Diverse Inter- and Intra-patient Circulating Tumor Cells (CTCs) Phenotypic Heterogeneity Identified in Metastatic Breast Cancer (MBC) Patients

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Background

- There is an unmet need for biomarkers to guide the treatment selection in MBC
- We previously described phenotypic CTC heterogeneity in metastatic castrate resistant prostate cancer (mCRPC), and showed that patients with high CTC heterogeneity have improved OS with chemotherapy, while patients with low CTC heterogeneity have longer OS with AR inhibitors (Scher et al. 2017 Cancer Research)
- Here, the same methodology was applied to evaluate the feasibility of CTC heterogeneity analysis in MBC patients

Methods

114 blood samples from MBC patients were processed for CTC analysis with HER2 and AR assay utilizing the Epic Sciences platform

1) Epic Sciences Platform

2) Single Cell Features

3) Unsupervised Clustering

4) Single Cell Capture and Sequencing

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 cytokertatin (OK), CD45, DAPI and one of the biomarkers (HER2 and AR), and scanned. CTC candidates are detected by a multiparametric digital pathology algorithm followed by human reader confirmation.
2. Following enumeration, CTCs were segmented and 20 morphological features and protein expression data was extracted.
3. CTCs undergo Principle Component Analysis (PCA) removing noise and redundant dimensions, and weighting features with more variance. Unsupervised approach (K-means) found 7 CTC subtypes from macro trends in high-dimensional biomarkers across all CTCs. Shannon index was used to score inpatient CTC heterogeneity.
4. Single cells are identified, relocated, captured, whole genome amplified (WGA), library prepared and low pass whole genome sequenced.

Observed CTC Phenotypic Subtypes by Clustering

CTC Subtypes Associated with Diagnostic Subtypes

- Tissue HER2+ pts were more enriched in cell type E
- Tissue HER2+ pts were more enriched in cell type G

Conclusions

- Diverse inter- and intrapatient phenotypic CTC heterogeneity was observed in this metastatic breast cancer cohort, consistent with findings seen in mCRPC and other MBC cohorts (Beverly H et al, ASCO 2018)
- Studies linking degree and patterns of CTC heterogeneity to therapeutic outcomes are ongoing

References: