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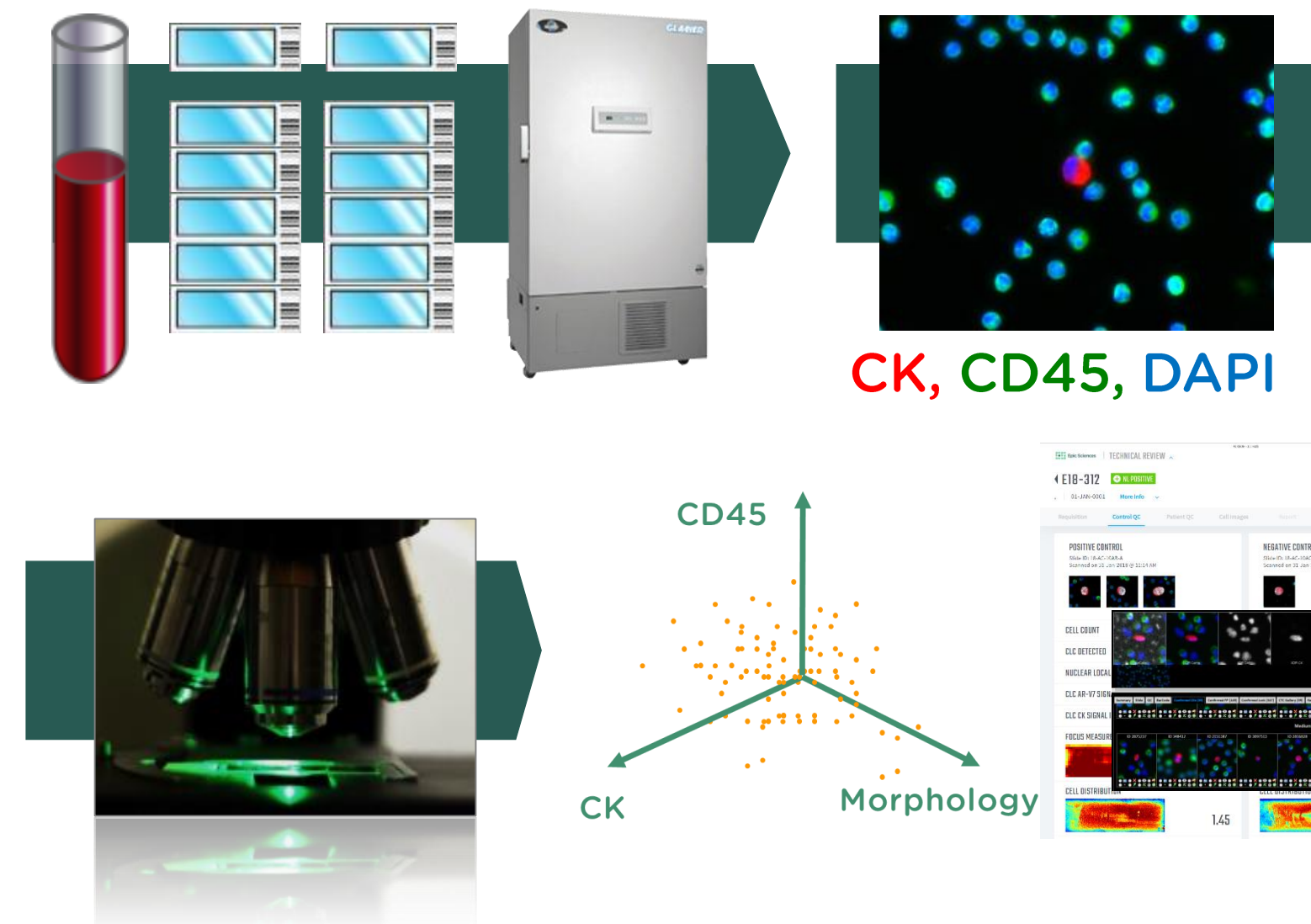
Background

- 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.

