Circulating tumour cell (CTC) characteristics associated with survival outcomes in metastatic castration-resistant prostate cancer (mCRPC) in a randomised trial of enzalutamide with or without [^{177}Lu]Lu-PSMA-617 (ENZA-p; ANZUP 1901)

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Background

- The randomised Phase II ENZA-p trial demonstrated that the addition of [^{177}Lu]Lu-PSMA-617 (LuPSMA) to enzalutamide (ENZA) improved PSA-progression-free survival (PSA-PFS) and overall survival (OS) compared with ENZA monotherapy in first-line mCRPC.
- ENZA and LuPSMA have distinct mechanisms of action and resistance, and therefore predictive biomarkers for this combination may differ from those relevant to either therapy alone.
- Previous studies examining LuPSMA in combination with other treatments, such as olaparib (LuPARP) and pembrolizumab (PRINCE) in mCRPC, have suggested that certain circulating tumor cell (CTC) features may be associated with PSMA PET volume and PSA responses to treatment.
- Here, we present findings from the CTC translational sub-study of the ENZA-p trial, which assessed CTC features using the Epic Sciences Assay to identify potential biomarkers of response to ENZA ± LuPSMA.

Study Design and Methods

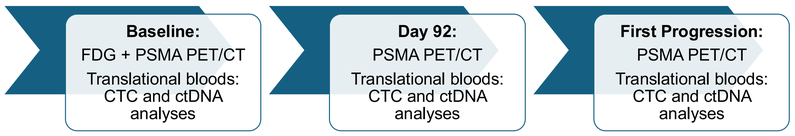

Figure 1. ENZA-p trial translational samples

Participants (pts) with progressive mCRPC were randomized 1:1 to ENZA alone or ENZA + LuPSMA. Translational research blood samples for central CTC analyses were collected in Streck Cell-Free DNA BCT® tubes at baseline, Day 92, and at the time of first confirmed PSA or radiologic progression.

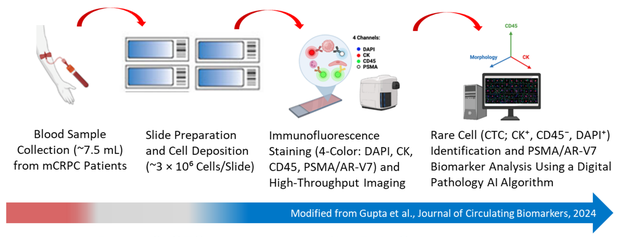

Figure 2. CTC analysis using the Epic Sciences Assay

CK⁺CD45⁻DAPI⁺ CTCs were enumerated from whole blood and characterized for PSMA and AR-V7 expression. The AR-V7 nuclear-to-cytoplasmic (N/C) ratio was calculated, with a cutoff of 4.96 used to define nuclear localisation. "Agnostic AR-V7" refers to AR-V7 positivity irrespective of subcellular localisation. Associations between CTCs per mL (CTC/mL) and the proportion of PSMA⁺ CTCs with PSA-PFS and OS were evaluated using Cox regression, with treatment arm effect modification assessed via an interaction term.

Baseline Participant Characteristics

Table 1: Baseline characteristics, n (%) or median (IQR).

	All (n=147)	ENZA (n=73)	ENZA + LuPSMA (n=74)
Age, years	71 (64 – 76)	71 (62 – 76)	71 (66 – 77)
Prognostic Biomarkers			
PSA, ng/ml	39 (13 – 75)	32 (13 – 83)	41 (16 – 75)
Haemoglobin, g/dL	131 (121 – 139)	131 (121 – 137)	132 (122 – 140)
LDH ≥ ULN	27 (18)	17 (23)	10 (14)
ALP ≥ ULN	67 (46)	35 (48)	32 (43)
Albumin < 35 g/L	13 (8.8)	6 (8.2)	7 (9.5)
Sites of Metastases			
Lymph nodes	92 (63)	46 (63)	46 (62)
Bone	134 (91)	65 (89)	69 (93)
Lung	12 (8.2)	5 (6.8)	7 (9.5)
Liver	3 (2.0)	2 (2.7)	1 (1.4)
PSMA PET			
SUVmax	37 (27 – 53)	40 (27 – 58)	37 (27 – 49)
SUVmean	7.7 (6.6 – 10.0)	7.7 (6.5 – 9.3)	7.7 (6.6 – 10.0)
Tumour Volume, mL	235 (76 – 713)	233 (75 – 770)	244 (96 – 583)

Proportion of PSMA+ CTCs decreases with treatment

Table 2. Summary of serial CTC features, median (IQR).

	Baseline		Day 92		Progression	
Treatment Arm	ENZA	ENZA + LuPSMA	ENZA	ENZA + LuPSMA	ENZA	ENZA + LuPSMA
Pts CTC evaluated (n)	73	74	60	76	37	32
CTC/mL whole blood	3.0 (0.8-8.6)	1.8 (0.6-6.5)	1.1 (0-4.0)	0.5 (0-3.3)	2.0 (1.0-5.0)	1.6 (0.7-3.0)
% with any PSMA⁺ CTC	56	59	33	18	65	44
% PSMA⁺ of total CTC*	46 (0-87)	50 (0-83)	1.1 (0-50)	0 (0-19)	35 (0-75)	1.4 (0-50)
Pts AR-V7 evaluated (n)	64	70	60	71	36	31
% Nuclear >4.96	13	1.4	5.0	1.4	19	13

*In patients with CTC >0

Serial CTC trends on treatment

- CTCs were assessed at baseline in 147/160 (92%) of patients that received treatment on the ENZA-p study, and 136 (85%) and 69 (43%) patients at Day 92 and Progression.
- CTC characteristics were similar between the treatment groups at baseline, except for AR-V7 positivity, which was higher in the ENZA alone arm (13% vs. 1.4% with nuclear N/C ratio >4.96).
- Median CTC/mL and the proportion of PSMA+ CTCs decreased at Day 92 in both arms, with most CTCs being PSMA- at Day 92. These trends were more notable in the ENZA + LuPSMA arm.
- At first progression, the proportion of PSMA+ CTCs remained low in the ENZA + LuPSMA arm compared to the ENZA alone arm (1.4% vs. 35% of total CTCs).

