Clinical Validation of CTC Subtype Frequency to Prognosis Overall Survival (OS) in Metastatic Castrate Resistant Prostate Cancer (mCRPC) Patients

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Background

- CellSearch® clinical sensitivity is limited in mCRPC by exhibiting low CTC counts in many patients despite poor outcomes.
- CellSearch® detects a narrow phenotype of CTCs: EpCam(+), CD45(-), CD45(+); which could reduce detection sensitivities.
- The Epic Sciences platform does not use enrichment, and detects an expanded range of CTC histology, including EpCam(+), CD45(-), CTCs Clusters, Apoptotic CTCs, and expanded CTC subtypes.
- Expanded CTC subtypes require clinical validation as individual features.
- To assess the clinical value of CTC subtypes, CTC burden of expanded CTC subtypes was associated to overall survival and evaluated in a large cross-sectional cohort of modern mCRPC practice.

Methods

- 221 blood samples from 175 unique patients were collected prior to initiating Androgen Receptor (AR) directed (n = 175) or placebo (n = 72) therapy for mCRPC.
- Samples were collected in CellSearch® and Epic Sciences platforms to enumerate EpCam(+), CD45(-), CTCs Clusters, Apoptotic CTCs, and Small CTCs (Figure A).
- Patients were followed for up to 13 µm.
- Paired CellSearch® blood draws were processed at MSKCC Clinical Laboratory per manufacturer recommendations. CellSearch® counts were capped at 20 CTCs per tube (from 7.5 µL of blood). For comparison, CellSearch® and Epic Sciences counts were normalized per milliliter; capped at 26.7 µL. Pairwise CellSearch® and Epic Sciences traditional CTC counts were collected from 175 patient samples.

CTC Histological Subtypes Detected on the Epic Sciences CTC Platform

1) Nucleated cells from blood sample placed onto slides and stored in 300 µL fixative (ideal)
2) Slides stained with cytokeratin, CD45, DAPI, and/or CD45
3) Slides scanned
4) CTC candidates detected by a high-parameter content digital pathology algorithm
5) Human reader confirmation of CTCs & quantitation of biomarker expression

CTCs enumerated in this study encompass several histological types:
- Traditional CTCs are detected as single cells positive for cytokeratin expression (Figures B-G).
- Some EpCam(-) CTCs are smaller than surrounding white blood cells (Figures C-G).
- EpCam(-) CTCs have distinctive nuclear malignant features and the presence of all morphological (Figure D-I).
- CTC Clusters consist of more than one adjacent CTC (Figures F-G).
- Apoptotic CTCs (Figures H) contain fragmented nuclei.
- All localization can be cytoplasmic (Figures H-K) or both, even within a single CTC Cluster (Figure G).

Clinical Sensitivity of CTC Detection: CellSearch® vs. Epic Sciences

CTC Cluster (present vsplied immunocytochemistry staining. *EpCam* is the sum of all CTC subtypes detected on the Epic Sciences platform per sample: Ck+, Ck-, CTC Clusters, Apoptotic, and Small CTCs.
- CellSearch® vs. Epic Sciences enumeration is matched samples shown side-by-side in a matched bar plot (Figure 6).
- * p < 0.05 by a multi-comparison (Fisher's exact test) CTC/mL.
- Note: CellSearch® counts were capped at 300 per tube (from 7.5 µL of blood) by MSKCC clinical laboratory. For comparison, CellSearch® and Epic Sciences counts were normalized per milliliter, capped at 26.7 µL.

CTC Histological Subtypes Prognosticate Overall Survival

- The Epic Sciences platform has increased clinical sensitivity for mCRPC CTC detection rate vs. CellSearch® (96% vs. 67%) and magnitude of enrichment (median 6.82/mL vs. 0.27/mL).
- All subtypes of CTCs detected by Epic Sciences: Ck+, Ck-, Small, Apoptotic, Clusters are prognosticators of shorter OS in univariate models.
- CTC subtypes: Ck(+), Ck(+) and Small CTCs as well as all Epic Sciences CTC Subtypes pooled, each add to the prognostication of OS in multivariate models.
- Characterization of non-traditional CTCs (Ck- and Small CTCs) provides increased clinical sensitivity and may provide key insights to cancer biology.

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Conclusions

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- All subtypes of CTCs detected by Epic Sciences: Ck(+), Ck(-), Small, Apoptotic, Clusters are prognosticators of shorter OS in univariate models.
- CTC subtypes: Ck(+), Ck(-) and Small CTCs as well as all Epic Sciences CTC Subtypes pooled, each add to the prognostication of OS in multivariate models.
- Characterization of non-traditional CTCs (Ck- and Small CTCs) provides increased clinical sensitivity and may provide key insights to cancer biology.