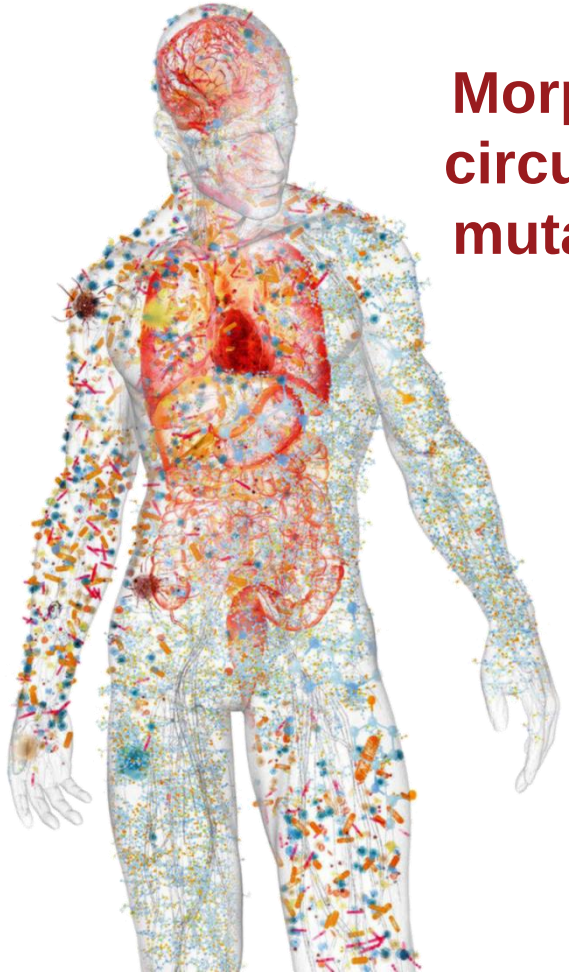

Morphologic and genomic characterization of circulating tumor cells in patients with ERBB2 mutant HER2 non-amplified metastatic breast cancer treated with neratinib

- **Eliminating the unknown of cancer**
- **Giving confidence in cancer care**

Stephanie N. Shishido, R. Masson, L. Welter, L. Xu, A. D'Souza, D. Spicer, Y. Jiang, L. Eli, R. Cutler, J. Lu, J. Hicks, and P. Kuhn

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ERBB2 mutant HER2 non-amplified breast cancer

- ***ERBB2* mutations** → aggressive breast cancer phenotypes
  - Extremely rare in the absence of gene amplification (2-4% of all cases).
  - Patients do not respond to HER2+ targeting standard of care treatment plans.

- **Neratinib**: an irreversible HER2/EGFR tyrosine kinase inhibitor
  - Clinical activity shown on heavily pre-treated *ERBB2* mutant breast cancer patients.
  - Combinational treatment of Neratinib with chemotherapy has shown a response rate of 55% for HER2+ breast cancer patients.

- Using the **HD-SCA workflow** we have evaluated the clinical response to Neratinib/Fulvestrant combinational treatment in relation to enumeration and characterization of circulating tumor cells (CTCs), as well as genomic analysis of the cfDNA.
  - 5 postmenopausal patients with metastatic *ERBB2* mutant/non-amplified breast cancer
    - Average of 5.4 lines of therapy prior to enrollment
  - Peripheral blood samples collected at multiple time points
  - Single cell genomic analysis by copy number variation (CNV) profiling and ERBB2 targeted mutational analysis
  - cfDNA genomic analysis by CNV and targeted mutational profiling using the OncoMine panel

