Diverse Inter- and Intra-patient Circulating Tumor Cells (CTCs) Phenotypic Heterogeneity Identified Across Multiple Metastatic Breast Cancer (mBCa) Cohorts

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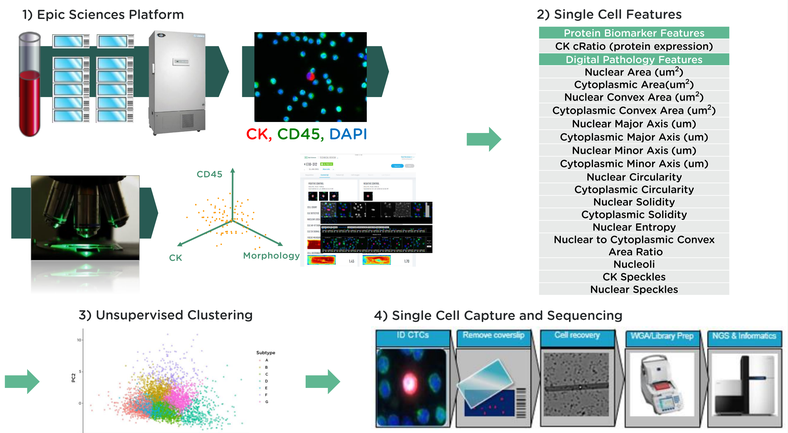
Background

- There is an unmet need for biomarkers to guide Tx selections for mBCa patients among choice of hormone therapies, targeted therapies and chemotherapy.
- We previously developed a quantitative measure of CTC phenotypic heterogeneity in mCRPC, and found that high heterogeneity patients are associated with relatively better overall survival on chemotherapy, while low heterogeneity patients have better survival with AR signaling inhibitors (Scher et al. 2017 Cancer Research).
- Here, the same methodology was applied to mBCa patient cohorts to ascertain the feasibility of CTC heterogeneity analysis in mBCa.

Methods

- 198 blood samples from mBCa patients were processed for CTC analysis utilizing the Epic Sciences platform. Following enumeration, multi-dimensional phenotypic characterization analysis was performed utilizing protein expression and digital pathology features.
- Features from each CTC (5158 CTCs from 198 patients, 107 HR+, 14 HER2+, 8 HR+/HER2+, 69 TNBC) were clustered using unsupervised approach (K-means) and the optimal number of clusters was determined using the elbow method with greater than 85% variance taken into account. Shannon index was used to assess the intra-patient heterogeneity. 157 CTCs from various classified CTC subtypes were single cell sequenced for copy number variations.

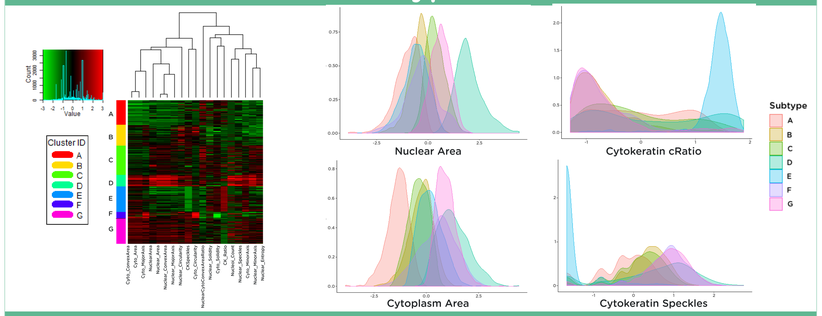


Schematic of Epic CTC Platform CTC enumeration, morphology, biomarker analyses and single cell sequencing workflow:

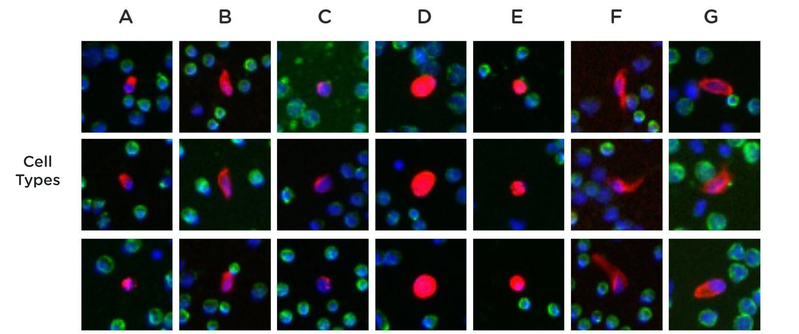
1. Nucleated cells from blood sample placed onto slides and stored in a -80°C biorepository. Slides are stained with cytokeratin (CK), CD45, DAPI and scanned. CTC candidates are detected by a multi-parametric digital pathology algorithm followed by human reader confirmation of CTCs and quantification of biomarker expression.
2. CTCs are segmented within the DAPI and CK channels and single cell features are extracted.
3. CTCs undergo Principle Component Analysis (PCA) removing noise and redundant dimensions, and weighing features with more variance. Machine learning clustering algorithms found 7 CTC subtypes from macro trends in high-dimensional biomarkers across all CTCs from all samples in cohort, and assigned each CTC to 1 of 7 subtypes. Heterogeneity is quantified by counting CTCs per "Cell Type" in each sample, then using a standard Shannon Index to quantify CTC phenotypic diversity per patient sample.
4. Single cells are identified, relocated, captured, whole genome amplified (WGA), library prepared and low pass whole genome sequenced for Large Scale Transitions (LST, a surrogate of chromosomal instability) and gene copy number alterations (CNA) (Greene et al. 2016 Plos One).

