Circulating tumor cell (CTC) morphologic sub-types present prior to treatment in the CARD trial identify therapy resistance

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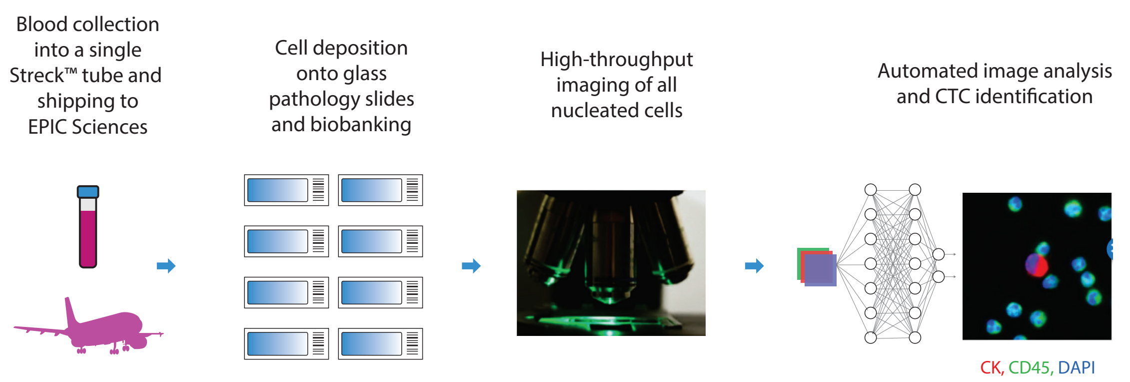
BACKGROUND

- In the prospective CARD trial (NCT02485691), cabazitaxel significantly improved radiographic progression-free survival and overall survival 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]
- There is a current unmet need to identify superior monitoring tools of treatment efficacy so that patients do not remain on ineffective therapies when they no longer clinically benefit from them.[2,3]
- Circulating tumor cell (CTC) counts are a validated pretreatment prognostic measure as well as a validated tool for response monitoring.[4–7]
- The EPIC Sciences platform allows for morphologic and molecular characterization of CTCs utilizing protein markers relevant to prostate cancer tumor biology, such as AR-V7, PSMA, or neuroendocrine (NE) markers, along with single-cell genomic characterization. This may enable disease subtyping and deconvolution of tumor heterogeneity from a blood sample.[8–11]
- The objective of this pre-planned CARD EPIC biomarker study was to analyze the morphology of CTC subtypes in a liquid biopsy.

METHODS

Figure 1. EPIC Sciences platform

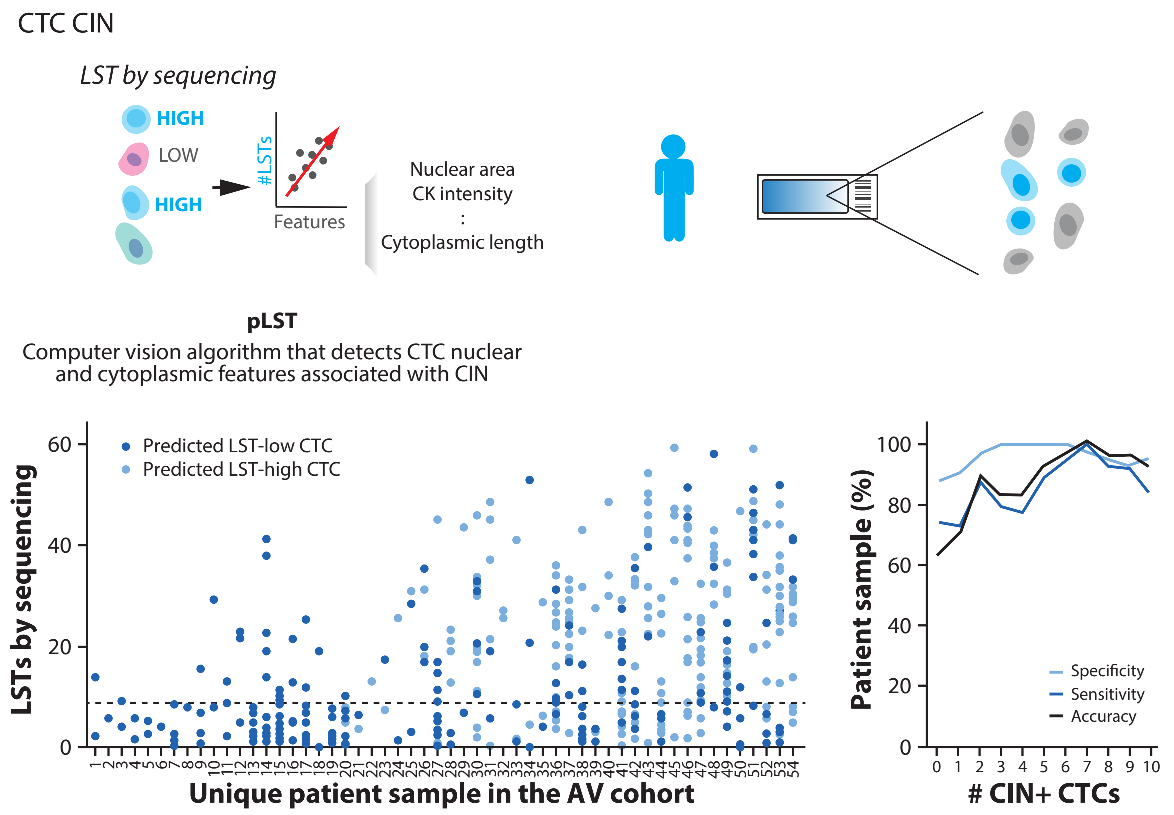
- Blood samples collected at screening, Cycle 2 Day 1 and end of therapy were sent to EPIC Sciences for CTC analysis[12]


