**Integrated CTC- and EV-based detection of PSMA protein and efficacy of $^{177}$Lu-PSMA-617 radioligand therapy.**

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**Background:** Blood-based predictive biomarkers of sensitivity to $^{177}$Lu-PSMA-617 are lacking, and may facilitate clinical decisions. Here, we studied whether integrated PSMA protein detection in circulating tumor cells (CTCs) and extracellular vesicles (EVs) is associated with outcomes in patients receiving $^{177}$Lu-PSMA therapy.

**Methods:** We enrolled 100 metastatic castrate-resistant prostate cancer (mCRPC) pts who were candidates for $^{177}$Lu-PSMA into a prospective biomarker trial. Blood samples were collected for CTC and EV analysis at baseline, at the time of response, and at progression. Baseline characteristics included serum PSA, alkaline phosphatase (ALP), hemoglobin, albumin, and radiographic tumor burden. PSMA+ CTCs were enumerated using an AI-empowered holographic imaging platform combined with in-flow protein marker analysis (Astrin Biosciences, St. Paul, MN); PSMA protein was quantified in plasma EVs using shotgun proteomics via mass spectrometry (Arafa et al., *Cancers* 2024; 16: 4261). We assessed the impact of PSMA+ CTCs and EV-derived PSMA protein on $PSA_{50}$ responses, PFS, and OS. Multivariable Cox regressions were used to adjust for baseline PSA, ALP, and hemoglobin. Exploratory analyses of other EV-derived proteins were also conducted.

**Results:** Of 100 enrolled pts, 47% had Gleason sum 9-10, 62% had > 10 bone mets, 12% had visceral mets, 72% had received ≥3 prior systemic therapies, and median PSA was 57 (range 1.5–5,000) ng/mL. High PSMA+ CTC counts ( > median) were associated with shorter overall survival (OS) (HR 2.71, 95%CI 1.18–6.21, p = 0.02). $PSA_{50}$ response rates were similar for those with high and low PSMA+ CTC counts (39% vs 42%, p = 0.8). Shotgun proteomics from plasma EV samples identified > 11 000 unique proteins, of which 12% represented the cell surfaceome. EV-PSMA protein correlated with baseline PSA, ALP, and tumor burden (all p < 0.05). High EV-PSMA protein ( > median) was associated with worse OS (1.81, 95%CI 0.97–3.35, p = 0.06). $PSA_{50}$ response rates were similar for those with high and low EV-PSMA protein (48% vs 42%, p = 0.5). After multivariate adjustment, nonsignificant trends for shorter OS persisted for pts with high PSMA+ CTCs (HR 1.71, 95%CI 0.72–4.05) and high EV-PSMA levels (HR 1.49, 95%CI 0.78–2.84). Worse OS was also observed in pts with high EV levels of B7-H3 (HR 2.85, 95%CI 1.58–5.14, p = 0.002), Trop-2 (HR 2.23, 95%CI 1.22–4.05, p = 0.008), and STEAP1 (HR 1.69, 95%CI 0.93–3.06, p = 0.08) proteins.

**Conclusions:** In mCRPC pts receiving $^{177}$Lu-PSMA, high PSMA+ CTC counts and high EV-derived PSMA levels portended poor survival. PSMA protein may be a novel blood-based biomarker of $^{177}$Lu-PSMA sensitivity, facilitating treatment decisions, with relevance for other PSMA-targeting strategies. The robust detection and prognostic impact of additional cell-surface proteins (*e.g.* B7-H3, Trop-2, STEAP1) may fuel the development of alternative novel therapeutics.