Development and Validation of Circulating Tumor Cell Enumeration (Epic Sciences) as a Prognostic Biomarker in Men with Metastatic Castration Resistant Prostate Cancer

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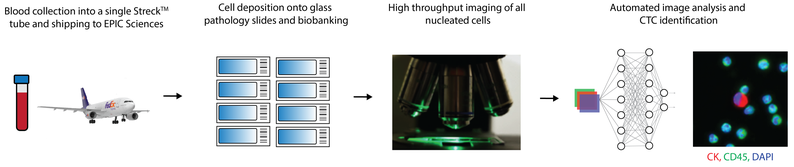
PRIMARY OBJECTIVE OF THIS STUDY

The **primary objective** of this pre-specified retrospective analysis is to assess CTC counts (Epic Sciences platform) as a biomarker of prognosis in patients treated with AR signalling inhibitors (ARSi) and compare to counts measured on the FDA approved predicate CellSearch™ Circulating Tumor Cell kit

THE EPIC SCIENCES NON-ENRICHMENT CTC DETECTION AND ENUMERATION TECHNOLOGY

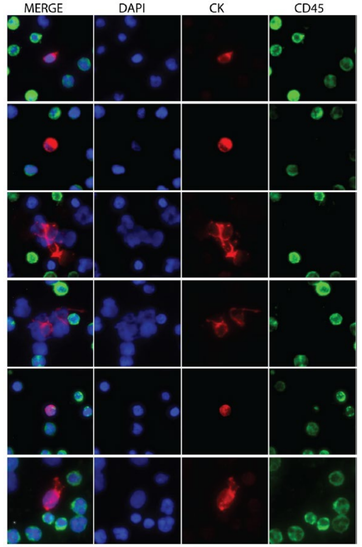


Summary of CTC detection technologies and available large clinical validations/studies

CTC Detection Technology	Method	CTC Definition (Epithelial)	Clinical Validation/Studies (CTC counts only) (Studies of > 100 patients only)
CellSearch™ Circulating Tumor Cell Kit	Affinity Capture by EpCAM ferrofluid	EpCAM captured, CK+, CD45-	Validation in multiple ph III trials
Epic Sciences	Non-enrichment, all nucleated cells plated onto slides and IF imaged. CTCs detected *in silico*.	CK+, CD45-, DAPI+ (this study)	Scher HI et al. ASCOGU 2021 (This study) de Bono J et al. ASCOGU 2021 (abstract #161)
Other	Affinity capture, microfluidics, size based	Variable depending on platform	Limited or none

ENUMERATING CTCS IN THIS STUDY

CTC Enumeration in This Study
- Cytokeratin positive (epithelial)
- CD45 negative (leukocyte)
- Intact nucleus
- Clusters of CTCs are counted as 1 event
- Count is normalized to volume



PATIENT SELECTION

MSKCC Discovery Cohort

216 mCRPC blood samples from patients with progressing mCRPC starting prior to starting an ARSi (2012-2016)

→ 45 samples taken >30 days prior to therapy start

171 unique patients with baseline blood-draws
End Pts: Overall Survival

91.4% had detectable CTCs

PROPHECY Trial Validation Cohort

120 men with progressing high risk mCRPC starting an ARSi in the PROPHECY trial

→ 2 men withdrew consent
11 failed QC

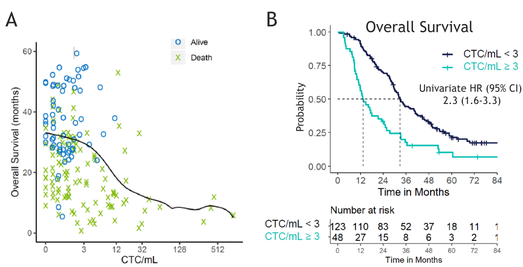
107 unique patients with baseline blood-draws
End Pts: OS, PFS, CellSearch method comparison

83.2% had detectable CTCs

Analysis Methods: The Kaplan-Meier estimate and Cox proportional hazards models adjusting for known prognostic features were used to assess the prognostic significance of CTC number. Analyses were completed by independent statisticians and the analysis of the Validation cohort was pre-specified based on the findings in the Discovery cohort.

CTC COUNTS ARE A PRE-TREATMENT PROGNOSTIC BIOMARKER IN THE DISCOVERY AND VALIDATION COHORTS

MSKCC Discovery Cohort



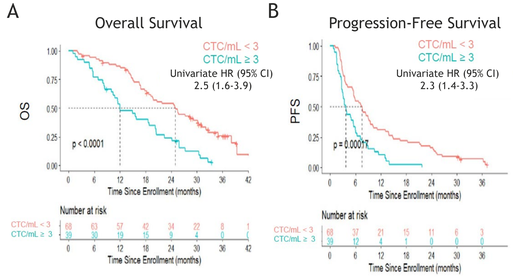
Multivariable Risk Adjusted Hazard Ratios in the Discovery Cohort (Epic Sciences)

	CTC/mL ≥ 3 HR** (95% CI); P	Fold Increase CTC (log2 + 1) HR** (95% CI); P
MSKCC Discovery Cohort	1.9 (1.3, 2.9); P = 0.002	1.2 (1.1, 1.4); P = 0.001

**adjusted for therapy line, sites of spread, LDH, PSA, Alkaline phosphatase.

A) Plot of survival times versus CTC/mL value. **B)** Kaplan-Meier estimate for OS. A natural cutoff of approximately 3 CTCs/mL is identified from (A). **C)** Multivariable Cox Proportional Hazards model of OS adjusting for standard baseline prognostic features as a dichotomized (< 3 versus ≥ 3 CTC/mL) and continuous variable (Fold change or log2 + 1 transform).

