The Impact of PSMA Positive Circulating Tumor Cells in Men with Metastatic Castrate-Resistant Prostate Cancer (mCRPC)

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Background and Objectives

- PSMA is encoded by the FOLH1 gene, catalyzes the cleavage of the peptide bond in glutamate-containing peptides (1), and is highly upregulated in mCRPC (>80%) (2).
- PSMA targeting molecules have been developed and derivatized with PET/CT detectable radio ligands, such as ^{68}Ga or ^{19}F in prostate cancer (2), and PSMA targeted radioligand therapies and thus PSMA(+) mCRPC represents a unique phenotype of mCRPC with therapeutic implications (3).
- In men with mCRPC, PSMA-targeted radioligand therapy with Lu177-PSMA-617 has drastically improved clinical outcomes including overall survival (3).
- Monitoring PSMA status on CTCs as part of a liquid biopsy could serve as an important predictive and pharmacodynamic biomarker in real-time (4-5).
- A liquid biopsy technique that detects and characterizes PSMA expression and heterogeneity could be useful in guiding optimal therapy and identifying individuals who will benefit the most from PSMA-directed therapies, including radioligand therapy or immunotherapeutic approaches.
- **The study's objectives were to investigate: 1) Prognostic association: PSMA positivity and heterogeneity by clinical characteristics (prior and post-therapy); and 2) Heterogeneity: describe the distribution of PSMA+/-CTC at baseline and progression in mCRPC patients treated with abi/enza (PROPHECY trial # NCT02269982).**

Methods

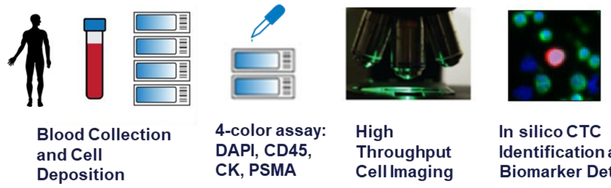
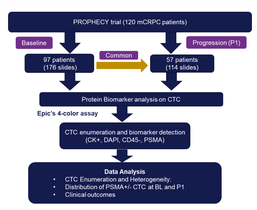


Figure 1. Epic Sciences platform identifies Circulating Tumor Cells.
- A whole blood sample was collected from the patient and shipped overnight to the Epic facility.
- RBCs were lysed; nucleated cells were resuspended;~3M nucleated cells from each sample were plated on a microscope slide.
- The slides were stained with a cocktail of antibodies: Pan cytokeratin (CK), CD45, and DAPI to assess CTC enumeration, and the fourth channel to evaluate PSMA protein expression (Abcam, clone ERP6253; cat#: 133579).
- Stained slides scanned by Epic's rapid automated fluorescent scanning method.
- Epic's proprietary algorithm analyzes cellular parameters, including PSMA expression and cell morphology, to differentiate candidate CTCs from surrounding white blood cells (WBCs). Candidate CTCs are identified and displayed in a report and are confirmed by two trained technicians.

Study Design and Baseline Characteristics



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Baseline characteristics	All Men (n=97)	CTC=0 (n=19)	PSMA Negative (<2 CTCs/mL optimal cutoff) (n=62)	PSMA Positive (≥2 CTCs/mL optimal cutoff) (n=16)
Age, median years (range)	73 (44-92)	71 (57-92)	74 (44-86)	71 (48-87)
Race: white/black/other (%)	83/12/5	84/11/5	84/11/5	75/19/6
Gleason Sum 8-10 (%)	82	74	81	56
Karnofsky Score ≥90 (%)	74	74	81	50
High Risk Features				
Hemoglobin <12 g/dl (%)	38	37	34	56
Elevated alkaline phosphatase (%)	43	11	47	69
Elevated serum LDH (%)	33	21	23	88
Prior abiraterone or enzalutamide (%)	36	47	36	25
Presence of liver or lung metastasis (%)	26	26	27	19
Presence of clinically significant pain requiring opiates (%)	28	26	24	44
CellSearch5 CTC > 5 cells per 7.5 mL (%)	43	16	39	94
Radiographic progression at entry(%)	78	68	76	100
PSA doubling time <3 months (%)	62	47	66	63
Prior docetaxel for mHSPC (%)	19	16	18	25
M1 stage at diagnosis (%)	29	26	24	50
>20 bone metastases (%)	33	5	32	69
Median Baseline PSA ng/ml (range)	18 (0.08-4190)	16 (0.23-482)	14 (0.08-1100)	58 (0.32-4190)
Epic nuclear AR-V7 positive (%)	8	0	7	25

Figure 2: Workflow and patient demographics of mCRPC men treated with ARSi from the PROPHECY trial (NCT02269982). A) In this prospective study, 154 mCRPC blood samples were used for CTC enumeration and PSMA expression detection, including baseline (BL, N=97) and paired progression (P1, N=57). **B)** The table summarizes the patient characteristics for the overall cohort for CTC biomarker detection. The prognostic association between PSMA (+) CTCs and clinical outcomes was performed, and the optimal threshold for PSMA positivity CTC was >2 CTC/mL.

