EMORY UNIVERSITY SCHOOL OF MEDICINE

Robust characterization of response & resistance to 177Lu-PSMA-617 in mCRPC by plasma-based epigenomic profiling

Abstract #5074

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BACKGROUND

Metastatic castration-resistant prostate cancer (mCRPC) is an advanced stage of prostate cancer with poor outcomes and limited therapeutic options.

The FDA-approved radiopharmaceutical therapy 177Lu-PSMA-617 targets PSMA, offering a novel approach for mCRPC treatment.

However, response to therapy is heterogeneous, and resistance mechanisms remain poorly understood.

Benchmarking molecular signatures to clinical outcomes could provide critical insights into predicting response and resistance to therapy.

We applied a multimodal epigenomic liquid biopsy platform to profile tumor-specific transcriptional activation and resistance pathways in plasma from patients treated with 177Lu-PSMA-617.

METHODS

Baseline plasma samples were collected from patients with mCRPC at the time of PSMA PET imaging and initiation of 177Lu-PSMA-617 therapy.

Epigenomic profiling of genome-wide signals from promoters, enhancers, and DNA methylation was performed on 1mL of plasma (N=81, ctDNA ≥0.5%).

We tested the association of androgen receptor (AR) activity, neuroendocrine-ness (NEPC-ness), and pathway activities with response using Cox proportional hazards models (CoxPH).

Androgen receptor (AR) activity assessed by enhancer signals at AR binding sites, NEPC-ness was assessed via gene-activity of NEPC marker genes, and pathway activities were calculating using gene set variation analysis (GSVA) scores based on gene-proximal epigenomic signals.

Response to 177Lu-PSMA-617 was assessed by clinical-radiographic progression free survival (crPFS), PSA-PFS, time to next treatment (TTNT) and overall survival (OS).

RESULTS

Figure 1. Overview of Precede's epigenomics liquid biopsy platform

Comprehensive epigenomic platform offers dynamic resolution into target & pathway biology from 1 mL of plasma

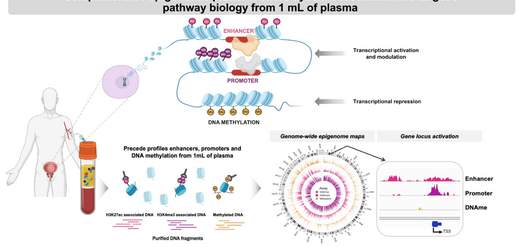


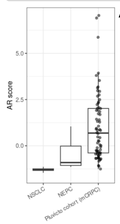
Table 1 – Clinical Cohort Overview

COHORT DESCRIPTION		Total N=81
Patient characteristics		
Age in years, median(range)		72.2 (52.8-85.7)
Race, number (%)	White	70 (86.2%)
	Black or AA	6 (7.4%)
	Hispanic	3 (3.7%)
Disease characteristics		
Stage at Diagnosis, number (%)	Localized	40 (49.4%)
	Metastatic	41 (50.6%)
Sites of Disease on PSMA PET, number (%)	Lymph Node	54 (66.7%)
	Bone	80 (98.8%)
	Lung	10 (12.4%)
	Liver	14 (17.3%)
	Other	11 (13.6%)
# of Prior Line of Systemic Therapies for Metastatic Disease, median (range)		4 (2-7)
PSA at Plasma in ng/mL, median (range, N=81)		91.7 (0-2144)
PSMA PET SUV_{mean}, median (range, N=41)		6.9 (2.8-18.3)
Days from PSMA PET scan to plasma collection, median (range, N=41)		66 (20-164)
Clinical Outcomes		
PSA-PFS in days, median (range)		122.5 (22-587)
crPFS in days, median (range)		126 (26-593)
Time to next treatment in days, median (range)		168 (26-597)
Overall Survival in days, median (range)		260 (26-942)

Cell free DNA derived from tumors exists in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against H3K27ac marking active enhancers, H3K4me3 marking active promoters and DNA methylation are used to enrich for associated DNA fragments from 1mL plasma and sequenced to define genome-wide epigenomic maps that capture the underlying transcriptional state of the tumor cells.[1]

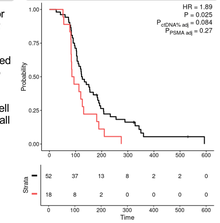
Figure 3. Plasma epigenomic profiles of AR & neuroendocrine programs reveal heterogeneity driving response to 177Lu-PSMA-617 therapy

A) AR score associates with expected cancer histology

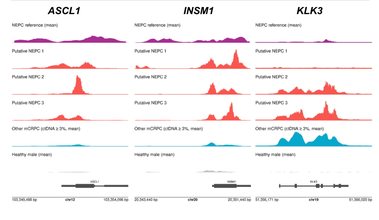


A An androgen receptor (AR) pathway activity score based on enhancer signal at canonical AR-regulated genes was applied to the mCRPC patient plasma samples (≥1.7% ctDNA), as well as cohorts of non-small cell lung cancer (NSCLC) and neuroendocrine prostate cancer (NEPC) plasma samples for comparison.