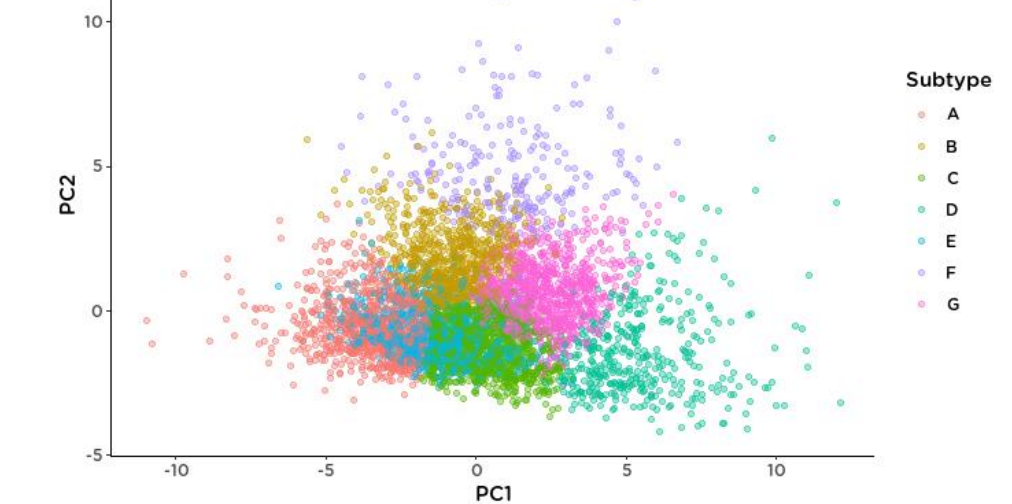
1) Epic Sciences Platform



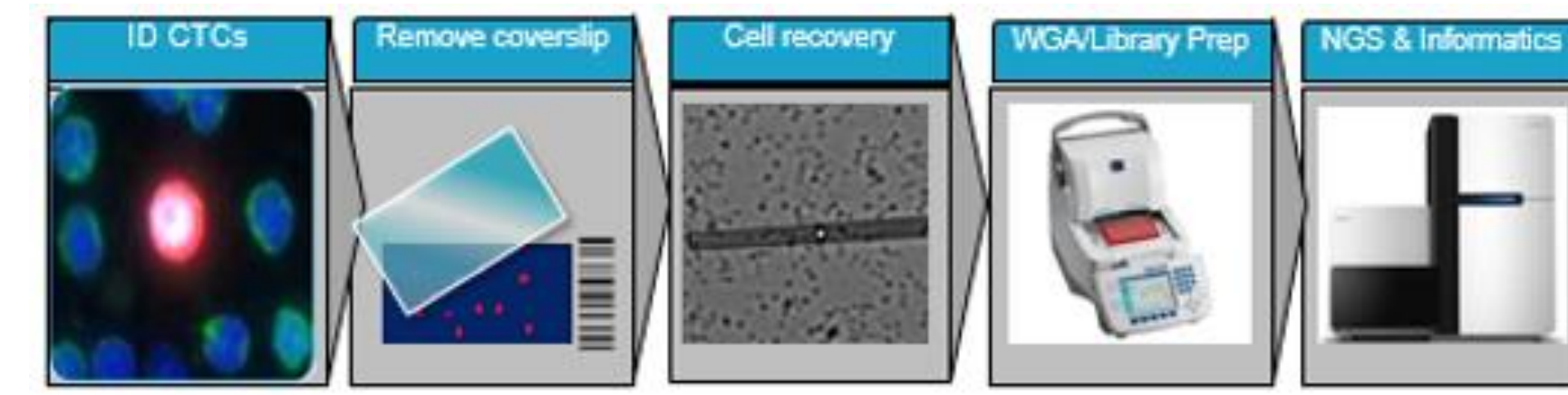
2) Single Cell Features

Protein Biomarker Features
CK cRatio (protein expression)
Digital Pathology Features
Nuclear Area (um ²)
Cytoplasmic Area(um ²)
Nuclear Convex Area (um ²)
Cytoplasmic Convex Area (um ²)
Nuclear Major Axis (um)
Cytoplasmic Major Axis (um)
Nuclear Minor Axis (um)
Cytoplasmic Minor Axis (um)
Nuclear Circularity
Cytoplasmic Circularity
Nuclear Solidity
Cytoplasmic Solidity
Nuclear Entropy
Nuclear to Cytoplasmic Convex Area Ratio
Nucleoli
CK Speckles
Nuclear Speckles