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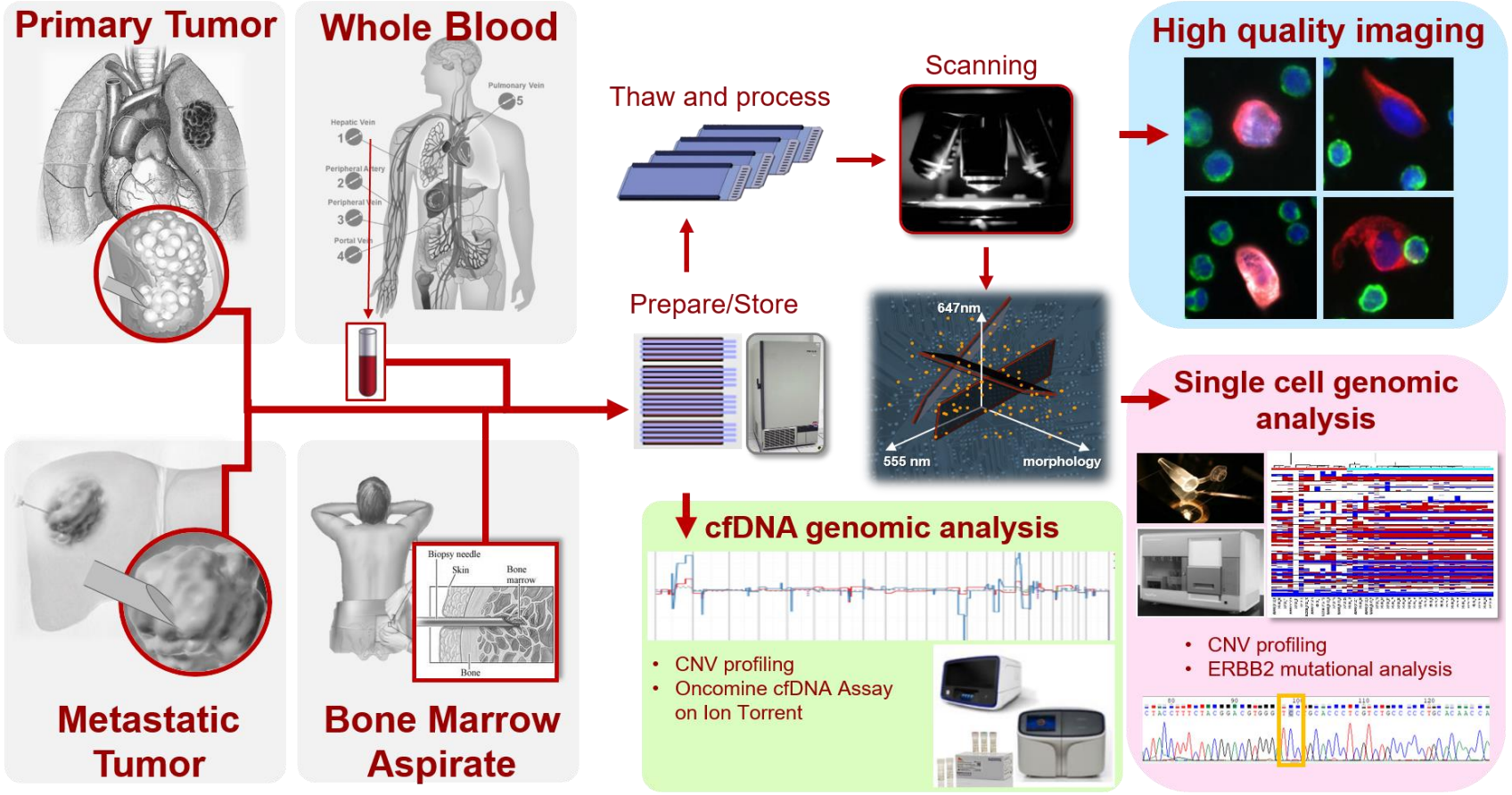
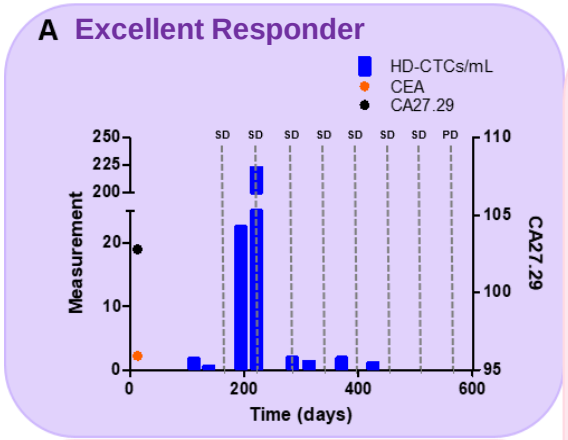
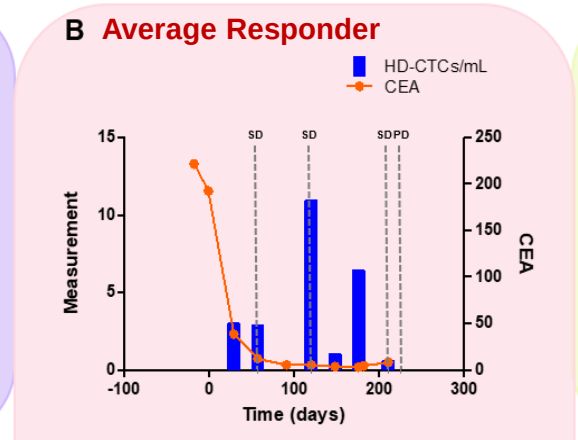
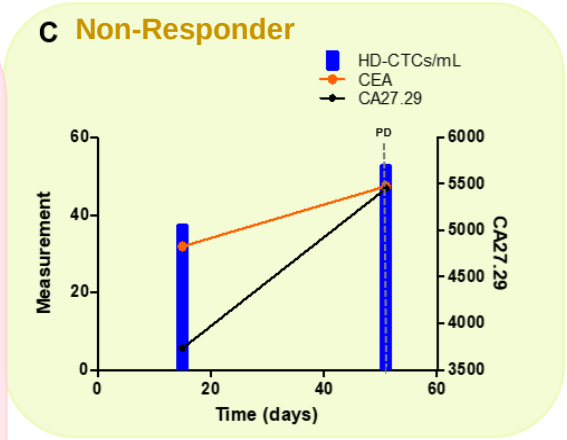
The HD-SCA workflow