PSMA Detection in CTC and Cell Line Cells

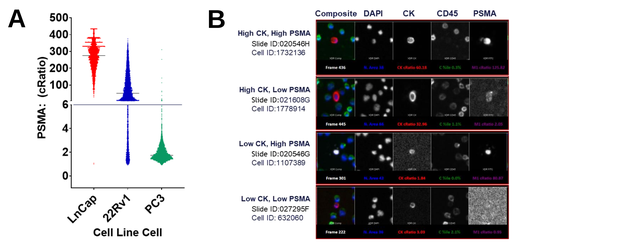


Figure 3. PSMA protein expression distributions in process control cell line cells and mCRPC patients' samples. A) PSMA testing was performed using LNCaP as a positive cell, PC3 as a negative cell, and 22Rv1 as a medium control cell. cRatio represents the ratio of a cell's intensity on a specified channel divided by the average of the median cell intensities for all frames in the same channel. **B)** CTC expressing high CK high PSMA, high CK low PSMA, low CK high PSMA, and low CK low PSMA in mCRPC patients were displayed as a CTC gallery.

Heterogeneity of CTC Enumeration and PSMA Expression in Pre- and Post-AR Therapy

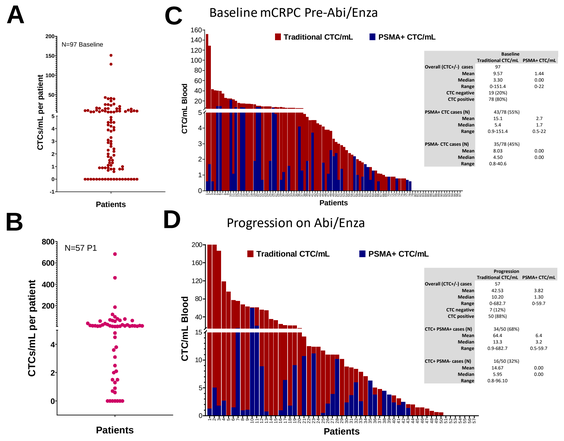


Figure 4. CTC enumeration and PSMA protein expression heterogeneity in pre- and post-abi/enza therapy. A and B) CTC enumeration at baseline (N = 97) and progression (N = 57), expressed as a CTC/mL. **C and D)** At baseline and progression, traditional CTC (CK+, CD45-, and DAPI with intact nucleus) and PSMA positive CTC/mL were shown. At pre-treatment, the overall CTC prevalence was 80% (78/97), where 55% (43/78) of the cases harbored PSMA positive CTCs (at least 1 CTC). Similarly, on progression on abi/enza, 88% (50/57) of cases detected CTCs with 68% (34/50) PSMA CTC positivity. **E)** PSMA protein expression distribution in single cell CTCs before and after therapy.

Results and Discussion

PSMA Positivity: Baseline versus Progression

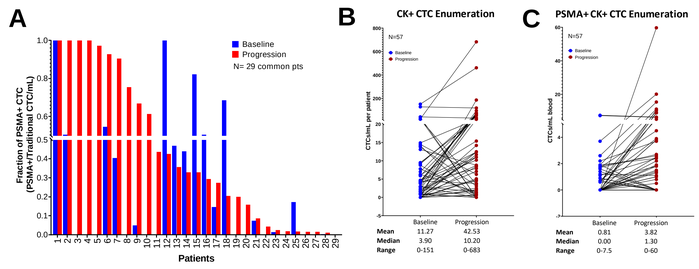


Figure 5. PSMA positivity comparison between common baseline and progression cases. A) Selection criteria; at progression, only PSMA+ CTC cases were included in the comparison, whereas at baseline, both PSMA positive and negative CTC cases were included. As a result, 34% of cases had over 50% PSMA+ CTC at progression; however, only 17% of cases harbored it at baseline. **B)** Case-by-case comparison of CTC/mL at baseline and progression (N =57). **C)** Case-by-case comparison of PSMA+ CTC/mL at baseline and progression (N =57).

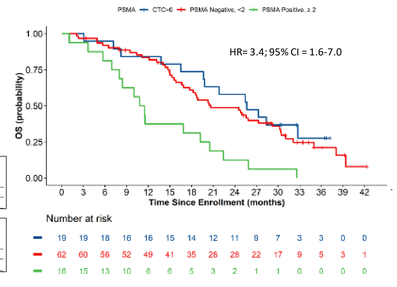
Clinical Outcomes: Relationship between PSMA+ CTC Enumeration with Overall Survival (OS) and Radiographic Progression-Free Survival (rPFS)