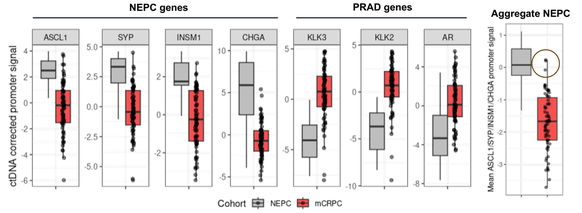
B) High AR scores associate with worse response to 177Lu-PSMA-617



D) Regulatory signal at canonical NEPC and PRAD loci in putative NEPC samples suggests subtype heterogeneity



C) Epigenomic signal at NEPC genes reveals putative NEPC samples missed by histology



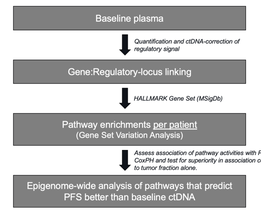
B Samples were stratified into AR-high (≥top quartile) and AR-low (<top quartile) groups. A CoxPH model was fit to evaluate the association between group and crPFS. HR values measure relative risk of AR-high group compared to AR-low group. We also tested whether AR group added significant explanatory power over ctDNA% or over predicted PSMA PET SUV_{mean} via analysis of deviance tests comparing a CoxPH model fit using AR group and ctDNA group (≥/< median) or PSMA group (Fig 2B) to CoxPH models fit using only ctDNA group ($P_{cDNA\%\ adj}$) or PSMA-group ($P_{PSMA\ adj}$). AR score provided no incremental benefit beyond PSMA scores for stratifying outcome.

D ctDNA-normalized promoter epigenomic signal at *ASCL1*, *INSM1* and *KLK3*. Putative NEPC samples 1/2/3 display higher activation at *ASCL1* and *INSM1* compared to the remainder of the mCRPC cohort. In addition, while putative NEPC 1 has low signal at *KLK3*, putative NEPCs 2 and 3 have high signal at the AR-regulated *KLK3* gene, suggesting potential mixed histology.

C Normalized and ctDNA-corrected promoter epigenomic signal at genes associated with neuroendocrine (NEPC) vs prostate adenocarcinoma (PRAD) subtypes. An independent cohort of NEPC samples was used as reference. Circle in the rightmost boxplot highlights mCRPC patients that may potentially present with neuroendocrine disease. Only plasma samples with ctDNA ≥3% were used for this analysis.

Figure 5. Wnt, metabolic and immune signaling are associated with response to 177Lu-PSMA-617 therapy

A) Pathway analysis workflow

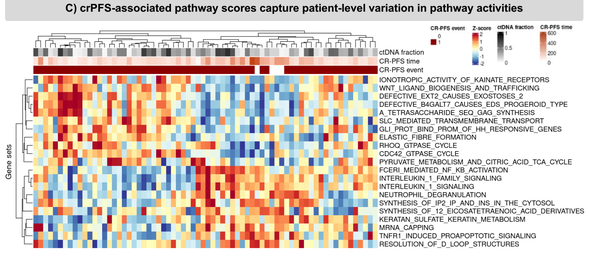


A Analysis workflow used to assess pathway activity scores per patient (≥3% ctDNA) from individual epigenomic features (enhancers) by adapting the gene set variation analysis (GSVA[4]) method.

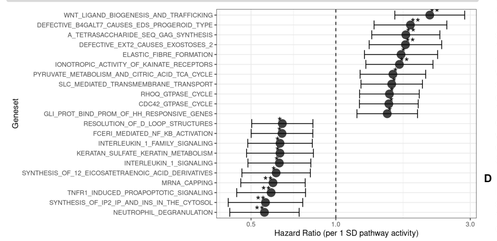
B For each pathway (MSigDb REACTOME[5,6]) scored, we tested whether the continuous pathway activity value had a significant association with the clinical endpoints measured via CoxPH models. Furthermore, we tested whether these associations provided additional explanatory value beyond ctDNA% via analysis of deviance. Pathways shown are those that pass statistical significance (FDR ≤10%) for at least one endpoint and FDR ≤20% for at least two endpoints.

B) Pathway activities associate with response to treatment across multiple clinical endpoints

C) crPFS-associated pathway scores capture patient-level variation in pathway activities



D) Less aggressive PRAD is associated with 177Lu-PSMA-617 crPFS



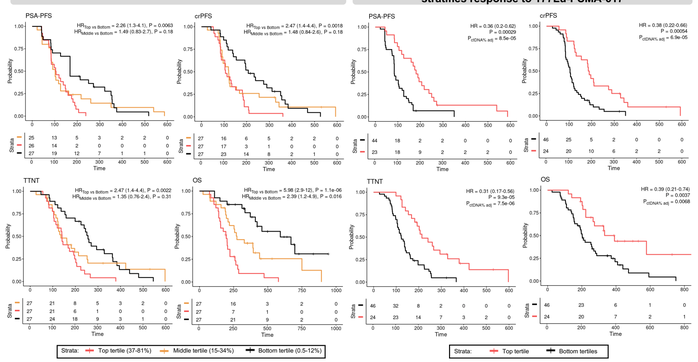
C Heatmap display of GSVA-derived pathway activity scores in baseline plasma samples for pathways found to have a significant association with crPFS (FDR ≤10%). Scores were converted to Z-scores to facilitate inter-pathway comparisons. Clustering was performed on distance matrices using Spearman (1-ρ).