Higher baseline CTC/mL and proportion of PSMA+ CTCs were associated with worse outcomes

Associations with treatment outcomes

- Rates of nuclear AR-V7 positivity were low overall, precluding further meaningful analysis
- No associations with treatment outcomes were seen for Day 92 or Progression analyses

PSA-Progression-free survival

- 122 PSA progression events occurred (69 ENZA, 53 ENZA + LuPSMA) at a median follow-up of 34 months (95% CI 32 – 36).
- Median PSA-PFS was 11 (95% CI 9 - 11), 8 (95% CI 4 - 10), and 13 months (95% CI 11 - 17) for the overall analysis, in the ENZA and ENZA + LuPSMA arms, respectively.

Overall Survival

- 91 deaths occurred (50 ENZA arm, 41 in the ENZA + LuPSMA)
- Median OS were 26 (95% CI 22 - 31) and 33 months (95% CI 27 - 35) in the ENZA and ENZA + LuPSMA arms, respectively.

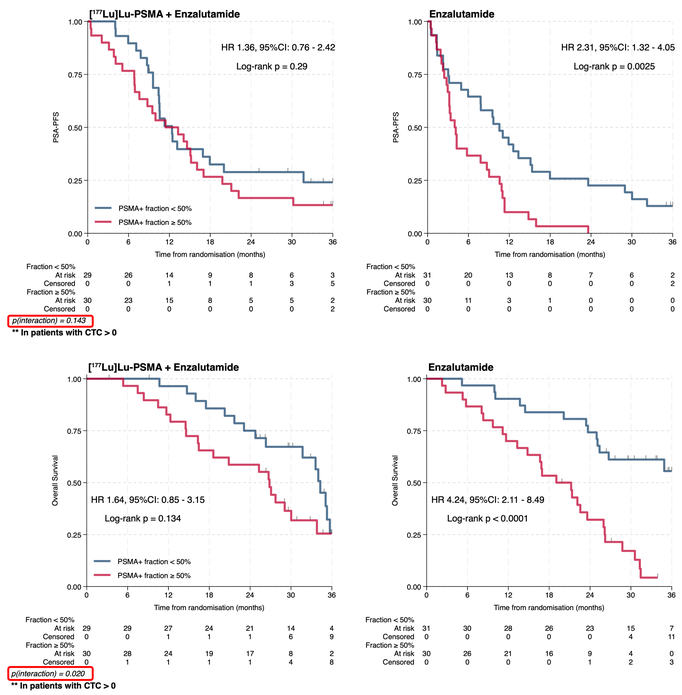

Figure 3. Kaplan Meier plot by treatment arm for PSMA⁺ CTC fraction ≥ 50 vs. < 50% by treatment arm for PSA-PFS (top) and OS (bottom)

Table 3. Baseline CTC characteristics and outcome associations, n (%) or median (IQR).

	Overall	ENZA	ENZA + LuPSMA
CTC Enumeration			
Pts evaluated (n)	147	73	74
CTC/mL whole blood	2.5 (0.7 – 8.6)	3.0 (0.8 – 8.6)	1.8 (0.6 – 6.5)
CTC/mL ≥ 5	53 (36)	29 (40)	24 (32)
PFS-PFS HR (95% CI) Interaction p=0.33	2.09 (1.45 – 3.02)	2.45 (1.49-4.02)	1.86 (1.06-3.24)
OS HR (95% CI) Interaction p=0.45	2.19 (1.44-3.32)	2.42 (1.39-4.24)	1.82 (0.96-3.42)
PSMA Status			
Pts evaluated (n)	147	73	74
PSMA⁺ CTC > 0*	85 (71)	41 (67)	44 (75)
% PSMA⁺ CTC*	49 (0 – 85)	46 (0 – 87)	50 (0 – 83)
% PSMA⁺ CTC ≥ 50*	60 (50)	30 (49)	30 (51)
PFS-PFS HR (95% CI) Interaction p=0.14	1.65 (1.12-2.43)	2.31 (1.32-4.05)	1.36 (0.76-2.42)
OS HR (95% CI) Interaction p=0.02	2.62 (1.65-4.18)	4.24 (2.11-8.49)	1.64 (0.85-3.15)
AR-V7 Status			
Pts evaluated (n)	134	64	70
% Nuclear >4.96	9 (6.7)	8 (13)	1 (1.4)
% Agnostic >4.96	17 (13)	13 (20)	50 (7.1)

*In patients with CTC >0

Conclusions

- Baseline CTC/mL ≥ 5 vs <5 was prognostic for shorter PSA-PFS and OS, independent of treatment arm.
- PSMA⁺ fraction ≥50% vs <50% was associated with shorter PSA-PFS and OS in the ENZA arm, but not in the ENZA + LuPSMA arm.
- These data suggest that PSMA⁺ CTC fraction ≥ 50% may be predictive of a benefit for adding LuPSMA to ENZA in pts with detectable CTCs.
- Further analyses with molecular imaging parameters and ctDNA are underway to assess if further liquid biopsy characterisation has additive predictive value to PET imaging biomarkers.

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