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

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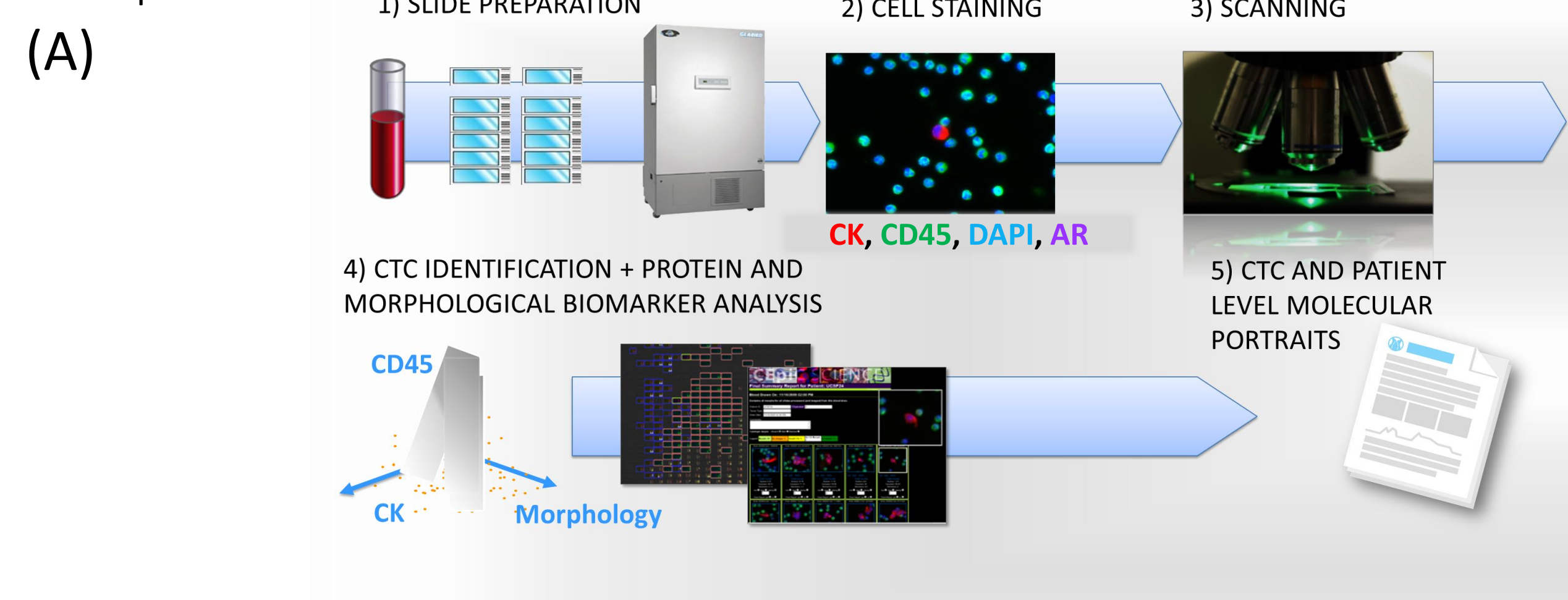[2] Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan-Kettering Cancer Center, New York, NY
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Background

- 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(+), CK(+), DAPI(+), CD45(+); which could reduce detection sensitivities.
- The Epic Sciences platform does not use enrichment, and detects an expanded range of CTC histology, including CK(+) CTCs, CK(-) CTCs, CTC Clusters, and Apoptotic CTCs.
- 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 179 unique patients were collected prior to initiating Androgen Receptor (AR) directed (n = 150) or taxane (n = 71) therapy for mCRPC.
- Samples were analyzed with the Epic Sciences platform to enumerate CK(+) CTCs, CK(-) CTCs, CTC Clusters, Apoptotic CTCs, and Small CTCs (Figure A).
- Patients were followed for up to 2.3 yrs.
- Paired CellSearch® blood draws were processed at MSKCC Clinical Laboratory per manufacturer recommendations. CellSearch® counts were capped at 200 CTCs per tube (from 7.5 mL of blood). For comparison, CellSearch® and Epic Sciences counts were normalized per milliliter, capped at 26.7/mL. Paired CellSearch® and Epic Sciences traditional CTC counts were collected from 173 patient samples.