3) Unsupervised Clustering



4) Single Cell Capture and Sequencing

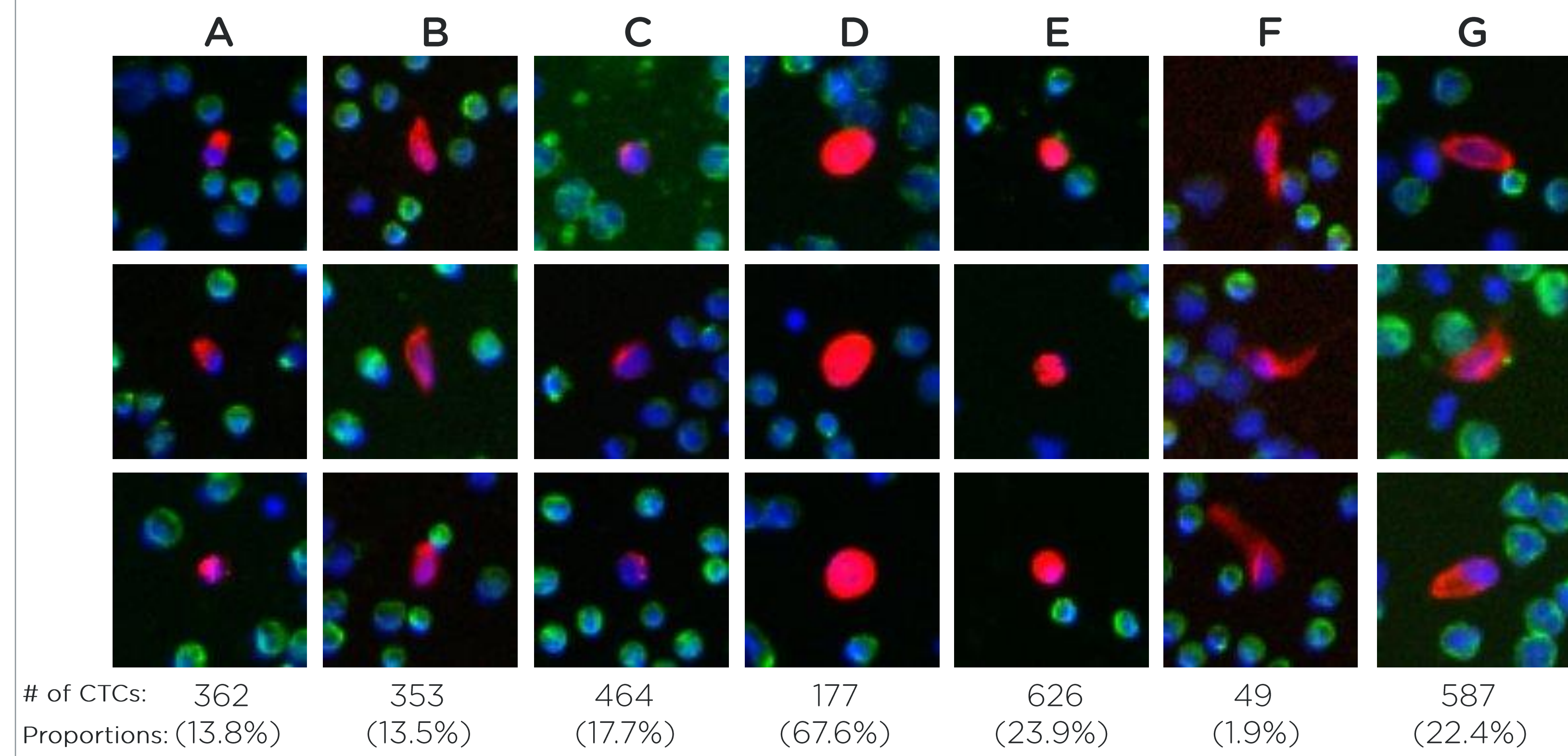


Schematic of Epic CTC Platform CTC enumeration, morphology, biomarker analyses and single cell sequencing workflow:

1. Nucleated cells from blood sample placed onto slides and stored in a -80°C biorepository. Slides are stained with cytokeratin (CK), CD45, DAPI and one of the biomarkers (HER2 and AR), and scanned. CTC candidates are detected by a multi-parametric 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 weighing 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 intra-patient CTC heterogeneity
4. Single cells are identified, relocated, captured, whole genome amplified (WGA), library prepared and low pass whole genome sequenced.