A Prevalence of PSMA/Traditional CTCs positivity at baseline and progression

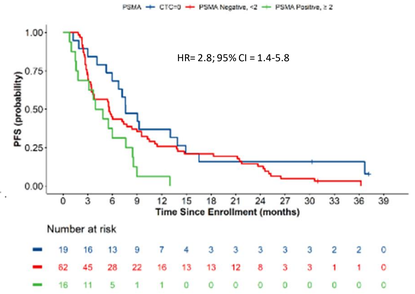
	Baseline N (%)	P1 N (%)
PSMA on median cutoff	97	57
CTC=0	19 (19.6)	7 (12.3)
PSMA Negative (PSMA<1)	47 (48.4)	18 (31.6)
PSMA Positive (PSMA>=1)	31 (32.0)	32 (56.1)
PSMA on optimal cutoff	97	57
CTC=0	19 (19.6)	7 (12.2)
PSMA Negative (PSMA<2)	62 (63.9)	27 (47.4)
PSMA Positive (PSMA>=2)	16 (16.5)	23 (40.4)

	Baseline N (%)	P1 N (%)
Traditional CTCs on median cutoff	97	57
CTC=0	19 (19.6)	7 (12.3)
CTC Negative (0<CTC<5)	41 (42.3)	14 (24.5)
CTC Positive (CTC>=5)	37 (38.1)	36 (63.2)
Traditional CTCs on optimal cutoff	97	57
CTC=0	19 (19.6)	7 (12.3)
CTC Negative (0<CTC<1)	10 (10.3)	3 (5.3)
CTC Positive (CTC>=1)	68 (70.1)	47 (82.4)

B OS Univariate Analysis: Kapan-Meier Curves by Baseline PSMA on optimal cutoff



C PFS Univariate Analysis: Kapan-Meier Curves by Baseline PSMA on optimal cutoff



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OS Multivariable Analysis: PSMA
adjusting on prior treatment, riskscore, continuous CTC variable

	Cutoff (>=)	N	Event	Hazard Ratio	LB	UB
PSMA Positive (optimal cutoff)	2	88	67	2.96	1.13	7.79

PFS Multivariable Analysis: PSMA
adjusting on prior treatment, riskscore, continuous CTC variable

	Cutoff (>=)	N	Event	Hazard Ratio	LB	UB
PSMA Positive (optimal cutoff)	2	88	86	2.28	0.90	5.76

E

CTC Biomarker Combination	Number	Percent (%)
CTC=0/ARV7-/CIN-/NE-	15	15.5
CTC=0/ARV7-/CIN+/NE-	4	4.1
PSMA-/ARV7-/CIN-/NE-	47	48.5
PSMA-/ARV7+/CIN-/NE-	2	2.1
PSMA-/ARV7-/CIN+/NE-	11	11.3
PSMA-/ARV7+/CIN+/NE-	2	2.1
PSMA+/ARV7-/CIN-/NE-	1	1.0
PSMA+/ARV7-/CIN+/NE-	6	6.2
PSMA+/ARV7+/CIN-/NE-	3	3.1
PSMA+/ARV7-/CIN+/NE+	5	5.2
PSMA+/ARV7+/CIN+/NE+	1	1.0
Total	97	100.0

Figure 6. Depicts the association between PSMA+ CTC enumeration and OS and rPFS. The associations of PSMA+ CTC enumeration with overall survival (OS) and radiographic progression-free survival (rPFS) were explored using Cox modeling. **A)** The prevalence of PSMA and traditional CTC positivity at baseline and progression, with the median and optimal cutoffs represented. **B)** The median OS for CTC 0 (reference), PSMA-CTC, and PSMA+CTC, respectively, were 26, 21, and 11 months. **C)** For CTC 0 (reference), PSMA-CTC, and PSMA+CTC, the median rPFS was 8, 6, and 4 months, respectively. Both PSMA+ and – groups are CTC positive. **D)** OS and rPFS multivariate analysis of PSMA using optimal cutoff. **E)** CTC biomarker group incidence by PSMA optimal cutoff (AR-v7, chromosomal instability (CIN), and neuroendocrine (NE) at baseline (N=97 cases).

Conclusions

- ✓ We quantified PSMA CTC heterogeneity in mCRPC men before and following progression on abi/enza therapy, finding an increase in PSMA CTC detection upon progression on AR therapy.
- ✓ PSMA+CTCs were found to be a poor predictor of both OS (hazard ratio (HR) = 3.4; 95% CI = 1.6-7.0) and rPFS (HR = 2.8; 95% CI = 1.4-5.8) in univariate analyses.
- ✓ Adjusting for prior therapy, Halabi risk score, and CTC, HRs for OS and rPFS PSMA+ CTC+ were 3.0 (95% CI = 1.1–7.8) and 2.3 (95% CI = 0.9–5.8) in multivariate analyses.
- ✓ We observed PSMA expression heterogeneity regardless of CTC CIN, NEPC or AR-V7 phenotype.
- ✓ The CTC and PSMA+CTC enumerations were adversely prognostic, and this assay could be useful in selecting patients for PSMA-targeted therapies in the early stages of disease progression.
- ✓ The relationship between PSMA CTC enumeration and heterogeneity characterization with clinical outcomes in the context of PSMA-directed therapy will be further explored in future research.

References

1- Jones W et al, Cancers (Basel) 2020. 2- Lawhn-Heath C et al, Radiology 2021. 3- Sartor O et I, NEJM 2021. 4- Armstrong et al, JCO 2019. 5- Gupta et al, MCR 2021.

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