(A)

1) SLIDE PREPARATION
2) CELL STAINING
3) SCANNING
CK, CD45, DAPI, AR
4) CTC IDENTIFICATION + PROTEIN AND MORPHOLOGICAL BIOMARKER ANALYSIS
5) CTC AND PATIENT LEVEL MOLECULAR PORTRAITS
CD45
CK   Morphology

Schematic of Epic Sciences CTC Platform CTC enumeration, morphology, biomarker, & FISH analyses workflow:

1) Nucleated cells from blood sample placed onto slides and stored in a -80°C biorepository
2) Slides stained with cytokeratin (CK), CD45, DAPI, AR N-Term
3) Slides scanned
4) CTC candidates detected by a multi-parametric digital pathology algorithm
5) Human reader confirmation of CTCs & quantitation of biomarker expression

Patient Demographics

Patient Primary Therapy	
Number of Unique Patients	179
Age, years	68 (45 – 91)
Primary Treatment	
Prostatectomy	84 (47%)
Radiation	34 (19%)
Brachytherapy	7 (4%)
None	54 (30%)

Characteristic	All Samples
Number of Baseline Samples	221
Age, years	68 (45 – 91)
Prior Hormone Therapies*	
1 - 2 lines	81 (37%)
3 lines	46 (21%)
≥ 4 lines	94 (42%)
Chemotherapy Status	
Chemo-naive	136 (62%)
Chemo-exposed	85 (38%)
Metastatic Disease	
Bone Only	63 (29%)
Lymph Node (LN) Only**	24 (10%)
Bone & LN	77 (35%)
Bone & Visceral ± LN**	35 (16%)
Laboratory Measures	
PSA, ng/mL	37.7 (0.1 – 3728.2)
Hgb, (g/dl)	12.0 (7.0 – 15.0)
ALK, (unit/L)	110 (25 – 2170)
LDH, (unit/L)	222.5 (123 – 1293)
ALB, (g/dl)	4.2 (3.1 – 4.9)

*Includes patients with GnRH agonists and antagonists, antiandrogens and next-generation hormonal therapies (abiraterone acetate and enzalutamide) patient was exposed to prior to initiation on the baseline therapy
** Includes patients with other soft tissue disease

Patient Line of Therapy

Total Samples	1st Treatment Decision	2nd Treatment Decision	3rd + Treatment Decision
A or E Baseline Blood Draw (n=150)	No Prior A or E (1st Line) n = 64	Previous A or E (2nd Line) n = 36	Previous A & E (3rd+ Line) n = 5
		Previous T (2nd Line) n = 8	Previous AR Tx & T (3rd+ Line) n = 37
T Baseline Blood Draw (n=71)	No Prior A or E (1st Line) n=12	Previous A or E (2nd Line) n = 12	Previous A & E (3rd+ Line) n = 10
			Previous AR Tx & T (3rd+ Line) n = 37