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

CD, cluster of differentiation; CK, cytokeratin; CTC, circulating tumor cells; DAPI, diamidino-2-phenylindole; hrs, hours.

Figure 2. CTC chromosomal instability[13,14]

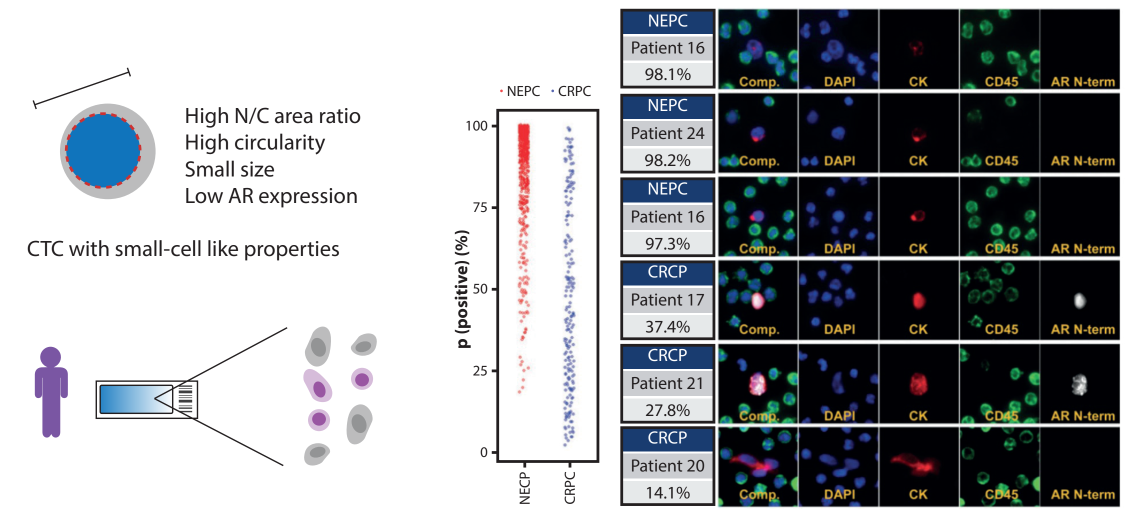
- Analytical cut-off of three or more for CTC (CIN)+ identifies patients samples harboring genomically instable CTCs with high specificity[13,a]


CTC CIN
LST by sequencing
HIGH
LOW
HIGH
#LSTs
Features
Nuclear area
CK intensity
Cytoplasmic length
pLST
Computer vision algorithm that detects CTC nuclear and cytoplasmic features associated with CIN
Predicted LST-low CTC
Predicted LST-high CTC
LSTs by sequencing
Unique patient sample in the AV cohort
Patient sample (%)
Specificity
Sensitivity
Accuracy
CIN+ CTCs

[a] Data and figure reproduced Schonhoft JD, et al. Cancer Res 2020.
AV, analytical validation; CIN, chromosomal instability; CTC, circulating tumor cells; LST, large-scale transitions; pLST, predicted Large Scale Transitions.

