CTC counts as a biomarker of prognosis and response in metastatic castration-resistant prostate cancer (mCRPC) from the CARD trial

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BACKGROUND

- In the prospective CARD trial (NCT02485691), cabazitaxel significantly improved radiographic progression-free survival (rPFS) and overall survival (OS) versus abiraterone or enzalutamide in patients with metastatic castration-resistant prostate cancer who had received docetaxel and progressed within 12 months with the alternative androgen-signaling-targeted inhibitor.[1]
- Currently, monitoring prostate-specific antigen (PSA) changes and evidence of progression by imaging are the recommended tools by which most clinicians base their decisions. However, while sensitive, PSA is not specific in a significant fraction of cases, and imaging (such as bone scans) are imperfect and are subject to interpretation bias.[2]
- Therefore, a current unmet need is to identify better tools for monitoring treatment efficacy so that patients do not continue to receive ineffective therapies for longer than necessary.
- Monitoring changes in circulating tumor cell (CTC) count along with assessing the molecular and genomic phenotypes of CTCs could address this unmet need.[3,4]

OBJECTIVE

- The primary objective of the pre-planned CARD EPIC biomarker study was to assess CTC counts as a biomarker of prognosis and response to treatment.

METHODS

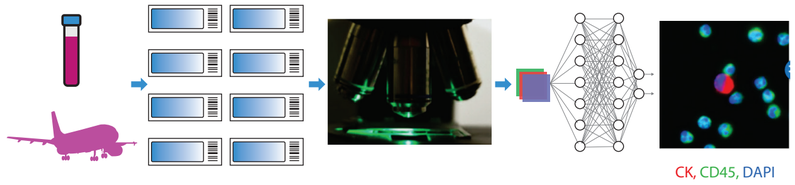
EPIC SCIENCES TECHNOLOGY FOR CTC DETECTION

Blood collection into a single Streck™ tube and shipping to EPIC Sciences

Cell deposition onto glass pathology slides and biobanking

High throughput imaging of all nucleated cells

Automated image analysis and CTC identification



CK, CD45, DAPI

HOW DOES EPIC SCIENCES METHODOLOGY DIFFER FROM OTHER TECHNOLOGIES?

CTC detection technology	Method	CTC definition	Clinical Validation/Studies (CTC counts; studies of > 100 patients only)
CellSearch™ CTC Kit	Affinity Capture by EpCAM ferrofluid	EpCAM captured, CK+, CD45-	Validation in multiple phase III trials
Epic Sciences	Non-enrichment, all nucleated cells plated onto slides and immunofluorescence imaged. CTCs detected in silico.	CK+, CD45-, DAPI+ (this study)	Scher HI, et al. ASCO-GU 2021 (poster 157) de Bono J, et al. ASCO-GU 2021 (current study)
Other	Affinity capture, microfluidics, size based,	Variable depending on platform	Limited or none

The association between CTC counts, defined as any CK+, CD45- cell, and clinical outcomes were analyzed using a Cox proportional hazards model for time to events (rPFS, OS).
CD, cluster of differentiation; CK, cytokeratin; CTC, circulating tumor cell; DAPI, diamidino-2-phenylindole; OS, overall survival; rPFS, radiographic progression-free survival.

RESULTS

CARD CTC SAMPLES

681 blood samples sent to EPIC Sciences

- 25 samples from patients that failed screening
- 35 failed quality check
- 2 wrong time point
- 3 samples were duplicated by time point

Evaluable samples
237 screening samples
213 C2 Day 1
166 end of treatment

Frequency of CTC detection in the CARD trial

	CTC/mL – median (range)	Samples with CTCs, n/N (%)
Screening	2.03 (0–410.2)	203/237 (86%)
C2	2.56 (0–174.4)	178/213 (84%)
End of treatment	2.23 (0–493.2)	132/166 (80%)