CTC Histological Subtypes Detected on the Epic Sciences CTC Platform

Composite | CK | DAPI | CD45 | AR
(B) (C) (D) (E) (F) (G) (H) (I)
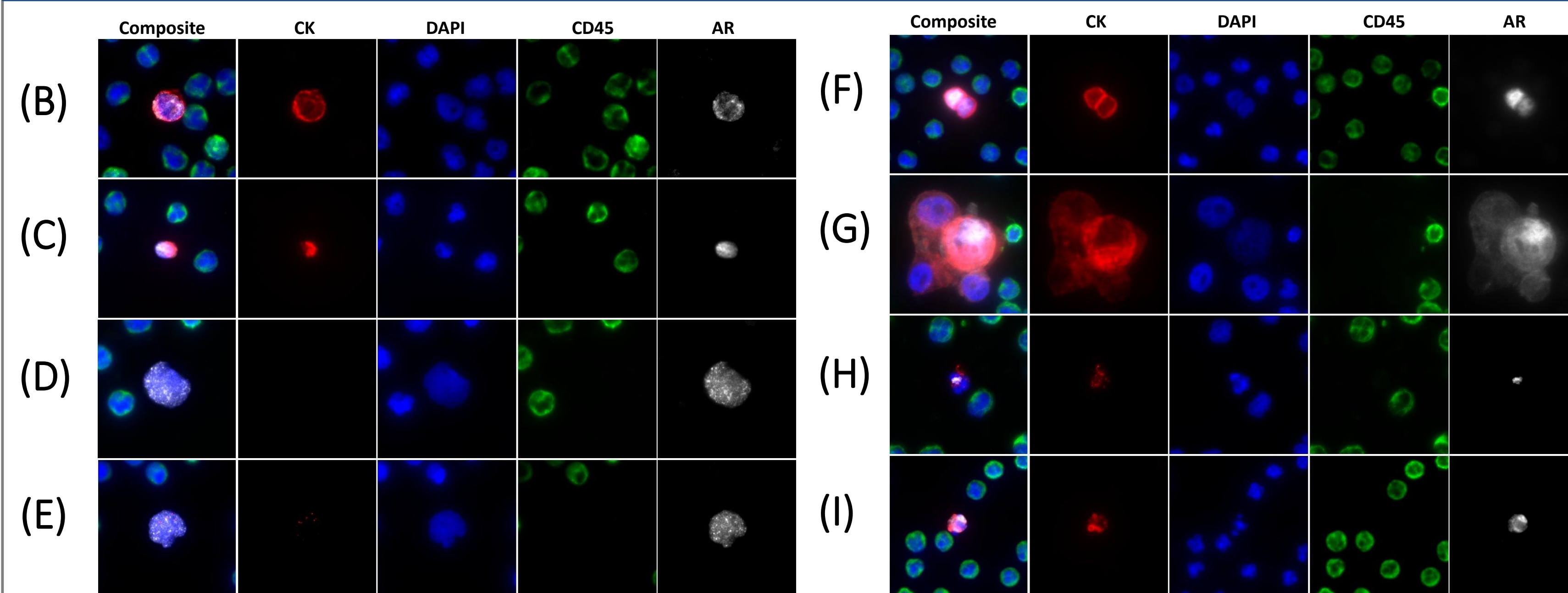
CTCs enumerated in this study encompass several histological types.

- "Traditional" CK(+) CTCs are detected as single cells positive for cytokeratin expression (Figures B-C).
- Some CK(+) CTCs are smaller than surrounding white blood cells (Figure C).
- CK(-) CTCs have distinctive nuclear malignant features and/or the presence of AR N-terminal domain (Figures D-E).
- CTC Clusters consist of more than one adjacent CTC (Figures F-G).
- Apoptotic CTCs (Figures H-I) contain fragmented nuclei.
- AR localization can be cytoplasmic (Figure B), nuclear (Figures D-F, H-I) or both, even within a single CTC Cluster (Figure G).

