Background

- Upregulation of HER2 and androgen receptor (AR) are mechanisms of acquired resistance to endocrine therapy.
- Measurement of these proteins and their localization requires metastatic biopsies, which are costly, invasive, and prone to under-sampling.
- A CTC-based test could expand the clinical utility of these biomarkers.

MBC blood samples were characterized for CTC prevalence, HER2 and AR expression on treatment and at time of disease progression using the Epic Sciences platform.

Methods

A. The Epic Sciences CTC Platform

1) Epic Sciences Platform

2) Single Cell Capture and Sequencing

3) CTC ISOLATION

4) SINGLE CELL WGA

5) LIBRARY PREPARATION

6) WHOLE GENOME SEQUENCING & BIOMARKERS

B. Example Cell images

C. Consort

131 blood samples from 82 MBC Pts:

- 73 ER/PR+; HER2-
- 32 ER/PR-, HER2-
- 20 ER/PR+; HER2+
- 6 ER/PR; HER2+

131 Samples tested for HER2 expression

128 samples tested for AR expression

C. Consort

HER2+ CTCs Are Identified in Tissue HER2- Pts

- HER2+ HER2+ (N = 73)
- HER2+ HER2- (N = 32)
- HER2+ HER2- (N = 20)
- HER2- HER2- (N = 6)

77% (20/27) samples had CTCs, Median CTC/mL = 1.2
46% (20/43) samples had HER2+ CTCs
27% (20/75) samples had AR positive CTCs
9% (20/228) samples had both HER2+ and AR positive CTCs

CTC Counts Are Mostly Independent of MBC Subtypes

- MBC Subtypes by Tissue Histo

- Total CTCs

- Prevalence of Biomarker+CTC in Samples w. CTCs

Case Study: HER2+ and AR+ CTCs Disappear Post Trastuzumab in a Tissue HER2- Pt

- HER2 by Tissue

- CTC HER2+ and AR+ at draw 1

- Started Trastuzumab right after draw 1 and continued for 135 days

- CTC HER2- and AR- at draw 2 (80 days after draw 1)

- Started CDK4+ inhibitor 184 days after draw 1

- Patient deceased 247 days after draw 1

Conclusions

- The majority (77.1%) of metastatic breast cancer patients had detectable CTCs.
- Diverse expression of HER2 and AR were observed and these endocrine therapy resistance markers could potentially guide subsequent therapy selection.
- Prospective evaluation of HER2 and AR on MBC pts’ CTCs as predictive biomarkers of benefit from inhibitors of these proteins is needed.

Single Cell CTC Genomics Confirm HER2 Amplification in Tissue HR+/HER2- Pts

A. Single Cell NGS Workflow

- Isolate Single CTCs

- Subject to Amplification

- Low Pass Hybrid Capture Sequencing

- Quantify Copy Number Alterations (CNAs)

B. Case Study (Pt# 40-01, Tissue IHC ER+/HER2-)

- HER2 Channel

- DAPI

- CD45

1. Single cell genomics identifies CTCs with HER2 gene amplification
2. 63% of CTCs in this patient had HER2 protein expression.
3. Large scale transitions: a surrogate of chromosomal instability; it measures the number of chromosome breaks between adjacent regions of at least 10 Mb.

Heterogeneous Genome Profiles Are Observed Across Multiple Patients

- Chromosomes

- Y Chromosome

- X Chromosome

- 19 CTCs from 6 pts (1-6 CTCs per pt) were single cell sequenced for copy number alterations (CNA)

- CTC genome complexity was observed for multiple patients

- Common breast cancer related gene copy number gain (FOXP1, ERBB2) and loss (PTEN, CDH1) were observed in all patients.