References:
Scher HI, et al. Phenotypic Heterogeneity of Circulating Tumor Cells Informs Clinical Decisions between AR Signaling Inhibitors and Taxanes in Metastatic Prostate Cancer. Cancer Res. 2017 Oct 15;77(20):5687-5698
Greene SB, et al. Chromosomal Instability Estimation Based on Next Generation Sequencing and Single Cell Genome Wide Copy Number Variation Analysis. PLoS One. 2016 Nov 16;11(11):e0165089.

Morphological Features Associated with CTC Subtypes



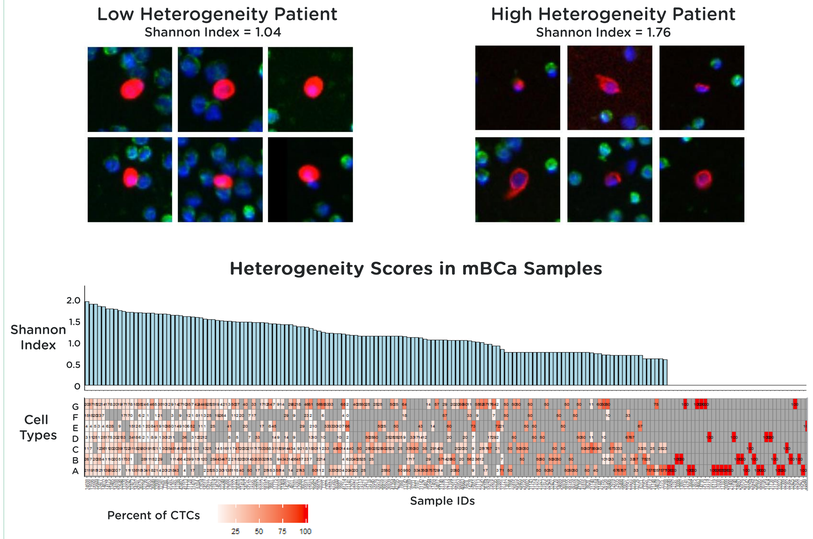
Example Cell Images of CTC Subtypes



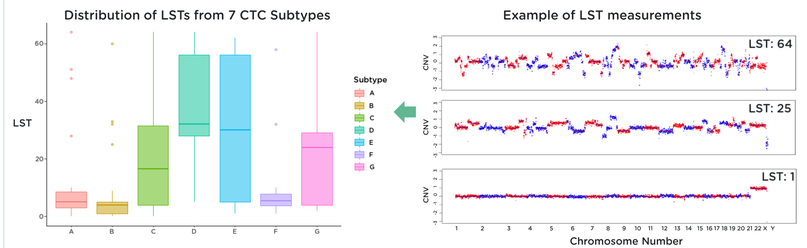
CTC Subtypes are Associated with Breast Cancer Pathological Types

- HR+ patients have relatively more cell type C and G.
- Her2+ patients have relatively more cell type B and G.
- HR+/Her2+ patients have relatively more cell type E.
- TNBC patients have more heterogeneous cell subtypes.

High CTC Phenotypic Heterogeneity is Common in mBCa Blood Samples



Phenotypic CTC Subtypes are Associated with Distinct Genomic Alterations



% of CTCs with Gene Copy Number Alterations

	A	B	C	D	E	F	G
n of CTCs	23	21	22	31	33	12	15
ERBB2 Gain	0.04	0.00	0.27	0.29	0.33	0.08	0.13
FGFR1 Gain	0.52	0.19	0.27	0.39	0.67	0.17	0.40
BRCA1 Loss	0.00	0.05	0.09	0.23	0.12	0.00	0.13
BRCA2 Loss	0.13	0.14	0.18	0.32	0.36	0.17	0.13
CDH1 Loss	0.13	0.29	0.50	0.84	0.52	0.25	0.47
PTEN Loss	0.13	0.19	0.00	0.03	0.03	0.17	0.27
TP53 Loss	0.17	0.24	0.36	0.90	0.55	0.25	0.33

- LST: Large Scale Transitions. A measurement of *n* of breakpoints for segments of over 10 million base pairs.
- Cell subtype C, D, E and G had higher LSTs than rest three subtypes.
- Unique gene copy number alterations were observed for 7 CTC subtypes.

Conclusions

- Diverse inter- and intra-patient phenotypic CTC heterogeneity is observed across multiple cohorts with specific genome profiles detected for different CTC subtypes.
- We seek to determine if patients with low heterogeneity might be better candidates for hormonal and targeted therapy.
- Studies linking heterogeneity to therapeutic efficacy and patient outcome are ongoing.