SANUS

Jane Doe

Diagnosis
Metastatic breast cancer

Accession No.
SG-1234567

Date of Birth
02/08/1971

Gender
Female

Biospecimen
Blood ✓
Primary tumor ✓

Sample Analysis
CTC ✓
scDNA✓
scRNA ✓
ctDNA✓

Physician
Benjamin Folger

Institution
Mayo Clinic

FlowCell | BC genes

Tumor specimen
Breast
Chicago Cancer Center,
ABC-123, A2
Collected 3/4/2019
Processed 3/5/2019

Notes
The sample was analysed using a panel of biomarkers from tumor cell identification. The sample is positive for markers HER2, CD47, PIK3/AKT and PD-L1 suggesting a high probability of resistance to Chemo-, and Radiotherapy.

GENOMIC BIOMARKERS

Biomarker profile	CTC DNA	ctDNA	CTC RNA	Primary Tumor
EpCAM		✓		✓
Pan CK				
E-Cadherin				
HER2	✓	✓		✓
PR				
ER		✓		
CD44				✓
CD133	✓			✓
DLG7		✓		
CEA/CA19		✓		
CA-1/9/53			✓	
N Cadherin	✓			
SNAIL		✓		
TWIST				
ZEB	✓		✓	
SLUG		✓		
UPA, PAI		✓		
CD47	✓	✓		✓
BMI				
PD-L1	✓	✓		
BCRA1,2		✓		
ATM			✓	
TP53	✓			
KRAS				
PIK3/AKT	✓		✓	

Somatic	Potentially Actionable	Variant Allele Fraction
PIK3CA	pE545K Missense variant (exon 10) - GOF	45.2%
NF1	pK1036fs Frameshift - LOF	28.5%
MAP2K4	pK114fs Frameshift - LOF	21.2%
TP53	copy number loss	
BRCA2	copy number loss	
STAG2	copy number loss	

Germline	Pathogenic/Likely Pathogenic	Clinical significance
BRCA2	pV220fs Chr13:32903604	

IMMUNOTHERAPY BIOMARKERS

Tumor Mutational Burden	Microsatellite instability Status
4.2m/MB, 64%	Stable

CELL BIOMARKERS

CTC count	**Metastatic potential**
14/ 7.5 mL whole blood	Significant (68%)

Sample CTC images are shown in Figure 2, confirming the epithelial origin of the cells by cytokeratin staining (green). The historical record of CTC enemeration with CellSearch™ biomarkers EpCAM, CK, CD45- is shown in Figure 1. The CTC record indicated onset of relapse and has led to change of therapy from Gemcitabine to Paclitaxel.

DIAGNOSIS AND FDA APPROVED THERAPIES

PARP inhibitor	**Olaparib,** **Talazoparib**	BRCA2 p220fs loss of function BRCA2 copy number LOF consensus HER2 negative breast cancer: NCCN

RECOMMENDATIONS

The tumor showed a loss of heterozygosity in CDKN2A and is responding better to Taxol.
This patient has a pathogenic germline variant in BRCA2 with some loss of heterozygosity, indicating that this is a BRCA2 driven tumor. Therefore, combination with PARP inhibitor therapy may be appropppriate. BRCA genetic counseling is recommended for this patient and potentially affected family members.
RNA analysis is being performed and will be reported in the FlowCell online portal when complete.

SUPPLEMENTARY MATERIALS

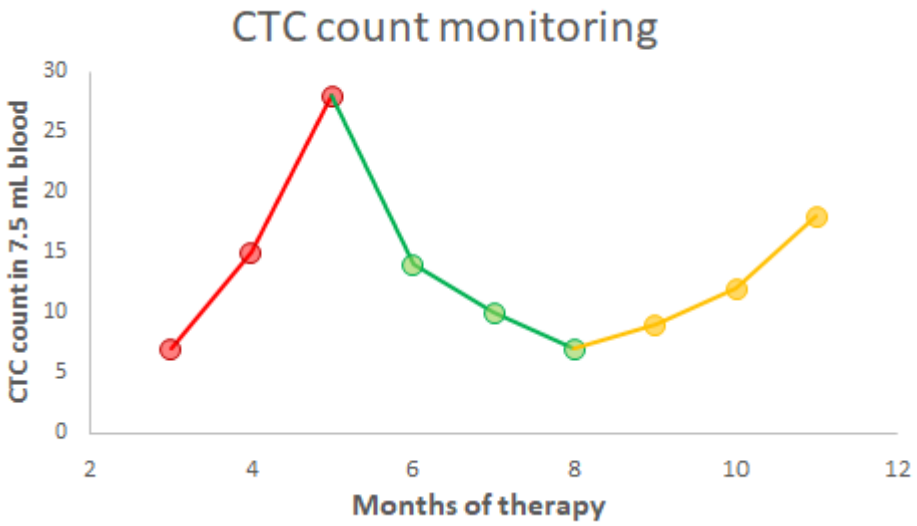

Figure 1: CTC count monitoring. Up to week 5 patient was treated with Gemcitabine (red line), resulting in increase of CTCs. A switch of treatment to Paclitaxel has cut the CTC count in half within a month of therapy change, however, the CTC count is increasing again. This is explainable because BRCA2 driver has not been addressed. A combination therapy of Taxol and PARP inhibitor may be suitable.