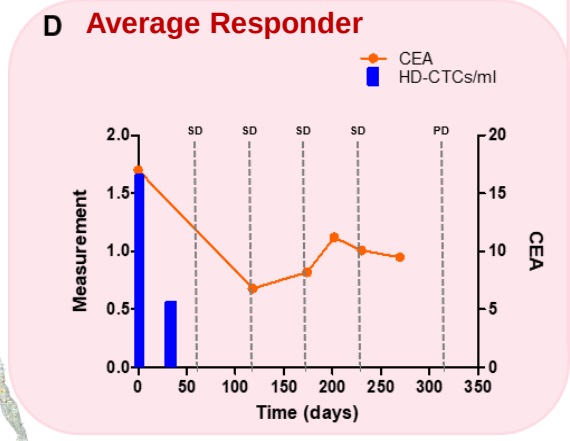


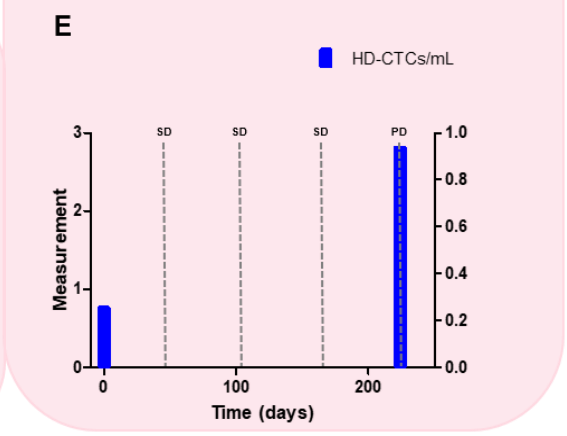
ERBB2 mutation and clinical timelines

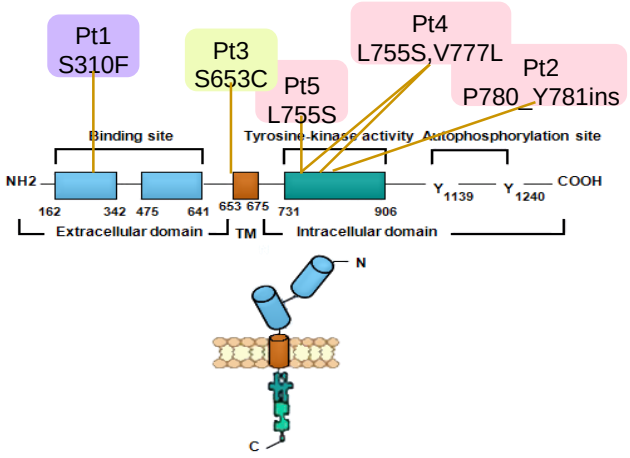




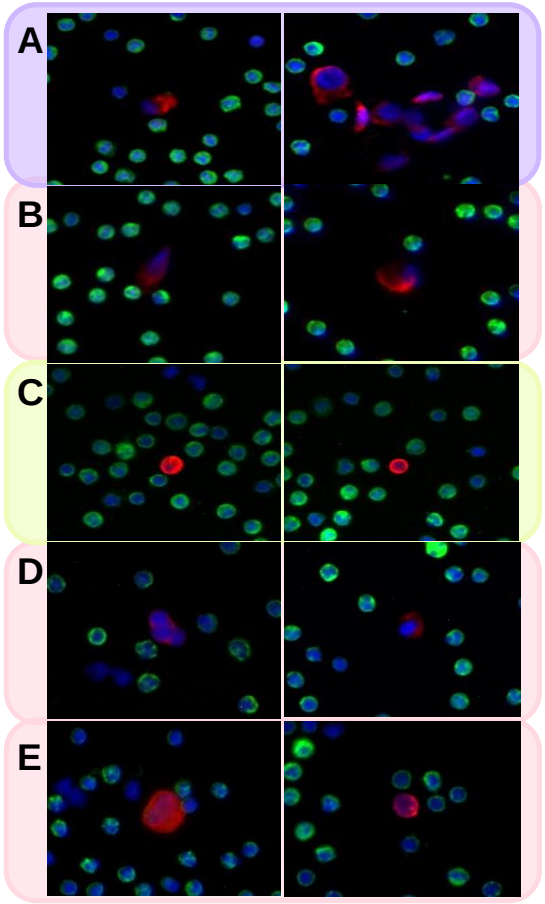








HD-CTC morphometrics per patient



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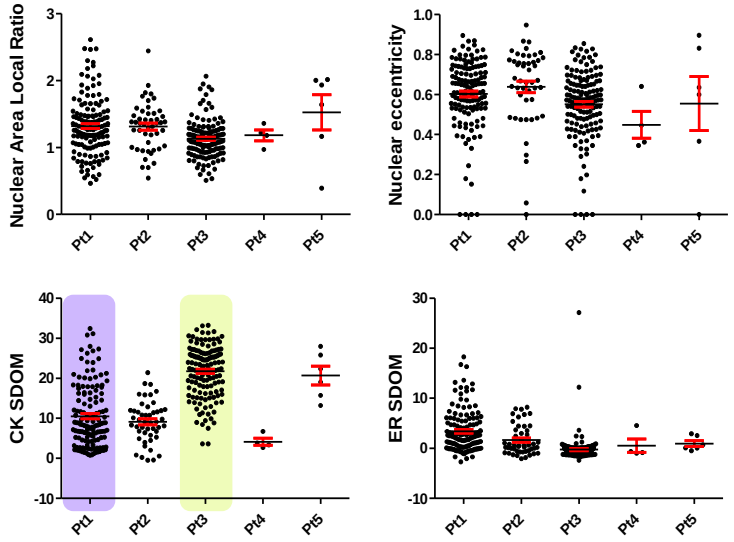