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

(J)
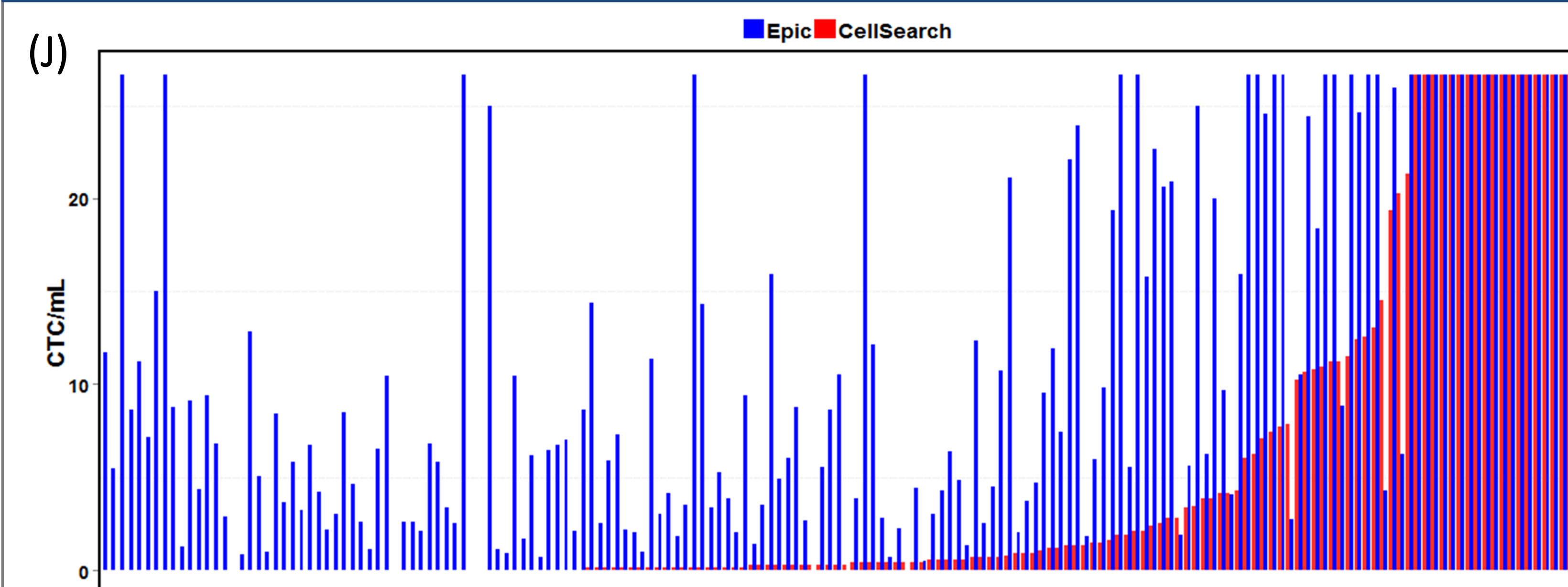
Epic | CellSearch

- 173 of 221 patient samples had matched CellSearch® testing.
- "Epic" 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 in matched samples shown side-by-side in a matched bar plot (Figure J).
