INTEGRATIVE DUAL ctDNA 5mC/5hmC METHYLOMICS AND CLONAL RECONSTRUCTION INFER TUMOR TRANSCRIPTION AND RESISTANCE PHENOTYPES IN METASTATIC PROSTATE CANCER

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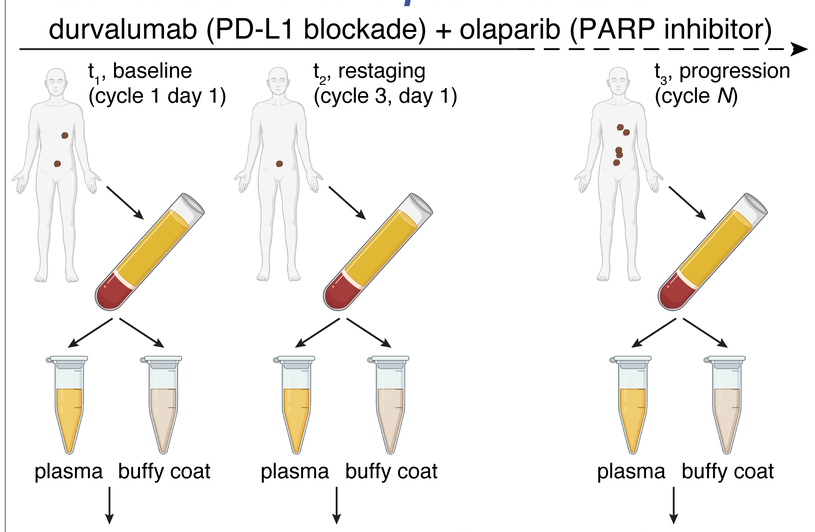
Serial plasma dual 5mC/5hmC profiling moved liquid biopsy beyond mutation detection toward transcriptome-like tumor-state and resistance monitoring in mCRPC

WHY THIS STUDY

Phase 2 cohort with serial plasma collection



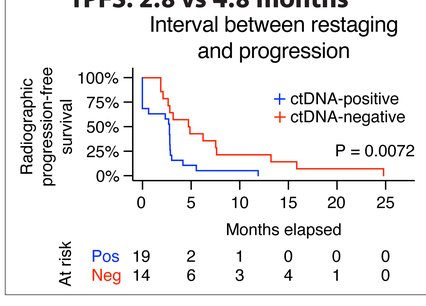
Cohort **33** *baseline plasma samples*

Serially **16** *complete 3-time-point series*

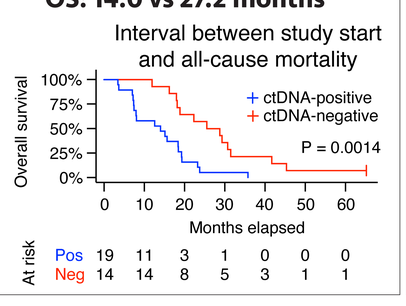
Controls **9** *buffy coat controls across 3 timepoints*

Genomics alone was prognostic but biologically incomplete; only a few recurrent baseline features showed associations with outcomes

rPFS: 2.8 vs 4.8 months

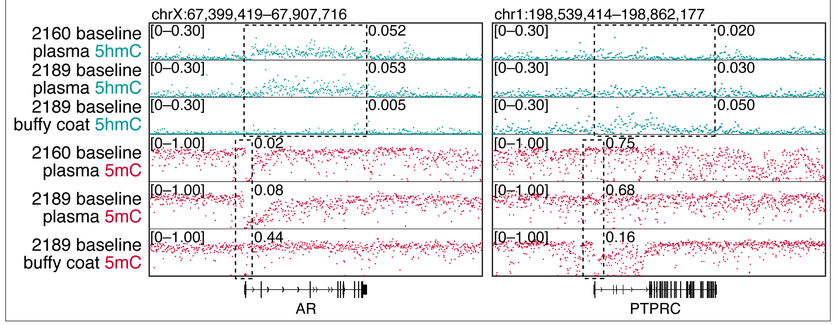


OS: 14.0 vs 27.2 months

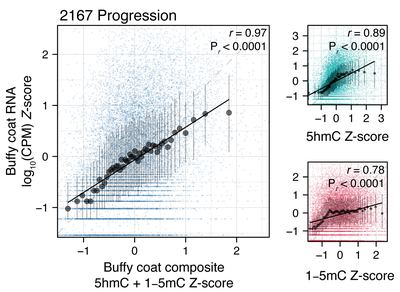


ASSAY LOGIC

In ctDNA, promoter 5mC falls while gene body 5hmC rises at active tumor genes

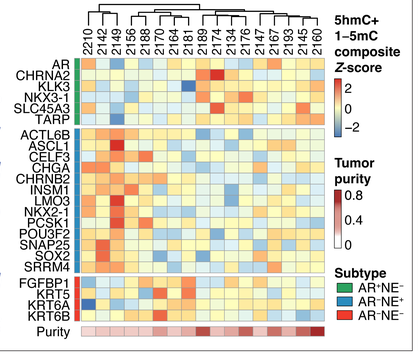


VALIDATION



Composite 5hmC + 5mC background-corrected, purity-adjusted Z-score tracked with gene expression better than either mark alone

Applied to baseline cfDNA, dual 5mC/5hmC recovered expected lineage states driven by androgen receptor, neuroendocrine and double-negative programs

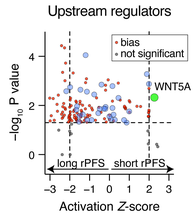


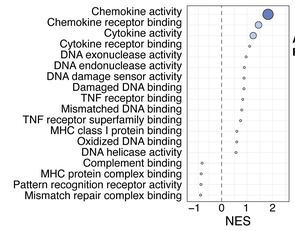
BASELINE BIOLOGY

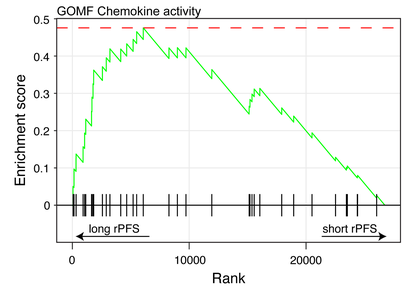
Poor responders showed a WNT5A-linked, less inflamed state

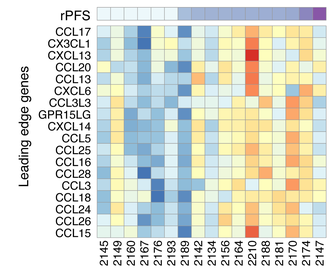
WNT5A stood out at baseline

In shorter-rPFS tumors, WNT5A emerged as the top activated upstream regulator, linking poor response to noncanonical WNT signaling.









LONGITUDINAL RESISTANCE

Two resistance routes emerged on longitudinal profiling

Progression on therapy

Adaptive / clonal remodeling

greater CCF shift
persistent AR / FOXP1

Rapid progression / intrinsic resistance

early progression on therapy
AR-independent / stem-like