Average Responder
Excellent Responder
Non-Responder

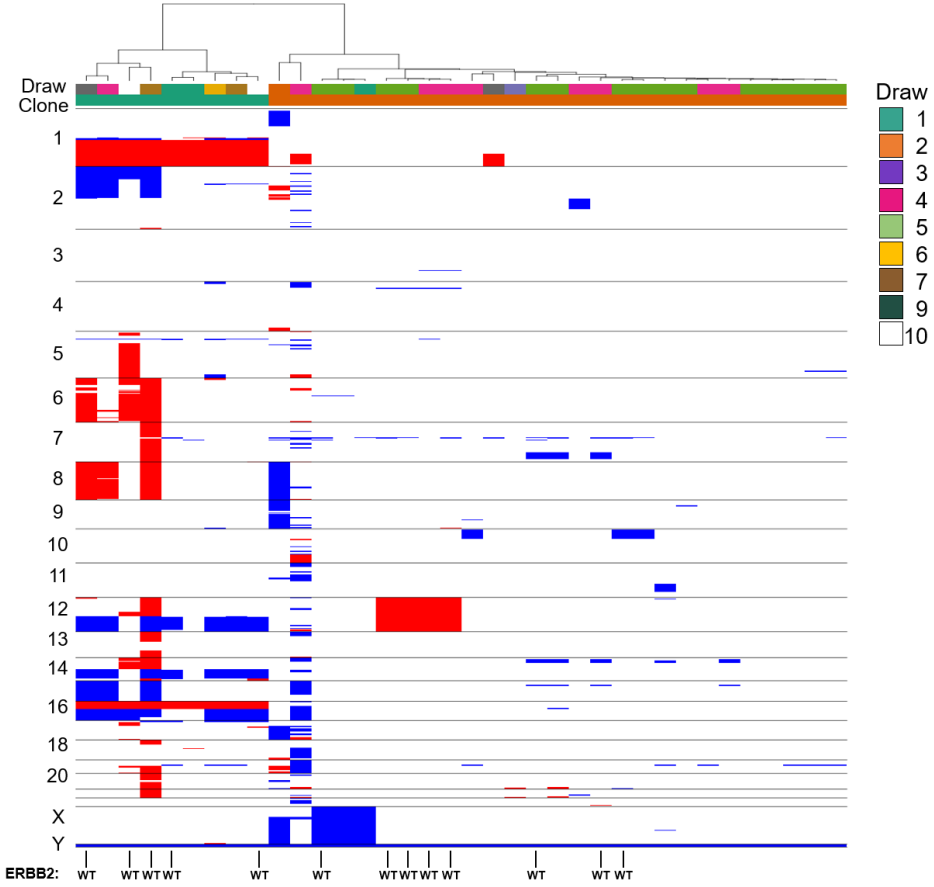
- Pt 4 and 5 had minimal number of cells detected limiting our observations.
- Pt 1 and 2 have a highly heterogeneous population of cells
- Pt 3 had a morphologically distinct population of HD-CTCs
  - More circular nuclei with higher CK expression

A-E) 40x images of HD-CTCs detected per patient.

F) Distribution of HD-CTC morphometric parameters by patient.

Single cell genomic analysis

- **Excellent Responder: Pt1**

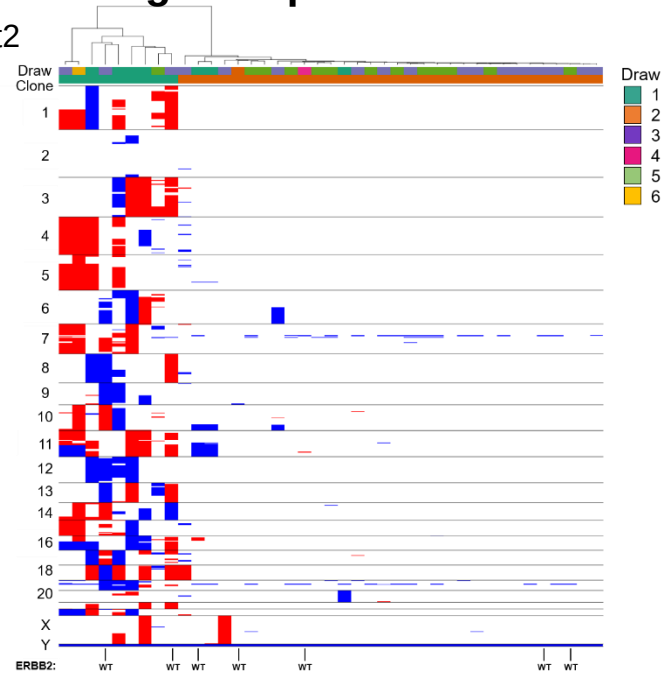


- Tumor Pathology Report: **ERBB2 S310F**, ARID1A Q479*, CDH1 l581FS*1, NOTCH2 (SEC22B-NOTCH fusion)
- CNV analysis of 54 HD-CTCs, 24 cells identified to have genomic alterations.
  - Subclones persist from draw 4 to 10.
  - 2 unique subclones identified in draw 5.
  - Deep alterations may be due to homologous recombination, leading to amplification or deletion of one arm of the chromosome.
- ERBB2 SNV for S310F: 13/13 WT