- The difference between Epic Sciences and CellSearch® enumeration is shown per sample (Figure K).
- Note: CellSearch® counts were capped at 200 per tube (from 7.5 mL of blood) by MSKCC clinical laboratory. For comparison, CellSearch® and Epic Sciences counts were normalized per milliliter, capped at 26.7/mL.
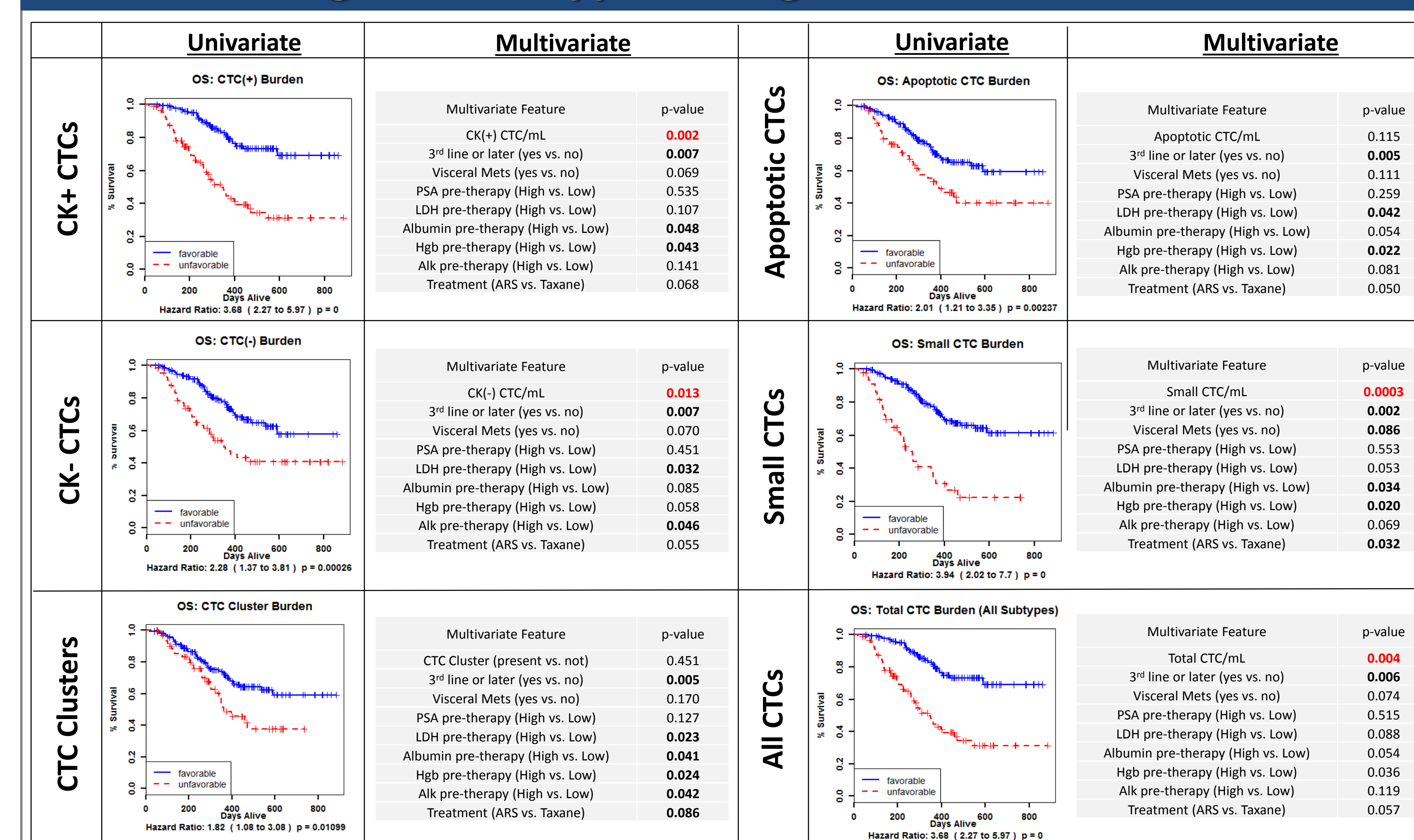
(K)