PROPHECY Validation Cohort



Multivariable Risk Adjusted Hazard Ratios in the Validation Cohort (Epic Sciences)

Endpoint	CTC/mL ≥ 3 HR** (95% CI); P	Fold Change CTC HR** (95% CI); P
OS	1.8 (1.1, 3.0): P = 0.03	1.3 (1.1, 1.6); P = 0.002
PFS	1.7 (1.1, 2.9); P = 0.03	1.3 (1.1, 1.5); P = 0.01

**HR adjusted for Halabi Prognostic Risk score (includes; sites of metastases, opioid use, LDH, ECOG, Albumin, Hemoglobin, Alkaline Phosphatase, PSA) See Halabi et al. JCO 2014; PMID24449231

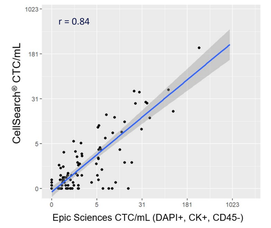
A&B) Kaplan-Meier estimate for OS and PFS using the 3 CTCs/mL cutoff identified above **C)** Multivariable Cox Proportional Hazards model of OS and PFS adjusting for standard baseline prognostic features as a dichotomized (< 3 versus ≥ 3 CTC/mL) and continuous variable (Fold change or log2 + 1 transform).

CONCLUSIONS

- The findings validate CTC number determined on the Epic Sciences platform as a prognostic biomarker prior to treatment with Androgen Receptor signaling inhibitors in two independent cohorts.
- CTC counts showed strong method agreement and correlation with counts determined using the FDA cleared CellSearch Circulating Tumor Cell kit which is approved for use as an aid to monitoring.
- In univariate and multivariable analyses, the associations with OS and PFS of CTC counts on both platforms were comparable in the Validation cohort.

COMPARISON WITH THE FDA CLEARED CELLSEARCH DEVICE

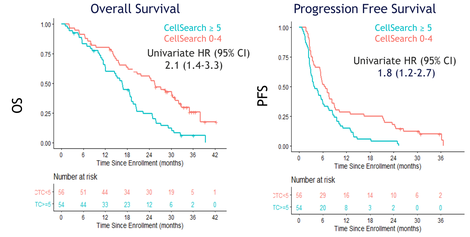
Epic Sciences CTC Counts in the PROPHECY Validation Cohort Show Strong Correlation and Agreement to the CellSearch Predicate With the Base CTC Definition



CTC Definition By Platform	
Epic Sciences	CK+, CD45- intact cell
CellSearch	EpCAM captured CK+, CD45- intact cell

Summary of Comparisons Between Platforms
- Strong correlation observed between platforms (r = 0.84)
- 94% of CTC counts within Bland-Altman limits
- Lin's concordance correlation coefficient (CCC) of 0.81 observed

Epic Sciences and CellSearch CTC Counts (CK+, CD45-) are Comparably Prognostic for OS/PFS in the Validation Cohort



Multivariable Risk Adjusted Hazard Ratios in the Validation Cohort

Technology	Endpoint	CTC ≥ 5 vs < 5 HR (95% CI); P	Fold Change CTC (continuous log2 +1) HR (95% CI); P
CellSearch	OS	1.7 (1.0, 2.9): P = 0.03	1.2 (1.1, 1.4); P = 0.002
CellSearch	PFS	1.5 (0.9, 2.3); P = 0.11	1.2 (1.0, 1.3); P = 0.007

**HR adjusted for Halabi Prognostic Risk score (includes; sites of metastases, opioid use, LDH, ECOG, Albumin, Hemoglobin, Alkaline Phosphatase, PSA) See Halabi et al. JCO 2014; PMID24449231

PATIENT DEMOGRAPHICS

Patient Demographics	MSKCC Discovery Set	PROPHECY Validation set
Unique Patients, no. (%)	171	107
Unique Blood Samples, no. (%)	171	107
Median Age in years (range)	68 (45,87)	73 (44,92)
Death events, no. (%)	137 (80.0%)	83 (77.6%)
Median Follow Up of Survivors in months (range)	60.3 (5.0, 84.8)	31 (3.4, 42.3)
Therapy Line - no. (%)		
pre-1st	103 (60.2%)	76 (71%)
pre-2nd	48 (28.1%)	31 (29%)
pre-3rd	20 (11.7%)	0 (0%)
Sites of Metastases – no. (%)		
Lymph Node Only	29 (17.0%)	4 (3.7%)
Bone Only	59 (34.5%)	25 (23.4%)
Lung Only	1 (0.6%)	0 (0%)
Multiple Sites	82 (48.0%)	76 (71.0%)
Prior Taxane Chemotherapy – no. (%)	12 (7.0%)	20 (18.7%)
Prior ARSi – no. (%)	56 (33.1%)	40 (37.4%)
Baseline lab values – median (range)		
PSA ng/mL	18.1 (0.1, 2006.1)	22.1 (0.1, 4194.9)
ALB g/L	4.2 (3.3, 4.9)	4.0 (2.7, 4.9)
ALK U/L	96 (42, 2170)	110 (91, 150)
HGB g/dL	12.6 (8.2, 15.7)	12.8 (8.7, 15.9)
LDH U/L	208 (124, 2115)	200 (100, 618)
WBC x 10^9/L	5.9 (2.6, 12.1)	6.4 (3.7, 22.3)
CellSearch® CTC count/7.5mL	n/a	4 (0, 12,972)
EPIC Sciences CTC count/mL	1.3 (0.0, 906.3)	1.3 (0.0, 916.2)

Abbreviations: PSA - prostate specific antigen, ALB - albumin, ALK - alkaline phosphatase, HGB - hemoglobin, LDH - lactate dehydrogenase, WBC - white blood cell

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