Single cell genomic analysis

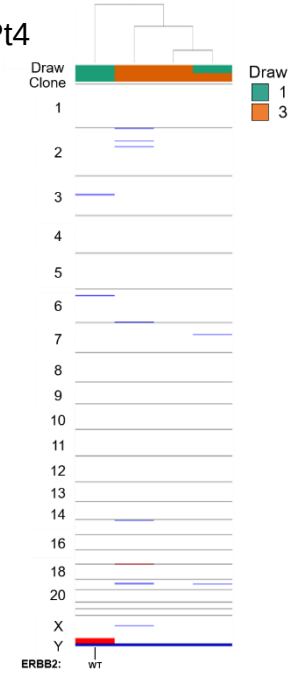
- **Average Responders:**

Pt2



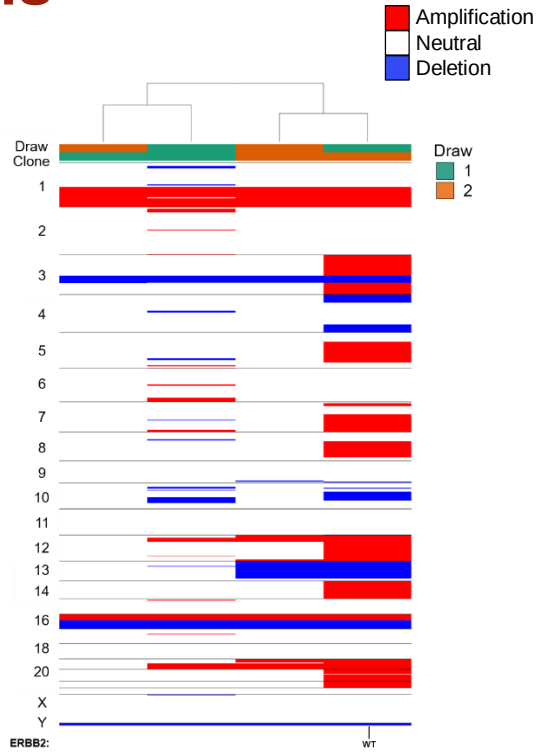
- Tumor Pathology Report: **ERBB2 (P780_Y781ins)**, ARID1A (Q1172*), CDH1 (L585fs*4), NUP93 (E14K)
- 9/21 HD-CTCs have genomic alterations
- Lack of clonality
- ERBB2 SNV for P780_781ins: 8/8 WT

Pt4



- Tumor Pathology Report: **ERBB2 (L755S, V777L),** PIK3CA (H1047A)
- Lack of genomic alterations
- ERBB2 SNV for V777L: 1/1 WT