D Here we show the HRs for all the REACTOME pathways found to be significantly associated with crPFS, not filtered on the significance of other endpoints as in (B).

Figure 2. Plasma epigenome-based detection of ctDNA and PSMA transcriptional activation associates with clinical outcomes

A) High ctDNA fraction is associated with worse outcome

B) PSMA PET SUV_{mean} predicted from the plasma robustly stratifies response to 177Lu-PSMA-617

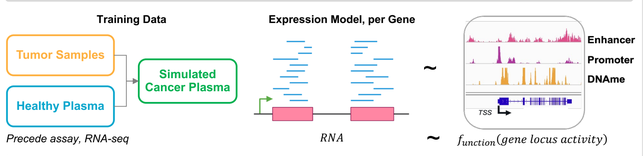


(Left) Patient plasma samples were stratified into ctDNA-high (≥ median) and ctDNA-low (< median) groups. We tested for a ctDNA group association across four clinical endpoints: progression-free survival (PFS) via PSA (PSA-PFS, recorded time from start of therapy to first PSA increase that is >25% and >2ng/mL above the nadir and is confirmed by a second value 3 weeks later), clinical/radiographic PFS (crPFS), time to next treatment (TTNT), and overall survival (OS). HR values measure relative risk of ctDNA-high group compared to ctDNA-low group.

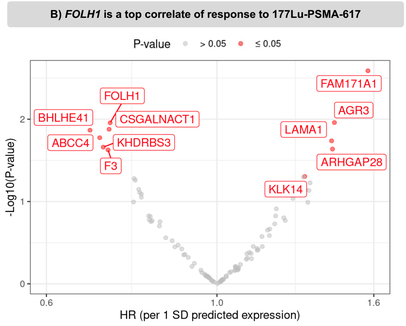
(Right) Analysis similar to (A) but we instead stratified patients using predicted PSMA PET SUV_{mean}[2]: Patients in the top tertile of PSMA values were compared to the bottom two tertiles via CoxPH. We also tested whether PSMA value added significant explanatory variance beyond ctDNA group by comparing models with ctDNA group to models with PSMA group and ctDNA group via analysis of deviance ($P_{ctDNA\%\ adj}$). Only plasma samples with ctDNA ≥3% were used for this assessment.

Figure 4. *FOLH1* and ECM gene expression are associated with 177Lu-PSMA-617 efficacy

A) Training models to predict tumor gene expression from plasma epigenomic signals



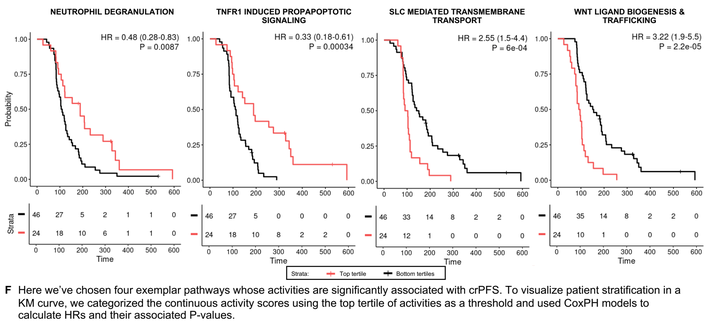
B) *FOLH1* is a top correlate of response to 177Lu-PSMA-617



A Schematic representation of the approach used to generate data to train gene locus expression models. Simulated cancer plasma is created by mixing tumor-derived chromatin with healthy plasma. (Right) For each gene, an expression model is trained to predict tumor RNA transcript levels as a function of gene locus activity in plasma, integrating enhancer signals, promoter accessibility, and DNA methylation (DNAme).[3]

B Gene expression models were applied to the baseline plasma samples to estimate gene expression for >300 genes. Continuous expression estimates were used in CoxPH models to test association with crPFS. Y-axis values are the P-values from analysis of deviance tests comparing CoxPH models fit using predicted expression and log2(ctDNA fraction) to CoxPH models fit using only log2(ctDNA fraction). Only samples with ctDNA ≥3% were used for this analysis.

F) Exemplar KM Curves for pathways activities associated with response to 177Lu-PSMA-617 via crPFS



F Here we've chosen four exemplar pathways whose activities are significantly associated with crPFS. To visualize patient stratification in a KM curve, we categorized the continuous activity scores using the top tertile of activities as a threshold and used CoxPH models to calculate HRs and their associated P-values.

CONCLUSIONS

Comprehensive epigenomic profiling of plasma cfDNA enables a minimally-invasive characterization of response and resistance mechanisms to 177Lu-PSMA-617 in mCRPC.

Wnt pathway activity and immune pathway activity as measured by the plasma epigenome is associated with resistance and sensitivity to 177Lu-PSMA-61, respectively.

By providing real-time insights into tumor biology and therapeutic efficacy, this platform supports precision medicine approaches for optimizing outcomes in PSMA-targeted therapies.

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References

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