C, cycle; CTC, Circulating Tumor Cells

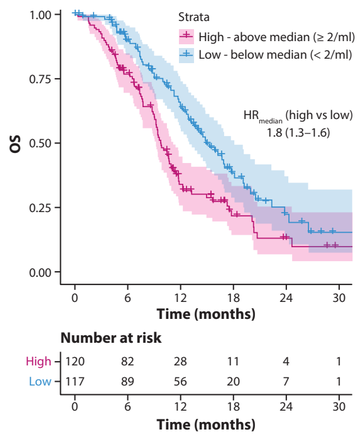
BASELINE CHARACTERISTICS

	Total (N = 255)	Evaluable CTCs at screening (n = 237)	Abiraterone or enzalutamide		Cabazitaxel	
			CTC/mL < 2 Median (n = 52)	CTC/mL ≥ 2 Median (n = 65)	CTC/mL < 2 Median (n = 65)	CTC/mL ≥ 2 Median (n = 55)
ECOG performance status, n (%)						
0 or 1	242 (94.9)	225 (94.9)	50 (96.2)	60 (92.3)	63 (96.9)	52 (94.5)
2	13 (5.1)	12 (5.1)	2 (3.8)	5 (7.7)	2 (3.1)	3 (5.5)
Timing of androgen-signaling-targeted inhibitor, n (%)						
After docetaxel	156 (61.2)	147 (62.0)	35 (67.3)	37 (56.9)	41 (63.1)	34 (61.8)
Before docetaxel	99 (38.8)	90 (38.0)	17 (32.7)	28 (43.1)	24 (36.9)	21 (38.2)
Time from androgen-signaling-targeted inhibitor, n (%)						
0–6 months	127 (49.8)	117 (49.4)	23 (44.2)	34 (52.3)	28 (43.1)	32 (58.2)
6–12 months	128 (50.2)	120 (50.6)	29 (55.8)	31 (47.7)	37 (56.9)	23 (41.8)
Best overall response, n (%)						
Not evaluable	3 (1.2)	3 (1.3)	0 (0)	0 (0)	0 (0)	3 (5.5)
Progressive disease	66 (25.9)	58 (24.5)	16 (30.8)	16 (24.6)	10 (15.4)	16 (29.1)
Partial response	29 (11.4)	27 (11.4)	1 (1.9)	5 (7.7)	15 (23.1)	6 (10.9)
Stable disease	112 (43.9)	105 (44.3)	23 (44.2)	25 (38.5)	33 (50.8)	24 (43.6)
Missing	45 (17.6)	44 (18.6)	12 (23.1)	19 (29.2)	7 (10.8)	6 (10.9)
ALP IU/L – median (range)	124 (35.0–2280)	126 (35.0–2280)	98.0 (38.0–1080)	141 (35.0–1980)	105 (44.0–743)	176 (44.0–2280)
LDH IU/L – median (range)	251 (50.2–3370)	251 (50.2–3370)	239 (50.2–929)	302 (127–3370)	219 (135–820)	266 (149–1090)
PSA ng/mL – median (range)	61.0 (1.07–15000)	61.0 (1.07–15000)	36.0 (2.28–948)	104 (1.45–2870)	36.3 (1.07–1190)	94.9 (2.65–15000)
CTC/mL – median (range)						
Baseline	–	2.03 (0–410)	0.717 (0–1.73)	6.00 (2.13–127)	0.600 (0–1.72)	7.41 (2.01–410)
Cycle 2	–	2.70 (0–174)	1.47 (0–16.7)	4.67 (0–174)	1.70 (0–94.3)	5.03 (0–110)
End of treatment	–	2.38 (0–493)	2.01 (0–270)	3.29 (0–90.7)	1.12 (0–23.6)	4.45 (0–493)

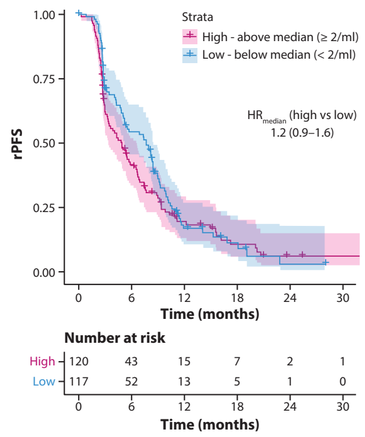
ALP, alkaline phosphatase; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PSA, prostate-specific antigen.

HIGH CTC COUNTS ARE PROGNOSTIC (OS)

OS (all arms combined)



rPFS (all arms combined)



Multivariable Risk Adjusted Hazard Ratios

Endpoint	*Total CTC/mL fold change (log2+1)		*Total CTC/mL ≥ vs < median (2/mL)	
	HR[a] (95% CI)	P	HR[a] (95% CI)	P
OS	1.13 (1.02–1.25)	0.03	1.66 (1.15–2.39)	0.007
rPFS	1.00 (0.91–1.10)	0.98	1.12 (0.82–1.54)	0.48

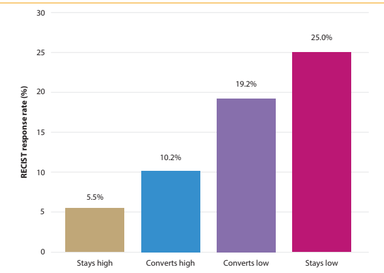
- CTCs are prognostic for OS as a continuous and dichotomized (median) variable in a multivariable model including standard prognostic features
- Prior validated cut-off of 3 CTC/mL was also prognostic (Scher HI et al. poster 157)

HRs were determined by a Cox proportional hazards model.
[a] adjusted for Therapy arm, PSA, LDH, ALP, Hb, Pain, Visceral metastases, ECOG score
ALP, alkaline phosphatase; CI, confidence interval; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; Hb, haemoglobin; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PSA, prostate-specific antigen; rPFS, radiographic progression-free survival.