Epic | CellSearch

Epic Sciences Detects More CTCs
CellSearch® Detects More CTCs
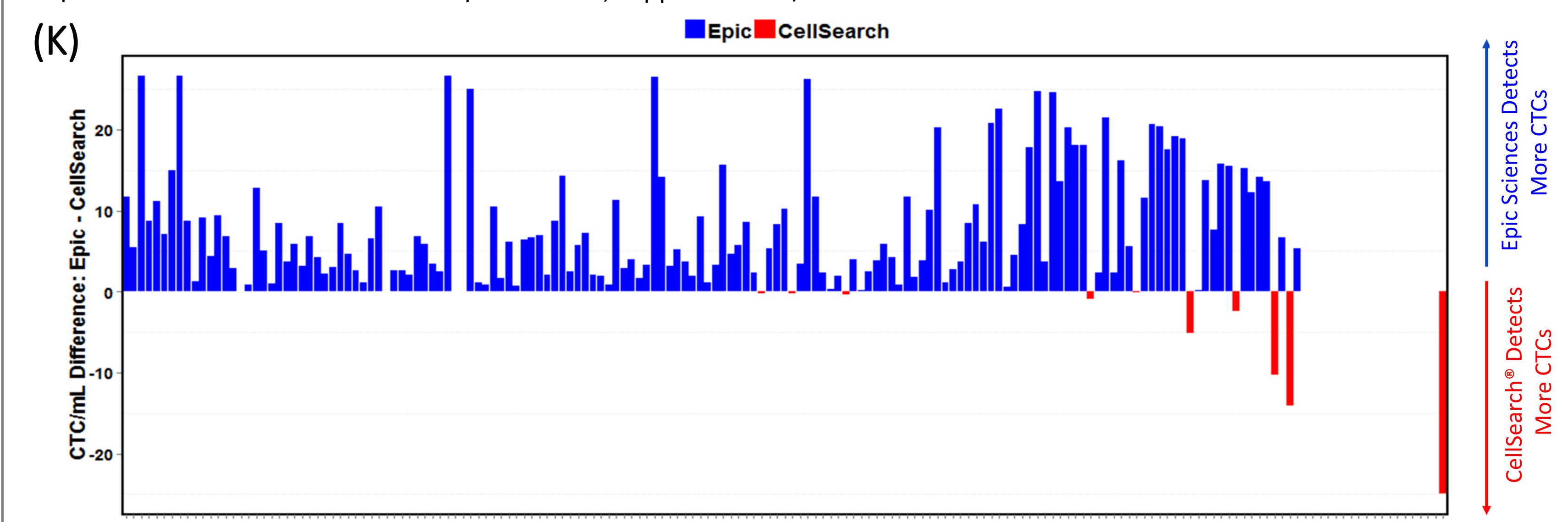
Platform Comparison of CTC Detection

(L)

	All Patients (n = 173)	1st line (n = 55)	2nd line (n = 44)	3rd line (n = 34)	4th+ line (n = 40)
CellSearch®: CTCs Present	116 (67%)	32 (58%)	24 (55%)	27 (79%)	33 (83%)
CellSearch®: CTCs Absent	57 (33%)	23 (42%)	20 (45%)	7 (21%)	7 (17%)
Epic Sciences: CTCs Present	166 (96%)	52 (95%)	43 (98%)	31 (91%)	40 (100%)
Epic Sciences: CTCs Absent	7 (4%)	3 (5%)	1 (2%)	3 (9%)	0 (0%)

(M)

All Matched Samples (n = 173)	CellSearch®	Epic Sciences
Median	0.27 / mL	6.82 / mL
Range	0 - 26.7* / mL	0 - 991 / mL

(N)

CellSearch® Favorable Count (< 5 CTC / 7.5mL = <0.67/1mL, n = 102)	CellSearch®	Epic Sciences
Median	0 / mL	4.37 / mL
Range	0 - 1 / mL	0 - 144 / mL

- CTC detection by line of therapy in mCRPC (Figure L).
- Median and range of CellSearch® and Epic CTC detection (Figure M).
- Enumeration in samples with CellSearch® count less than prognostic threshold of 5 CTCs/tube (0.67/mL) (Figure N).
- Note: Epic tested two slides (corresponding to roughly 1mL of blood) while CellSearch® assayed 7.5mL, potential bias against Epic in comparison.

*CellSearch® and Epic counts were normalized per milliliter, capped at 26.7/mL

CTC Histological Subtypes Prognosticate Overall Survival

CK+ CTCs

OS: CTC(+) Burden
favorable / unfavorable
Hazard Ratio: 3.68 (2.27 to 5.97) p ~ 0

Multivariate Feature	p-value
CK(+) CTC/mL	0.002
3rd line or later (yes vs. no)	0.007
Visceral Mets (yes vs. no)	0.069
PSA pre-therapy (High vs. Low)	0.535
LDH pre-therapy (High vs. Low)	0.107
Albumin pre-therapy (High vs. Low)	0.048
Hgb pre-therapy (High vs. Low)	0.043
Alk pre-therapy (High vs. Low)	0.141
Treatment (ARS vs. Taxane)	0.068

