Determination of tumor PSMA expression and response to Lutetium-PSMA in men with prostate cancer using a novel epigenomic liquid biopsy platform

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BACKGROUND

- 177Lu-PSMA-617 (Lutetium-PSMA) is an FDA-approved radiopharmaceutical therapy for men with metastatic castration-resistant prostate cancer (mCRPC).
- Treatment eligibility requires a PSMA PET scan to confirm tumor PSMA expression.
- With therapeutic strategies in development targeting an array of cell surface proteins, there is an emerging unmet need for minimally invasive biomarkers to simultaneously quantify multiple tumor drug target expression and predict therapeutic response.
- We performed comprehensive epigenomic profiling on 1mL of plasma from 72 men with mCRPC and demonstrated an accurate cell-free (cf)DNA-based readout of tumor PSMA expression and response to Lutetium-PSMA.

METHODS

- We collected plasma at the time of PSMA PET scan in men with mCRPC and analyzed the PET images to quantify total tumor SUV_{mean}, the radiographic feature that most strongly correlates with response to Lutetium-PSMA.
- In the same patients, we collected plasma at the time of Lutetium-PSMA treatment initiation and collected detailed clinical outcomes data.
- We profiled genome-wide cfDNA epigenomic signals across histone modifications associated with active enhancers and promoters, and DNA methylation, on these plasma samples and identified putative gene-regulatory loci at the *FOLH1* (PSMA) locus.
- Using these signals, we trained a model to predict PSMA PET SUV_{mean} and validated performance in an independent set of samples held out of model training.
- The results below are limited to patients with cfDNA tumor fraction >3%.

References:

1. Baca S et al., *Nature Medicine* (2023)

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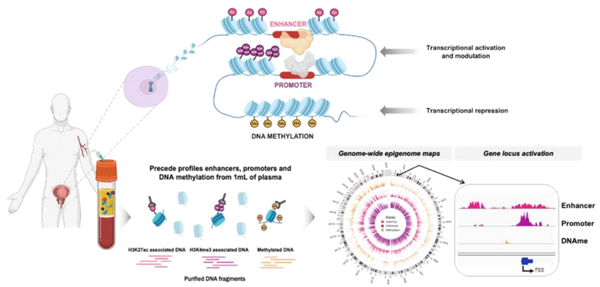
RESULTS

Figure 1. Comprehensive epigenomic platform offers dynamic resolution into target and pathway biology from 1mL of plasma

(A) – Overview of the Precede Biosciences liquid biopsy platform



(B) – Clinical study workflow of the mCRPC cohort

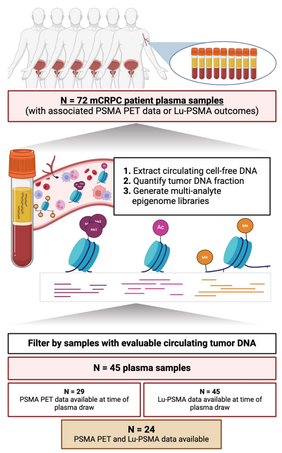


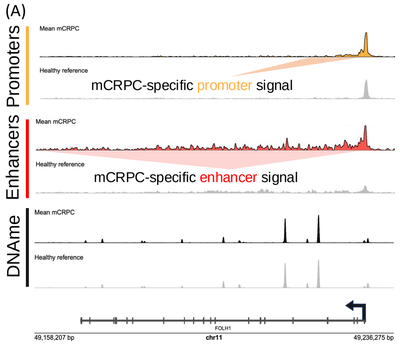
Table 1. Clinical cohort overview

COHORT DESCRIPTION	Total N = 45
Patient characteristics	
Age in years, median(range)	72 (53.4–88)
Race, number (%)	
White	38 (84.4%)
Black or AA	4 (8.9%)
Hispanic	1 (2.2%)
Disease characteristics	
Stage at Diagnosis, number (%)	
Localized	28 (62.3%)
Metastatic	17 (37.8%)
Sites of Disease on PSMA PET, number (%)	
Lymph Node	31 (68.9%)
Bone	43 (95.6%)
Lung	7 (15.6%)
Liver	9 (20%)
Other	10 (22.2%)
# of Prior Line of Systemic Therapies for Metastatic Disease, median (range)	4 (2–7)
PSA at Plasma in ng/mL, median (range, N= 29)	148.9 (0–2126)
PSMA PET SUVmean, median (range, N=29)	6.2 (2.8–16.8)
Days from PSMA PET scan to plasma collection, median (range, N=29)	62 (-62–133)
Clinical Outcomes	
PSA-PFS in days, median (range)	84 (22–587)
crPFS in days, median (range)	106 (26–593)
Time to next treatment in days, median (range)	140 (26–613)
Overall Survival in days, median (range)	203 (26–613)

(A) Cell free (cf)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. **(B)** Clinical study overview of mCRPC patient plasma samples evaluated in the current study.

Figure 2. The *FOLH1* locus has robust enhancer and promoter signal in mCRPC patients compared to healthy volunteers correlated with PSMA PET SUV_{mean}

(A)



(B)

Group	N	Mean ctDNA%	Mean PSMA PET SUV_{mean}
mCRPC – PSMA PET Low	14	32 (3-75)	4.7 (2.8-6.1)
mCRPC – PSMA PET High	15	37 (5-78)	9.2 (6.2-16.8)

(A) Epigenomic signal at the *FOLH1* locus shows increased active promoter and active enhancer marks in mCRPC patients compared to healthy volunteers. Enhancer, promoter, and DNA methylation signal in mCRPC patients was normalized, smoothed, and averaged together (within analyte) with the mean signal from a cohort of male healthy volunteers. **(B)** Epigenomic signal at the *FOLH1* locus shows increased active promoter and active enhancer marks in mCRPC patients with high PSMA PET SUV_{mean} (≥ median, N = 15) compared to mCRPC patients with low PSMA PET SUV_{mean} (< median, N = 14).