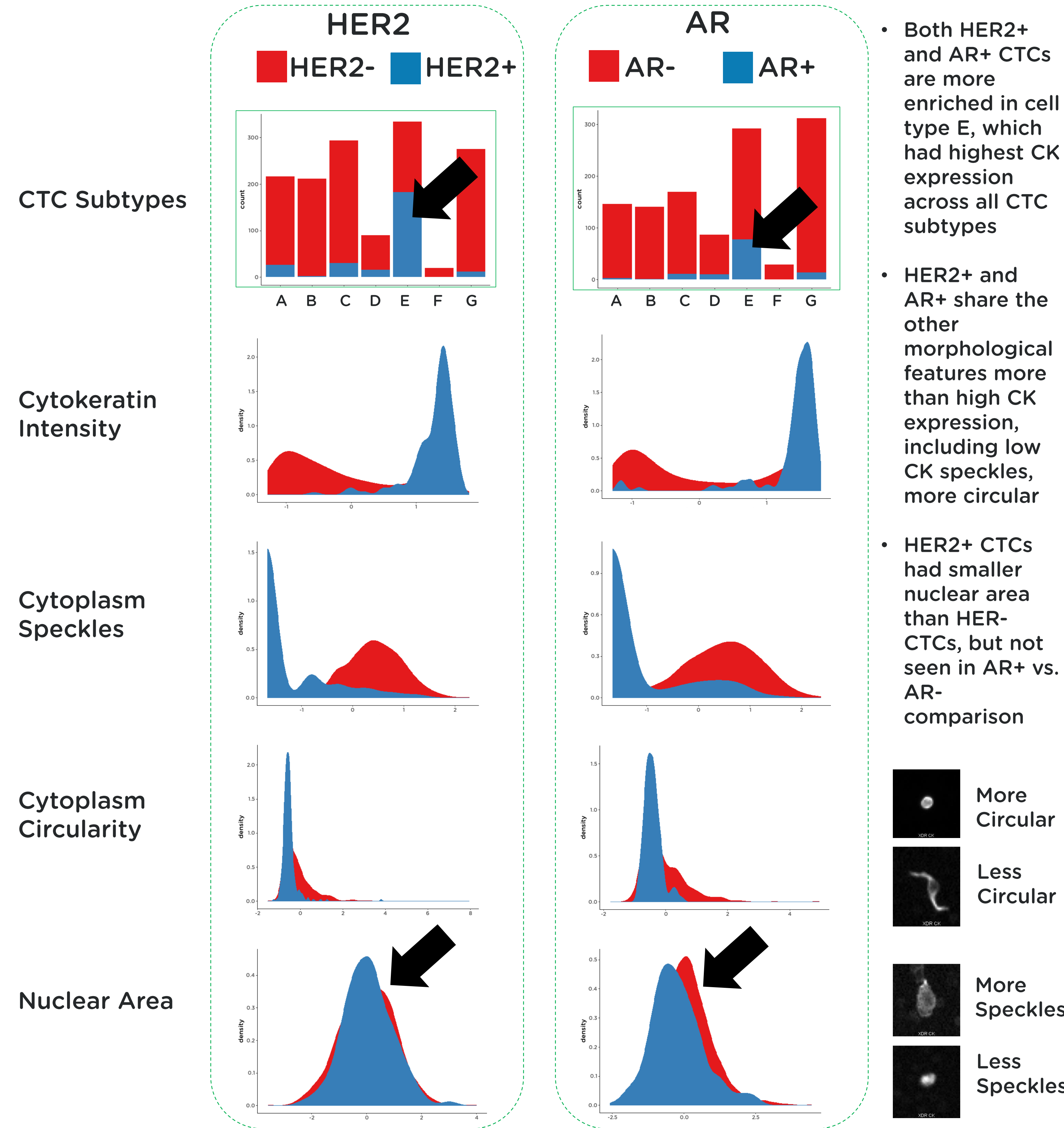
References:
 Scher HI, et al. Phenotypic Heterogeneity of Circulating Tumor Cells Informs Clinical Decisions between AR Signaling Inhibitors and Taxanes in Metastatic Prostate Cancer. *Cancer Res.* 2017 Oct 15;77(20):5687-5698
 Greene SB, et al. Chromosomal Instability Estimation Based on Next Generation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