CK- CTCs

OS: CTC(-) Burden
favorable / unfavorable
Hazard Ratio: 2.28 (1.37 to 3.81) p = 0.0026

Multivariate Feature	p-value
CK(-) CTC/mL	0.013
3rd line or later (yes vs. no)	0.007
Visceral Mets (yes vs. no)	0.070
PSA pre-therapy (High vs. Low)	0.451
LDH pre-therapy (High vs. Low)	0.032
Albumin pre-therapy (High vs. Low)	0.085
Hgb pre-therapy (High vs. Low)	0.058
Alk pre-therapy (High vs. Low)	0.046
Treatment (ARS vs. Taxane)	0.055

CTC Clusters

OS: CTC Cluster Burden
favorable / unfavorable
Hazard Ratio: 1.62 (1.08 to 3.06) p = 0.0109

Multivariate Feature	p-value
CTC Cluster (present vs. not)	0.451
3rd line or later (yes vs. no)	0.005
Visceral Mets (yes vs. no)	0.170
PSA pre-therapy (High vs. Low)	0.127
LDH pre-therapy (High vs. Low)	0.023
Albumin pre-therapy (High vs. Low)	0.041
Hgb pre-therapy (High vs. Low)	0.024
Alk pre-therapy (High vs. Low)	0.042
Treatment (ARS vs. Taxane)	0.086

Apoptotic CTCs

OS: Apoptotic CTC Burden
favorable / unfavorable
Hazard Ratio: 2.01 (1.27 to 3.16) p = 0.00237

Multivariate Feature	p-value
Apoptotic CTC/mL	0.115
3rd line or later (yes vs. no)	0.005
Visceral Mets (yes vs. no)	0.111
PSA pre-therapy (High vs. Low)	0.259
LDH pre-therapy (High vs. Low)	0.042
Albumin pre-therapy (High vs. Low)	0.054
Hgb pre-therapy (High vs. Low)	0.022
Alk pre-therapy (High vs. Low)	0.081
Treatment (ARS vs. Taxane)	0.050

Small CTCs

OS: Small CTC Burden
favorable / unfavorable
Hazard Ratio: 3.84 (2.50 to 7.7) p ~ 0

Multivariate Feature	p-value
Small CTC/mL	0.0003
3rd line or later (yes vs. no)	0.002
Visceral Mets (yes vs. no)	0.086
PSA pre-therapy (High vs. Low)	0.553
LDH pre-therapy (High vs. Low)	0.053
Albumin pre-therapy (High vs. Low)	0.034
Hgb pre-therapy (High vs. Low)	0.020
Alk pre-therapy (High vs. Low)	0.069
Treatment (ARS vs. Taxane)	0.032

All CTCs

OS: Total CTC Burden (All Subtypes)
favorable / unfavorable
Hazard Ratio: 3.48 (2.27 to 5.97) p ~ 0

Multivariate Feature	p-value
Total CTC/mL	0.004
3rd line or later (yes vs. no)	0.006
Visceral Mets (yes vs. no)	0.074
PSA pre-therapy (High vs. Low)	0.515
LDH pre-therapy (High vs. Low)	0.088
Albumin pre-therapy (High vs. Low)	0.053
Hgb pre-therapy (High vs. Low)	0.036
Alk pre-therapy (High vs. Low)	0.119
Treatment (ARS vs. Taxane)	0.057

Univariate thresholds for KM plots were chosen based on time-dependent survival ROC curves. CTC/mL was included in multivariate models as a log2-transformed continuous variable as previously reported in Scher et al 2009.

Conclusions

- The Epic Sciences platform has increased clinical sensitivity for mCRPC CTC detection rate vs. CellSearch®, (96% vs. 67%) and magnitude of enumeration (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.