy number alterations (CNSs) 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 sequencing coverage, NA=not available. # TMB of white blood cell (WBC) is defaulted to 0.

Patient	Mutation	Tissue	CTC	ctDNA
104	ESR1 p.D538G		1	0
104	PIK3CA p.E542K	1	0	1
104	PIK3CA p.E542K		1	0
104	PIK3CA p.E365K	0	1	1
104	PIK3CA p.E453K	0	0	1
104	PIK3CA p.E453K		0	1
104	PIK3CA p.E453K		0	1
104	PIK3CA p.E453K		0	1
139	ERBB2 G778ifdup	1		0
44	PIK3CA p.H1047R	1		0
44	ERBB2 G778ifdup	1		0
87	FGFR2 p.272if		1	1
24	FGFR4 p.N535K	1	0	1
24	FGFR4 p.N535K	1	0 *	1
24	PIK3CA p.109if	1	0 *	1
24	PIK3CA p.109if	1	0 *	1
2	ESR1 p.Y537S	1	1	0
17	PIK3CA p.H1047R	1	0	1
17	PIK3CA p.E418K	1	0*	1
113	PIK3CA p.E542K	0*	1	1

Table 4. Actionable Alterations not shared in CTC, ctDNA, tissue.

1 = Detected
0 = Not Detected
* = No Sequencing Coverage
Not Assayed

Figure 4

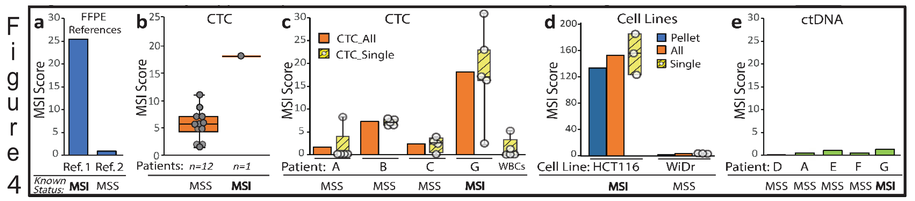
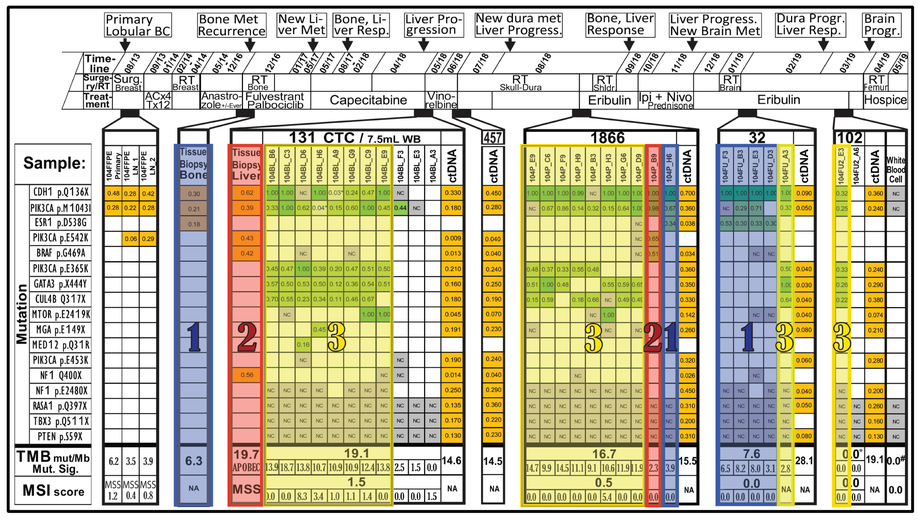


Figure 6. Tissue, ctDNA and single-cell CTC serial NGS in one lobular breast cancer patient undergoing a seq-uence of therapies from primary cancer to metastatic progression and hospice. Synchronous samples are grouped in boxes and time of collection is marked in the timeline by black horizontal bars. 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, cyclophosphamide, T= paclitaxel, Ipi=ipilimumab, Nivo=Nivolumab, NC=no sequencing coverage, NA=not available.
*TMB calculation for 1 lone CTC in a sample is defaulted to 0, #TMB of a white blood cell defaulted to 0.

Figure 6



MI-CTCSeq	10/15	67%
Tissue	11/15	73%
ctDNA	11/15	73%

Table 1. Patients with Actionable Alterations

Mutated Gene	# patients	%
PIK3CA	8	53%
ESR1	4	27%
ERBB2	2	13%
FGFR2	1	7%

Table 2. Genes with Actionable Alterations

# Patients	# Alterations
8/11 (73%)	14/22 (64%)

Table 3. Actionable Alterations not shared between CTC, ctDNA, and Tissue.

CONCLUSIONS

These data suggest the feasibility of the non-invasive interrogation of the circulating tumor cell genome of lobular breast cancer for the detection and monitoring of chemo-, endocrine, precisio 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 heterogeneity and evolution over a sequence of endocrine, chemo- and immuno-therapies.

Our data support continued investigation of the potential utility of single-cell Circulating Tumor Cell profiling to complement ctDNA and tissue 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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