ASSOCIATION OF CTC CHANGES WITH OS, rPFS AND RECIST RESPONSE

- Patients that maintain low CTCs at C2D1 have longer OS and a higher rate of Partial Response (PR)

Tumor response (all arms combined)



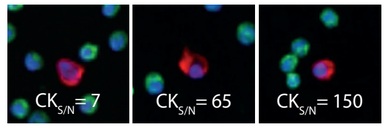
Multivariable Risk Adjusted Hazard Ratios

	rPFS		OS	
	HR[a] (95% CI)	P	HR[a] (95% CI)	P
CTC conversion over median[b]				
Stays high (n = 69)	(reference)		(reference)	
Converts low (n = 33)	0.89 (0.52–1.52)	0.67	0.66 (0.37–1.20)	0.18
Converts high (n = 45)	0.81 (0.49–1.33)	0.40	0.52 (0.30–0.90)	0.02
Stays low (n = 51)	0.79 (0.51–1.25)	0.32	0.46 (0.27–0.79)	0.004

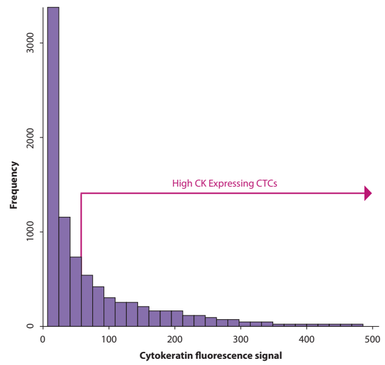
[a] HRs were determined by a Cox proportional hazards model adjusted for Therapy arm, PSA, LDH, ALP, Hb, Pain, Visceral metastases, ECOG score.
[b] Median CTC/mL in CARD is 2/mL (high represents above median, low represents below median CTC/mL); Stays high = always ≥ 2 CTC/mL; converts high = <2 CTC/mL at baseline and ≥ 2 CTC/mL at Cycle 2; converts low = ≥ 2 CTC/mL at baseline and < 2 CTC/mL at Cycle 2; stays low = always < 2 CTC/mL.
ALP, alkaline phosphatase; CI, confidence interval; CK, cytokeratin; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; Hb, haemoglobin; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PSA, prostate-specific antigen; RECIST, Response Evaluation Criteria in Solid Tumors; rPFS, radiographic progression-free survival.

CLEARANCE OF HIGH CK CTCs PREDICTS CABAZITAXEL ACTIVITY

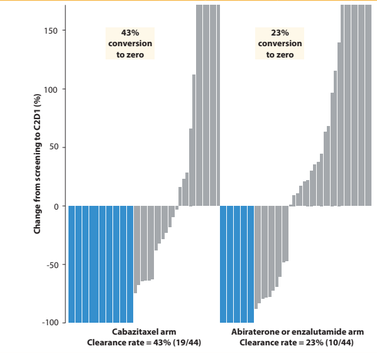
- Clearance of high CK-expressing cells is strongly associated with longer OS and rPFS



Histogram of CK Signal of 11,760 CTCs detected in CARD



- A higher rate of clearance of high CK CTCs is observed in the Cabazitaxel arm compared with the ARSi arm



Multivariable Risk Adjusted Hazard Ratios

	rPFS		OS	
	HR[a] (95% CI)	P	HR[a] (95% CI)	P
High CK CTC clearance				
Screening > 0, Cycle 2 > 0 stays positive (n=59)	(reference)		(reference)	
Screening > 0, Cycle 2 = 0 converts to zero (n = 29)	0.45 (0.25–0.79)	0.006	0.42 (0.23–0.77)	0.005
Screening = 0, Cycle 2 > 0 converts positive (n = 33)	0.86 (0.52–1.41)	0.55	0.35 (0.20–0.62)	< 0.001
Screening = 0, Cycle 2 = 0 stays at zero (n = 77)	0.67 (0.43–1.02)	0.06	0.33 (0.20–0.56)	< 0.001

[a] HRs were determined by a Cox proportional hazards model adjusted for Therapy arm, PSA, LDH, ALP, Hb, Pain, Visceral metastases, ECOG score.
ALP, alkaline phosphatase; C, cycle; CI, confidence interval; CK, cytokeratin; CTC, circulating tumor cell; D, day; ECOG, Eastern Cooperative Oncology Group; Hb, haemoglobin; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PSA, prostate-specific antigen; rPFS, radiographic progression-free survival; SE, standard error.

CONCLUSIONS

- This pre-planned analysis of the CARD trial confirms that baseline CTC counts, measured by EPIC Sciences platform, are prognostic and that early favorable changes are associated with response to treatment and survival.
- High cytokeratin expressing CTCs were more strongly associated with rPFS/OS in the CARD population and a ~2-fold higher rate of clearance was observed in the cabazitaxel arm.
- Ongoing work will analyze the association of CTC changes and subtypes as a surrogate (Prentice Criteria) for survival and early treatment benefit in comparison with PSA changes.
- Analysis of CTC subtypes (androgen receptor/ androgen receptor-V7+, chromosomal instability, heterogeneity, small-cell like [i.e. those losing epithelial lineage and genomic subtypes]) is ongoing.

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DISCLOSURES
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