

Uncertainty-aware functional profiling of metastatic prostate cancer from cell-free DNA

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Background: Functional molecular phenotypes that determine treatment response in metastatic castration-resistant prostate cancer (mCRPC) are poorly captured by genomic assays, while metastatic tissue biopsies are both invasive and difficult to acquire serially. We developed Triton and Proteus as a deep-learning framework to infer tumor gene expression from standard cell-free DNA (cfDNA) whole-genome sequencing (WGS), and integrated this framework with longitudinal genomic, imaging, and clinical profiling of patients receiving ¹⁷⁷Lu-PSMA-617.

Methods: Triton extracts base-pair-resolution promoter signals and regional promoter and gene-body fragmentation and nucleosome features from cfDNA. Proteus then integrates these features with sequence and gene annotations to predict expression as probability distributions, enabling uncertainty-aware withholding and confidence-based weighting. Proteus was trained and evaluated using patient-derived xenograft admixtures and patient cohorts with matched tumor RNA-seq across prostate, lung, and bladder cancers. Separately, the ¹⁷⁷Lu-PSMA-617 cohort comprised 158 patients with mCRPC; 548 longitudinal ultra-low-pass WGS samples were available from 150 patients. Baseline and on-treatment ctDNA, copy-number features, PSMA imaging, and outcomes were analyzed. Proteus was applied to a baseline WGS subset (n=120) and paired pre/post-treatment samples (n=7).

Results: In the external CRPC West Coast Dream Team cohort, Proteus maintained high transcriptome-wide concordance with tumor RNA-seq (mean $\rho=0.86$), recovered patient-specific gene variation better than prior cfDNA methods, and classified active Cancer Surfaceome and Tier 1 druggable genes with AUROCs of 0.92-0.94. Withholding of model-reported low-confidence calls improved mean positive and negative predictive values, sensitivity, and specificity. In the ¹⁷⁷Lu-PSMA-617 cohort, detectable cycle-2 ctDNA was associated with shorter PSA-PFS, SPECT-PFS, and overall survival. Higher chromosome 8 entropy and tyfonas were also associated with adverse outcomes, with focal MYC/RAD21 amplifications among recurrent chromosome 8 events. Among 115 patients with complete baseline Proteus and survival data, higher G2M-checkpoint and spermatogenesis scores were significantly associated with shorter overall survival after adjustment for tumor fraction. These proliferative and plasticity-associated programs coexisted with retained FOLH1/PSMA and alternative targets CD276/B7-H3 and TACSTD2/TROP2, arguing against uniform PSMA loss or wholesale neuroendocrine transition. Paired analysis identified post-treatment stress, metabolic, and plasticity programs and nominated TPBG as a candidate treatment-emergent target.

Conclusions: Longitudinal cfDNA WGS can jointly quantify disease burden, genome evolution, and confidence-aware tumor gene expression during radioligand therapy. This integrated functional liquid-biopsy framework identifies early response, prognostic, and candidate resistance features while enabling serial molecular assessment without repeated requiring repeated tissue biopsies.

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