Title: Integrated CTC- and EV-based detection of PSMA protein and efficacy of 177 Lu-PSMA-617

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Background: Blood-based predictive biomarkers of sensitivity to 177 Lu-PSMA-617 are lacking, and may facilitate clinical decisions.
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; PSMA protein was quantified in plasma EVs using shotgun proteomics via mass spectrometry. 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.
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.