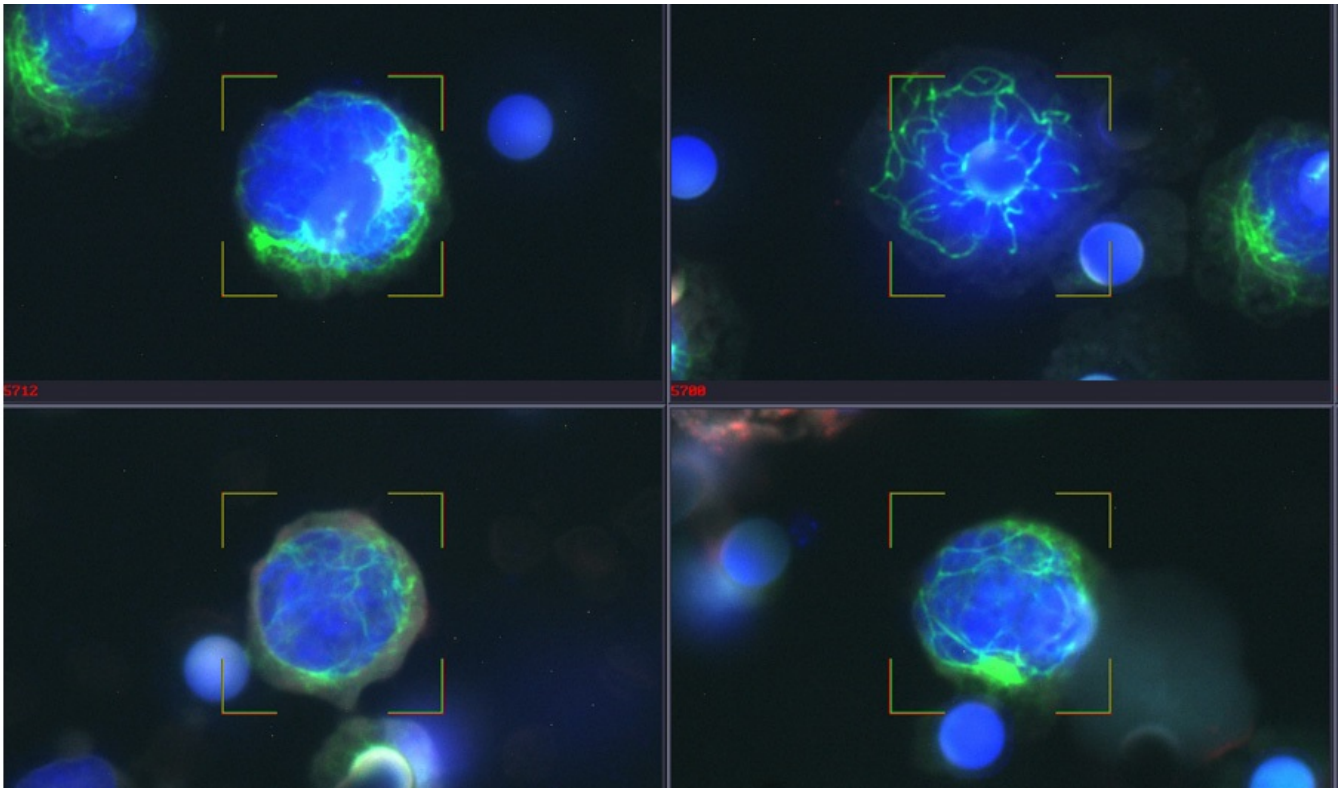

Figure 2: Sample CTC images. Staining of nuclei is with DAPI(Blue) and microtubules with Phalloidin(Green) Mutations in CTCs that correlate with solid tumor progression.

Notes and acronyms for the Distilled Data Report:

- tube standard volue = 7.5 mL
- MP = metastatic potential
- VA = variant allele
- VA = variant allele
- fs = frameshift
- CNL = copy number loss
- GOF = gain of function
- LOF = loss of function

Distilled Data Report

Patient ID: BC1234567
Oncogenes and checkpoints, HER2, CD47, PIK3/AKT, PD-L1
Liquid biopsy: (epCAM, ctDNA), (N-Cadherin, CTC DNA),(ZEB, CTC DNA, CTC RNA),(BRCA1, ctDNA)
CTC count: 14/tube, MP = 68%
CTC phenotype: epCAM clustering
Biopsy: epCAM,CD133, HER2, CD44, CD47, CD133
Mutations: (PIK3CA, pE545K, GOF, VA 45.2%), (NF1, pK1036fs, LOF, VA= 28.5%), (MAP2K4, pK114fs, LOF, VA=21.2%),(TP53, CNL), (BRCA2,CNL), (STAG2, CNL)
CDKN2A heterozygos loss, response to Taxol 68%