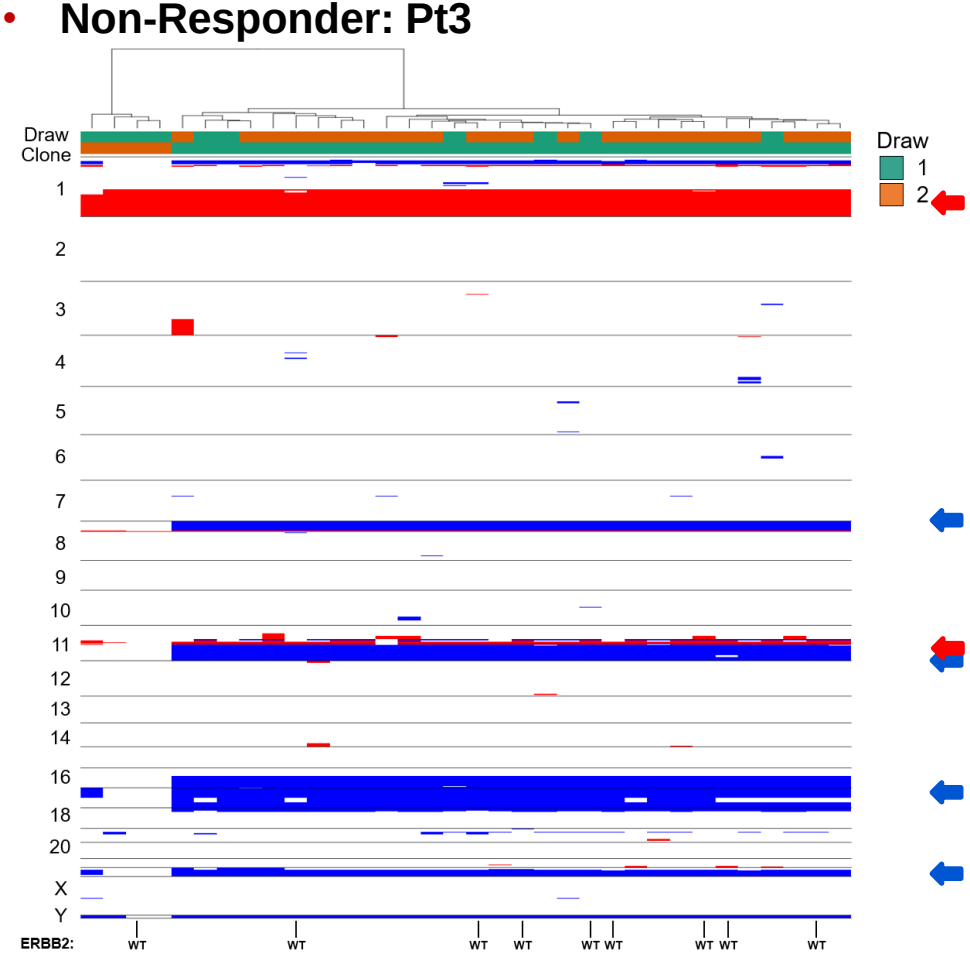
Pt5



- Tumor Pathology Report: **ERBB2 (L755S)**
- Subclonal population of cells
- ERBB2 SNV for L755S: 1/1 WT