Figure 3. CTC NE/small-cell like[9,14]

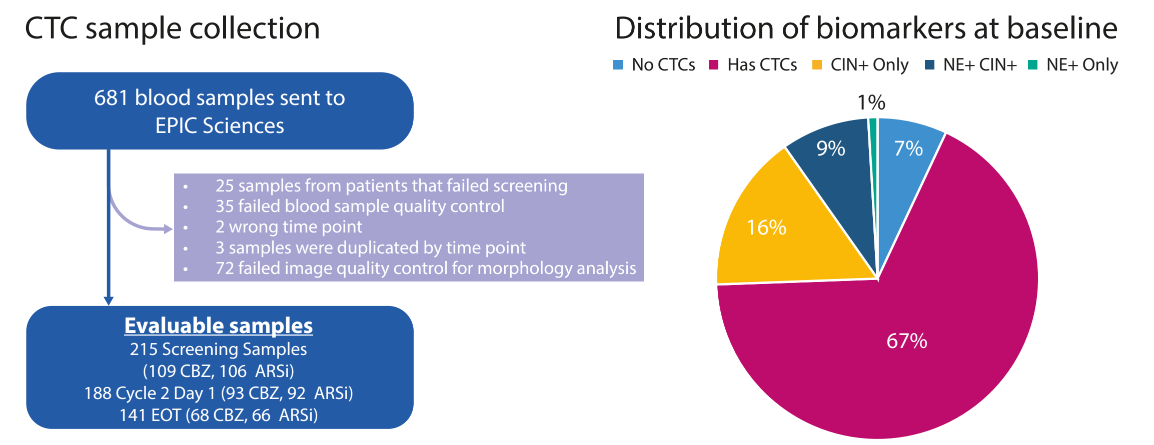
Trained classifier that identifies CTCs more like those from *de novo* NE prostate cancer[9,a]


High N/C area ratio
High circularity
Small size
Low AR expression
CTC with small-cell like properties
NEPC
CRPC
p (positive) (%)
NEPC
Patient 16
98.1%
NEPC
Patient 24
98.2%
NEPC
Patient 16
97.3%
CRPC
Patient 17
37.4%
CRPC
Patient 21
27.8%
CRPC
Patient 20
14.1%
Comp.
DAPI
CK
CD45
AR N-term

[a] Data and figure reproduced from Beltran H, et al. Clin Cancer Res. 2016.
AR, androgen receptor; DAPI= 4′-6-diamidino-2-phenylindole; CD, cluster of differentiation; CK, cytokeratin; Comp., comparison; CRPC, castration-resistant prostate cancer; CTC, circulating tumor cells; N/C, nuclear/cytoplasmic ratio; NE, neuroendocrine; NEPC, neuroendocrine prostate cancer.

RESULTS

Figure 4.


CTC sample collection
681 blood samples sent to EPIC Sciences
25 samples from patients that failed screening
35 failed blood sample quality control
2 wrong time point
3 samples were duplicated by time point
72 failed image quality control for morphology analysis
Evaluable samples
215 Screening Samples
(109 CBZ, 106 ARSi)
188 Cycle 2 Day 1 (93 CBZ, 92 ARSi)
141 EOT (68 CBZ, 66 ARSi)
Distribution of biomarkers at baseline
No CTCs
Has CTCs
CIN+ Only
NE+ CIN+
NE+ Only
1%
7%
9%
16%
67%

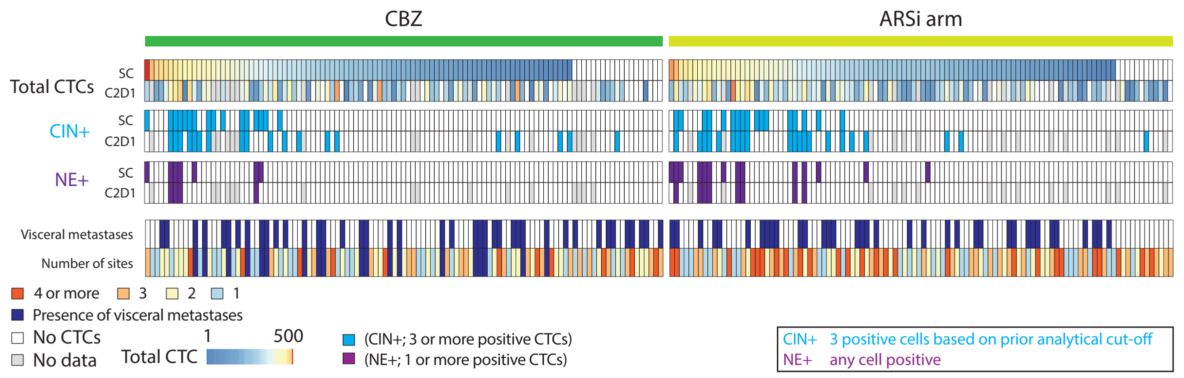
ARSi, androgen-signaling-targeted inhibitor; CBZ, cabazitaxel; CIN, chromosomal instability; CTC, circulating tumor cells, EOT, end of therapy; NE, neuroendocrine.