Observed CTC Phenotypic Subtypes by Clustering

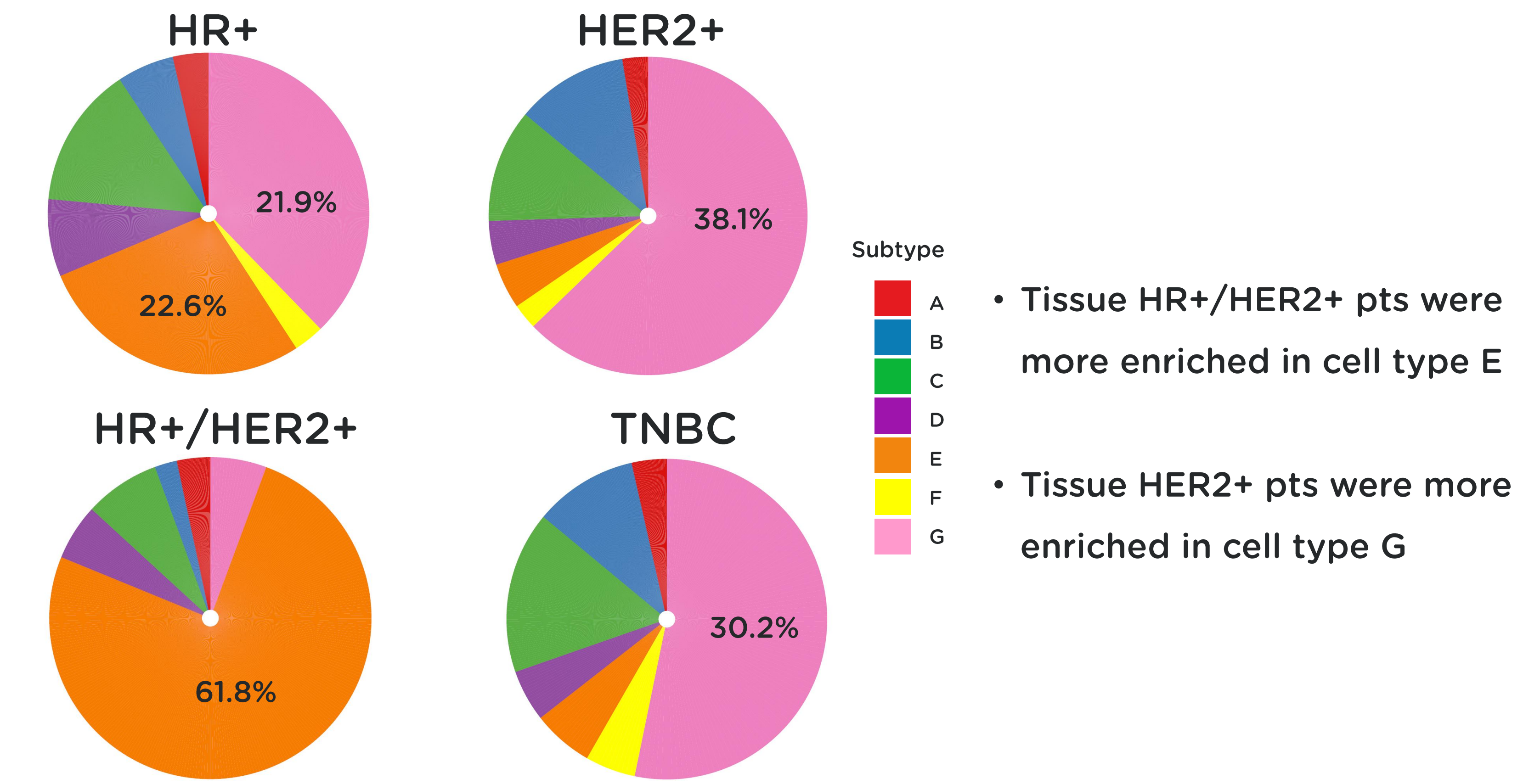


Identification of 7 Distinct CTC Phenotypes

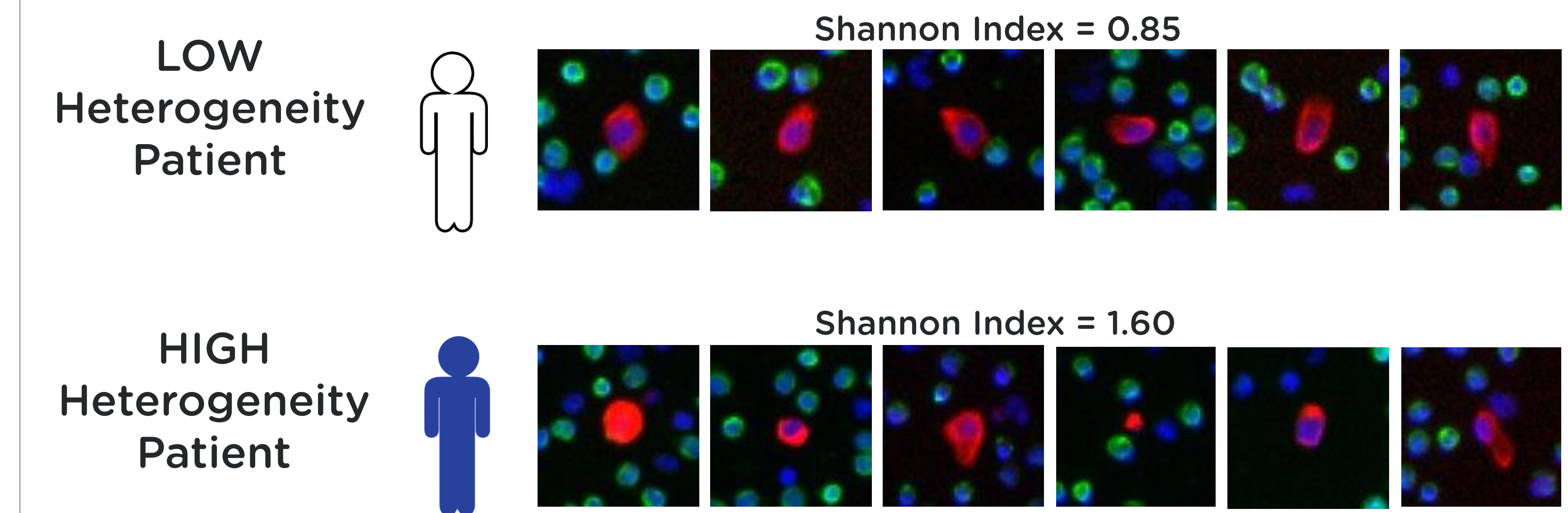
HER2+ and AR+ CTC Morphological Characteristics



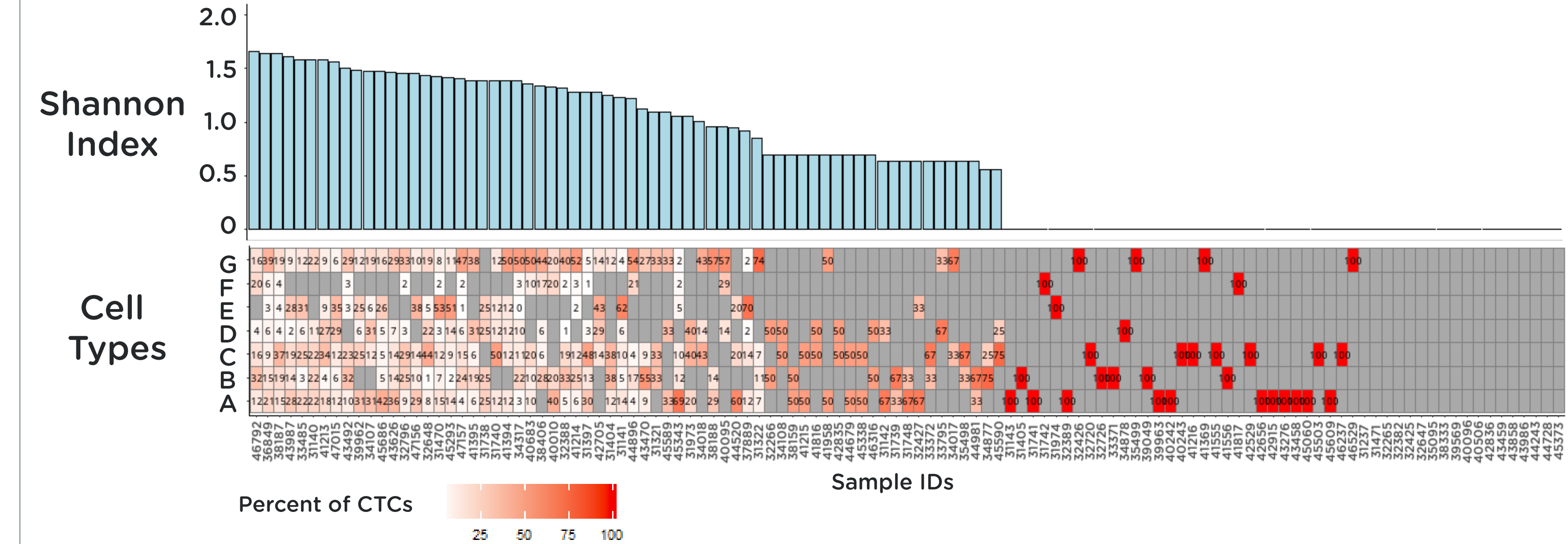
CTC Subtypes Associated with Diagnostic Subtypes



Diverse CTC Heterogeneity Observed in MBC Pts



Phenotypic Heterogeneity Scores in MBC Pts



Conclusions

- Diverse inter- and intra-patient 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