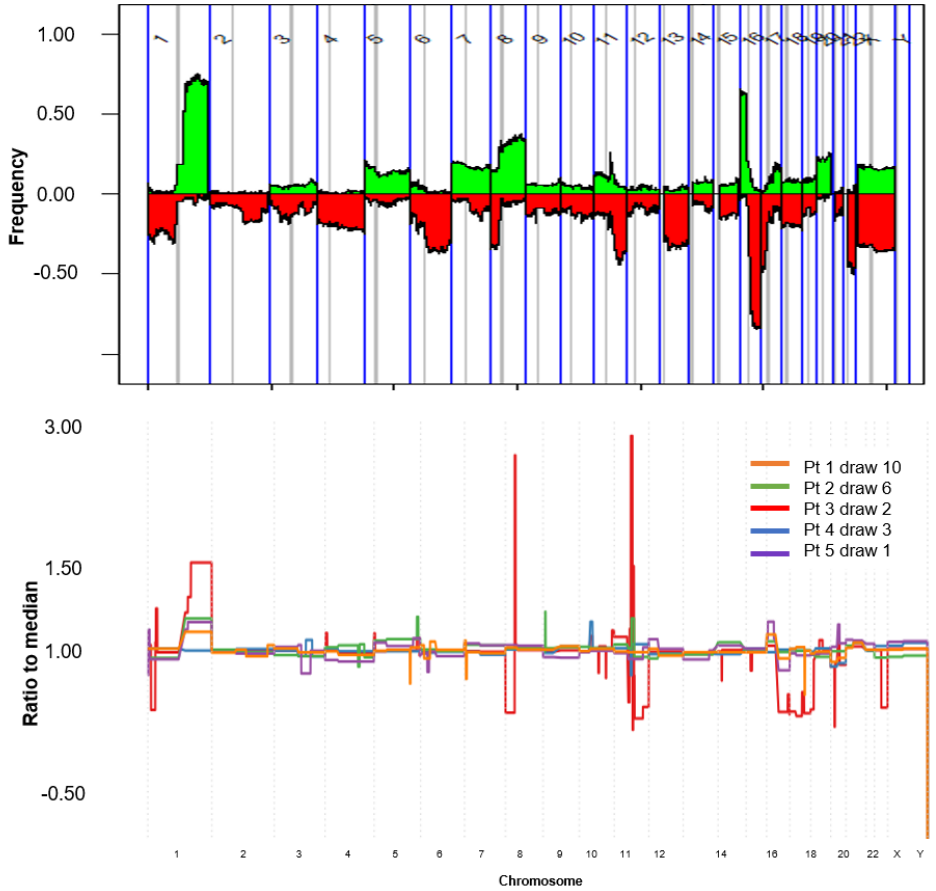
Single cell genomic analysis

- **Non-Responder: Pt3**



- Tumor Pathology Report: **ERBB2 (S653C),** PIK3CA (E545K), PTPN11 (E76K)
- Genomic analysis of 22 HD-CTCs isolated from Patient 3
- Clonal population of cells.
- The cell cycle appears to be completely unconstrained.
  - **FGFR1** amplified
  - **Cyclin-D** amplified
  - Heterozygous loss of chromosome 11q containing **CHK1** and **ATM**
  - Loss of **P53**
  - Loss on chromosome 22 containing **CHK2**.
- ERBB2 SNV for S653C: 9/9 WT

cfDNA genomic analysis



- CNV profile for luminal A tumors from The Cancer Genome Atlas (TCGA).
- The cfDNA CNV profiles match the classic Luminal profile
- The same clonal architecture identified in the HD-CTCs was also detectable in the cfDNA, with a greater tumor fraction present in follow up sampling for Patients 1, 2, 3, and 5.
- The cfDNA from Patient 4 samples had a low to nondetectable tumor fraction, suggesting the tumor DNA was washed out by normal cellular DNA.

cfDNA OncoMine

Excellent Responder: Pt1

Gene	Draw8	Draw10
ERBB2	-	**S310F**
TP53	R213* (**0.47%**)	R213* (**4.5%**)

- Increase in the fraction of detectable TP53 mutation during treatment
- Identification of ERBB2 mutation from tumor pathology report

Average Responder: Pt2

Gene	Draw6
ERBB2	**G776V**

Average Responder: Pt4

Gene	Draw1	Draw4
ERBB2	L755S, V777L	L755S, V777L
PIK3CA	H1047R	H1047R
TP53	H365fs	-

Average Responder: Pt5

Gene	Draw1
ERBB2	L755S

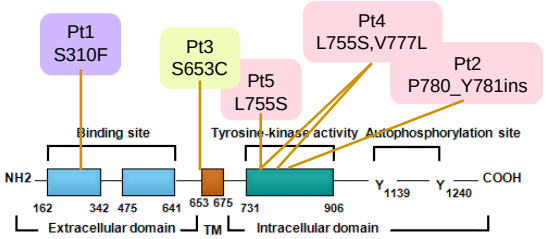
- New ERBB2 mutation identified in Pt2
- Analysis confirms the ERBB2 mutation from the tumor pathology report for Pt 4 and 5

Non-Responder: Pt3

Gene	Draw1	Draw2
ERBB2	-	**L755S**
ESR1	E380Q, Y537N	E380Q
PIK3CA	E545K (**2.7%**)	E545K (**30%**), **E726K**
TP53	R248Q	R248Q, **G245D**

- Increase in the fraction of detectable mutations during treatment
- Identification of new mutations in ERBB2, PIK3CA, and TP53

- *Oncomine panel does not detect mutations in transmembrane domain of ERBB2, potentially explaining the lack of the S653C mutation identified in the tumor.*



Summary

- The HD-SCA workflow provides a comprehensive view of the complete liquid biopsy from metastatic breast cancer patients with ERBB2 mutations

- The HD-SCA workflow may be used as a prognostic tool for therapeutic response to directly impact treatment decisions.
  - Stratify patients into treatment arms
    - Patients with concurrent aberrations in cell cycle checkpoints driven by TP53 mutations are associated with a lack of clinical benefit.
    - Monitor tumor heterogeneity by identifying the frequency of mutations at a single cell level
  - Monitor treatment response
    - Patients with stable disease had detectable CTCs that are morphologically and genomically heterogeneous
    - cfDNA analysis provides a general overview of the cellular population.

- Identified genomic aberrations in single CTCs and the cfDNA that may contribute to disease progression on Neratinib/Fulvestrant therapy.

Acknowledgements

Janice Lu

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