Table 1. Baseline demographics of the patients randomized in the CARD study

	Total	Evaluable for CTC CIN/NEPC	Patients with CTC CIN+ at SC (analytical cut off ≥ 3*)	Patients with presence of NE+ CTCs at SC
	(N = 255)	(N = 215)	(N = 33)	(N = 21)
ECOG PS, n (%)				
0 or 1	242 (94.9)	203 (94.4)	28 (84.8)	19 (90.5)
2	13 (5.1)	12 (5.6)	5 (15.2)	2 (9.5)
Timing of ARSi, n (%)				
After docetaxel	156 (61.2)	135 (62.8)	22 (66.7)	13 (61.9)
Before docetaxel	99 (38.8)	80 (37.2)	11 (33.3)	8 (38.1)
Time from ARSi, n (%)				
0–6 months	127 (49.8)	104 (48.4)	20 (60.6)	12 (57.1)
6–12 months	128 (50.2)	111 (51.6)	13 (39.4)	9 (42.9)
Best overall response, n (%)				
Not evaluable	3 (1.2)	3 (1.4)	0 (0)	0 (0.0)
PD	66 (25.9)	57 (26.5)	9 (27.3)	6 (28.6)
PR	29 (11.4)	25 (11.6)	2 (6.1)	0 (0.0)
SD	112 (43.9)	96 (44.7)	13 (39.4)	10 (47.6)
Missing	45 (17.6)	34 (15.8)	9 (27.3)	5 (23.8)
Median alkaline phosphatase, IU/L (range)	124 (35.0–2280)	127 (35.0–2280)	223 (35.0–2280)	309 (106–1980)
Median lactate dehydrogenase, IU/L (range)	251 (50.2–3370)	248 (50.2–3370)	351 (142–3370)	364 (173–3370)
Median PSA, ng/mL (range)	61.0 (1.07–15000)	61.9 (1.07–15000)	107 (6.30–994)	120 (2.52–1540)
Median NLR (range)	3.38 (0.84–108)	3.40 (0.840–108)	4.87 (1.31–16.5)	3.72 (1.31–12.5)
Median CTC/mL (range)	-	2.01 (0–410)	16.1 (3.30–410)	22.1 (2.33–410)
Median CIN+ CTC/mL (range)	-	0 (0–63.4)	6.72 (3.01–63.4)	11.4 (0.853–63.4)
Median NEPC+ CTC/mL (range)	-	0 (0–4.0)	1.00 (0–4.0)	1.00 (1.0–4.0)

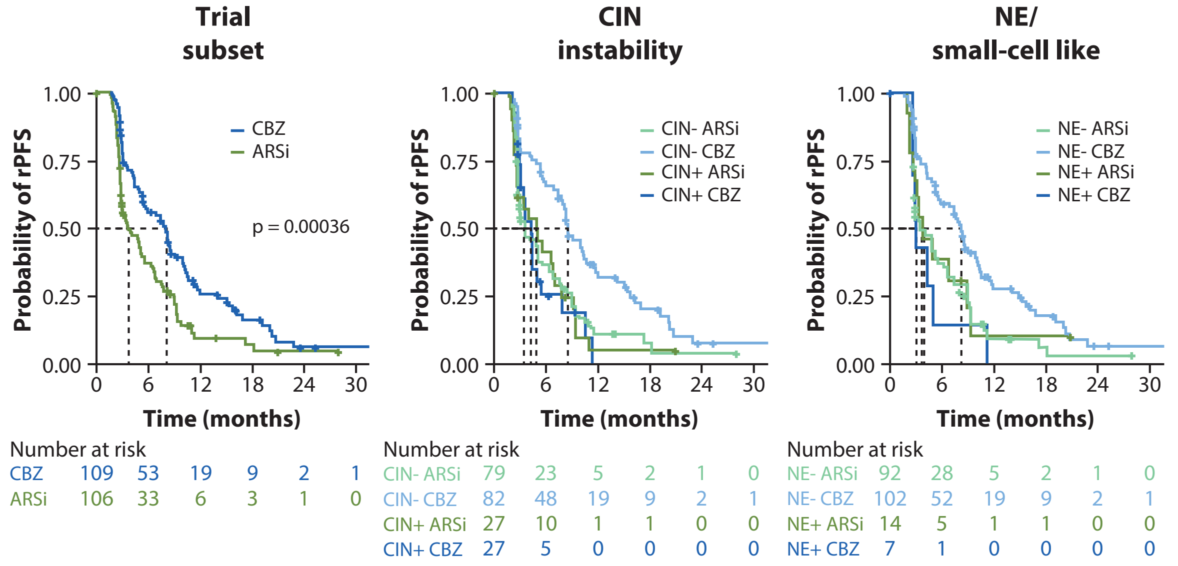
ARSi, androgen-signaling-targeted inhibitor; CIN, chromosomal instability; CTC, circulating tumor cells; ECOG PS, Eastern Cooperative Oncology Group performance status; NE neuroendocrine positive; NEPC, neuroendocrine prostate cancer; NLR, neutrophil to lymphocyte ratio; PD, progressive disease; PR, partial response; PSA, prostate-specific antigen; SC, screening; SD, stable disease.

Figure 5. Biomarker frequency[13]


CBZ
ARSi arm
Total CTCs
CIN+
NE+
SC
C2D1
Visceral metastases
Number of sites
4 or more
3
2
1
Presence of visceral metastases
No CTCs
No data
Total CTC
1
500
(CIN+; 3 or more positive CTCs)
(NE+; 1 or more positive CTCs)
CIN+ 3 positive cells based on prior analytical cut-off
NE+ any cell positive

ARSi, androgen-signaling-targeted inhibitor; C2D1, Cycle 2 Day 1; CBZ, cabazitaxel; CIN, chromosomal instability; CTC, circulating tumor cells; NE, neuroendocrine; SC, screening.

Figure 6. Presence of CTC morphologic subtypes associated with poorer rPFS despite cabazitaxel treatment

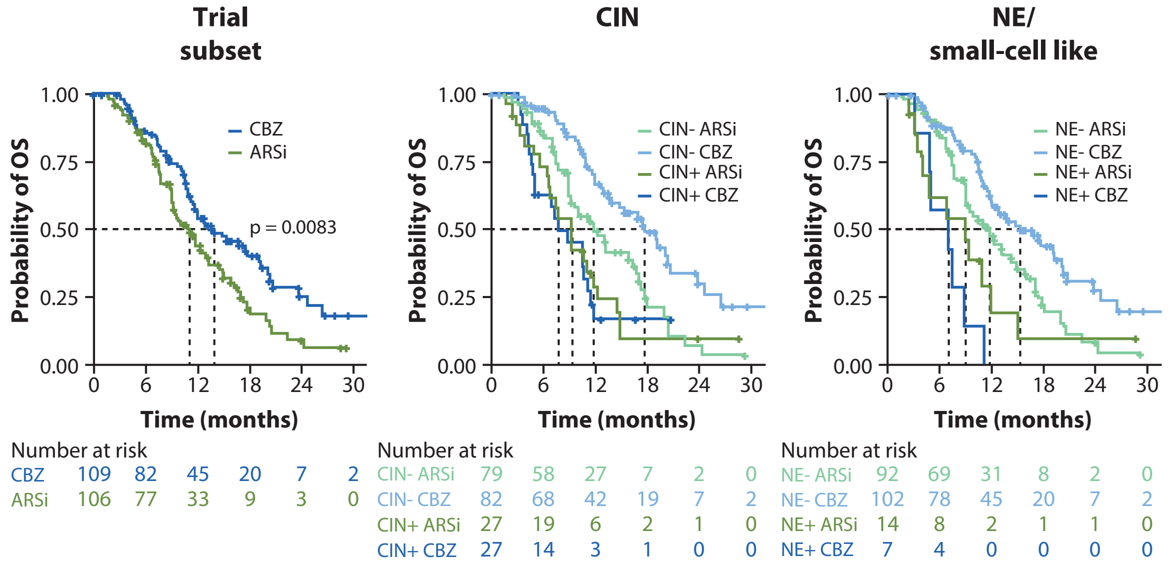

Trial subset
CIN instability
NE/ small-cell like
Probability of rPFS
Time (months)
CBZ
ARSi
p = 0.00036
CIN- ARSi
CIN- CBZ
CIN+ ARSi
CIN+ CBZ
NE- ARSi
NE- CBZ
NE+ ARSi
NE+ CBZ
Number at risk
CBZ 109 53 19 9 2 1
ARSi 106 33 6 3 1 0
CIN- ARSi 79 23 5 2 1 0
CIN- CBZ 82 48 19 9 2 1
CIN+ ARSi 27 10 1 1 0 0
CIN+ CBZ 27 5 0 0 0 0
NE- ARSi 92 28 5 2 1 0
NE- CBZ 102 52 19 9 2 1
NE+ ARSi 14 5 1 1 0 0
NE+ CBZ 7 1 0 0 0 0

Median rPFS (months)

	Trial subset	CIN+ vs CIN-	NE+ vs NE-
CBZ arm	8.0	4.2 vs 8.5	3.0 vs 8.2
ARSi arm	3.7	4.2 vs 3.4	3.9 vs 3.5

ARSi, androgen-signaling-targeted inhibitor; CBZ, cabazitaxel; CIN, chromosomal instability; CTC, circulating tumor cells; NE, neuroendocrine; rPFS, radiographic progression-free survival.

Figure 7. Presence of CTC morphologic subtypes associated with poorer OS despite cabazitaxel treatment


Trial subset
CIN
NE/ small-cell like
Probability of OS
Time (months)
CBZ
ARSi
p = 0.0083
CIN- ARSi
CIN- CBZ
CIN+ ARSi
CIN+ CBZ
NE- ARSi
NE- CBZ
NE+ ARSi
NE+ CBZ
Number at risk
CBZ 109 82 45 20 7 2
ARSi 106 77 33 9 3 0
CIN- ARSi 79 58 27 7 2 0
CIN- CBZ 82 68 42 19 7 2
CIN+ ARSi 27 19 6 2 1 0
CIN+ CBZ 27 14 3 1 0 0
NE- ARSi 92 69 31 8 2 0
NE- CBZ 102 78 45 20 7 2
NE+ ARSi 14 8 2 1 1 0
NE+ CBZ 7 4 0 0 0 0

Median OS (months)

	Trial Subset	CIN+ vs CIN-	NE+ vs NE-
CBZ arm	13.9	7.7 vs 17.5	7.7 vs 17.5
ARSi arm	11.0	7.7 vs 10.0	9.4 vs 9.9

ARSi, androgen-signaling-targeted inhibitor; CBZ, cabazitaxel; CIN, chromosomal instability; CTC, circulating tumor cells; NE, neuroendocrine; OS, overall survival.

Table 2. CTC CIN biomarker at SC or C2D1: Multivariable risk adjusted HR for rPFS and OS

	Cabazitaxel arm				ARSi arm			
	HR_{rPFS} (95% CI)	P	HR_{OS} (95% CI)	P	HR_{rPFS} (95% CI)	P	HR_{OS} (95% CI)	P
CIN+ at SC or C2D1	2.44 (1.28–4.64)	0.007	2.58 (1.28–5.22)	0.008	0.74 (0.37–1.45)	0.38	1.13 (0.53–2.40)	0.74
Total CTCs > median (2/mL) at SC or C2D1	0.92 (0.53–1.61)	0.77	1.30 (0.61–2.74)	0.50	1.14 (0.58–2.23)	0.71	1.59 (0.71–3.54)	0.26
NLR > 3.38	0.72 (0.43–1.19)	0.20	0.61 (0.33–1.13)	0.12	1.77 (1.02–3.08)	0.04	2.19 (1.16–4.12)	0.02
ALP ≥ vs < 142 U/L	0.99 (0.58–1.68)	0.97	1.15 (0.59–2.24)	0.68	0.97 (0.52–1.84)	0.94	0.54 (0.25–1.17)	0.12
PSA ≥ vs < 61 ng/mL	1.00 (0.61–1.65)	1.00	1.46 (0.80–2.66)	0.22	1.36 (0.79–2.34)	0.26	1.91 (0.99–3.68)	0.05
LDH ≥ vs < 251 U/L	0.97 (0.60–1.58)	0.90	1.35 (0.74–2.46)	0.33	1.20 (0.70–2.05)	0.51	2.88 (1.50–5.54)	0.002
Hb ≥ vs < 120 g/L	0.96 (0.54–1.73)	0.90	0.58 (0.28–1.18)	0.13	0.70 (0.42–1.17)	0.17	0.96 (0.54–1.70)	0.88
Visceral metastases (Y vs N)	1.41 (0.83–2.40)	0.21	1.09 (0.57–2.07)	0.80	1.27 (0.75–2.14)	0.37	1.78 (1.00–3.15)	0.05
ECOG PS (0 or 1 vs 2)	2.66 (0.58–12.21)	0.21	1.13 (0.14–9.50)	0.91	3.12 (0.86–11.27)	0.08	11.87 (2.71–51.96)	0.001
Presence of pain (Y vs N)	1.38 (0.77–2.46)	0.28	1.37 (0.64–2.93)	0.42	0.98 (0.54–1.78)	0.96	1.09 (0.58–2.05)	0.78

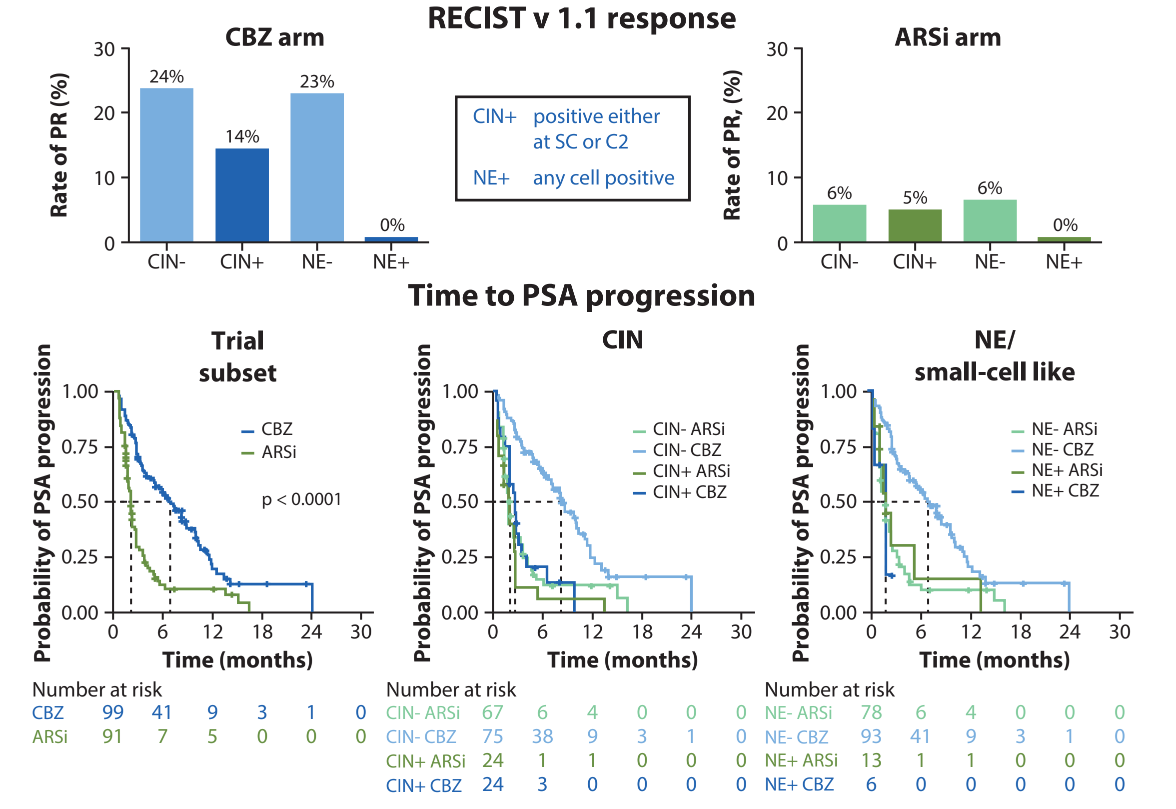
ALP, alkaline phosphatase; ARSi, androgen-signaling-targeted inhibitor; C2D1, Cycle 2 Day 1; CI, confidence interval; CIN, chromosomal instability; CTC, circulating tumor cells; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; HR, hazard ratio; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PSA, prostate-specific antigen; rPFS, radiographic progression-free survival; SC, screening.

Table 3. CTC NEPC biomarker at SC or C2D1: Multivariable risk adjusted HR for rPFS and OS

	Cabazitaxel arm				ARSi arm			
	HR_{rPFS} (95% CI)	P	HR_{OS} (95% CI)	P	HR_{rPFS} (95% CI)	P	HR_{OS} (95% CI)	P
NEPC+ at SC or C2D1	2.89 (1.00–8.37)	0.05	5.36 (1.80–15.96)	0.003	0.62 (0.26–1.46)	0.28	1.20 (0.46–3.14)	0.71
Total CTCs > median (2/mL) at SC or C2D1	1.12 (0.67–1.87)	0.67	1.64 (0.82–3.30)	0.16	1.11 (0.58–2.13)	0.75	1.60 (0.75–3.43)	0.22
NLR > 3.38	0.75 (0.45–1.24)	0.26	0.72 (0.39–1.33)	0.29	1.70 (1.00–2.87)	0.05	2.18 (1.15–4.12)	0.02
ALP ≥ vs < 142 U/L	0.94 (0.54–1.61)	0.81	1.04 (0.53–2.03)	0.91	1.00 (0.54–1.86)	1.00	0.54 (0.25–1.17)	0.12
PSA ≥ vs < 61 ng/mL	1.11 (0.67–1.84)	0.69	1.81 (0.98–3.34)	0.06	1.41 (0.82–2.41)	0.21	1.94 (1.00–3.77)	0.05
LDH ≥ vs < 251 U/L	0.85 (0.51–1.42)	0.54	1.14 (0.62–2.10)	0.68	1.23 (0.72–2.09)	0.45	2.82 (1.48–5.37)	0.002
Hb ≥ vs < 120 g/L	0.99 (0.54–1.79)	0.97	0.64 (0.31–1.34)	0.24	0.70 (0.42–1.17)	0.17	0.94 (0.53–1.66)	0.83
Visceral metastases (Y vs N)	1.34 (0.79–2.27)	0.28	1.21 (0.63–2.29)	0.57	1.23 (0.72–2.09)	0.45	1.81 (1.01–3.22)	0.05
ECOG PS (0 or 1 vs 2)	2.10 (0.42–10.45)	0.37	1.22 (0.15–10.18)	0.86	3.37 (0.91–12.55)	0.07	11.69 (2.65–51.64)	0.001
Presence of pain (Y vs N)	1.54 (0.86–2.78)	0.15	1.93 (0.89–4.18)	0.09	0.97 (0.54–1.77)	0.93	1.12 (0.59–2.13)	0.73

ALP, alkaline phosphatase; ARSi, androgen-signaling-targeted inhibitor; C2D1, Cycle 2 Day 1; CI, confidence interval; CTC, circulating tumor cells; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; HR, hazard ratio; LDH, lactate dehydrogenase; NEPC, neuroendocrine prostate cancer; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PSA, prostate-specific antigen; rPFS, radiographic progression-free survival; SC, screening.

Figure 8. Poor treatment responses are observed in patients harboring CIN/NE CTC morphologic subtypes, despite cabazitaxel treatment


RECIST v 1.1 response
CBZ arm
ARSi arm
Rate of PR (%)
24%
14%
23%
0%
6%
5%
6%
0%
CIN-
CIN+
NE-
NE+
CIN+ positive either at SC or C2
NE+ any cell positive
Time to PSA progression
Trial subset
CIN
NE/ small-cell like
Probability of PSA progression
Time (months)
CBZ
ARSi
p < 0.0001
CIN- ARSi
CIN- CBZ
CIN+ ARSi
CIN+ CBZ
NE- ARSi
NE- CBZ
NE+ ARSi
NE+ CBZ
Number at risk
CBZ 99 41 9 3 1 0
ARSi 91 7 5 0 0 0
CIN- ARSi 67 6 4 0 0 0
CIN- CBZ 75 38 9 3 1 0
CIN+ ARSi 24 1 1 0 0 0
CIN+ CBZ 24 3 0 0 0 0
NE- ARSi 78 6 4 0 0 0
NE- CBZ 93 41 9 3 1 0
NE+ ARSi 13 1 1 0 0 0
NE+ CBZ 6 0 0 0 0 0

ARSi, androgen-signaling-targeted inhibitor; C2, Cycle 2; CBZ, cabazitaxel; CIN, chromosomal instability; CTC, circulating tumor cells; NE, neuroendocrine; PR, partial response; PSA, prostate-specific antigen; RECIST, Response Evaluation Criteria in Solid Tumours SC, screening.

CONCLUSIONS

- Of 215 patients with baseline CTC in the CARD study, 25.1% were CIN+ and 9.8% were NE+.
- Presence of CIN+/NE+ CTCs was associated with poor survival and treatment response in the cabazitaxel arm; however, in the androgen-signaling-targeted inhibitor arm, poor survival and treatment responses were observed regardless of biomarker.
- Ongoing work will further molecularly characterize these CTC subtypes to guide treatment approaches in such patients.

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