Figure 3. Enhancer and promoter signal at *FOLH1* locus predicts PSMA PET SUV_{mean} in both cross-validation and in validation cohort

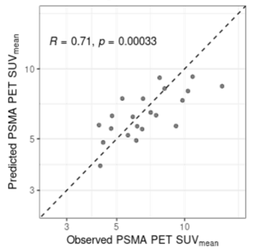
Validation schema

1. Use all samples in training cohort.
2. Empirically determine regions of robust *FOLH1* enhancer/promoter signal.
3. Train ML model to predict PSMA PET SUV_{mean} from enhancer/promoter signal.
4. Predict validation cohort.

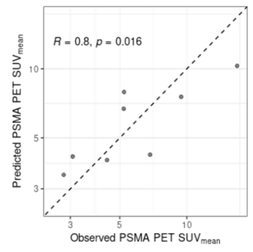
LOOCV schema

1. Leave out one sample out of 21 training samples.
2. Empirically determine regions of robust *FOLH1* enhancer/promoter signal.
3. Train ML model to predict PSMA PET SUV_{mean} from enhancer/promoter signal.
4. Predict left-out sample.
5. Repeat 21 times.

Training cohort LOOCV



Validation cohort

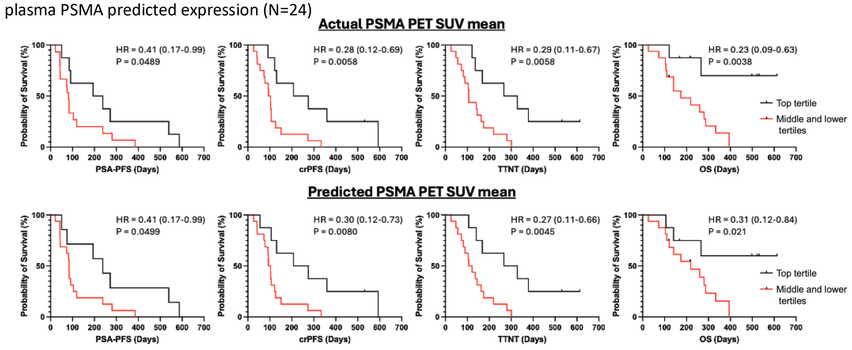


Cohort	N	Mean ctDNA%	Mean PSMA PET SUV_{mean}
Training	21	33.3 (3.9-65.4)	7.1 (4.2-14.7)
Validation	8	37.8 (3.3-78.7)	6.7 (2.8-16.8)

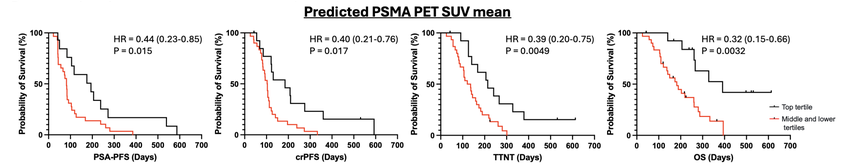
Enhancer and promoter signal at the *FOLH1* locus were used to train a machine learning (ML) model to predict PSMA PET SUV_{mean}. Samples were first split into training and validation cohorts, which were matched for ctDNA% and PSMA PET SUV_{mean} distributions. For model training, training cohort samples (ctDNA% ≥3) were used to identify robust, mCRPC-specific enhancer/promoter regions at the *FOLH1* locus. Signal at these regions were then used train a model to predict the corresponding PSMA PET SUV_{mean} quantifications. Performance was assessed via Pearson correlation in both a leave-one-out (LOO) cross-validation (CV) setting within the training cohort, as well as the held-out validation cohort using a final model trained on all data from the training cohort.

Figure 4. Plasma PSMA measurements are comparable to PET PSMA measurements in stratifying Lutetium-PSMA treatment outcomes

(A) Association of clinical endpoints with patient stratification based on either PET PSMA SUV_{mean} measurement or plasma PSMA predicted expression (N=24)



(B) Patients in the top tertile of plasma PSMA predicted expression in an expanded patient cohort demonstrate significantly improved clinical outcomes (N=45)



(A) Comparison of the hazard ratios (HR) based on predicted PSMA PET SUV_{mean} from liquid biopsy vs recorded PSMA PET SUV_{mean} values in patients with ctDNA ≥ 3% (N=24) across four clinical endpoints: 1) 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, PFS: progression free survival 2) crPFS: clinical-radiographic PFS, 3) TTNT: Time to next treatment following Lu-PSMA, and 4) OS: overall survival. Patients in the top tertile of SUV_{mean} values were compared to the bottom two tertiles using either observed or predicted SUV_{mean} values via log-rank test. Cox proportional hazards models fit using observed vs predicted PSMA PET SUV_{mean} do not differ significantly for each clinical endpoint (comparing Harrell's C-statistics, all P > 0.5). **(B)** Log-rank tests evaluating the four clinical endpoints based on assessment of plasma samples collected from N=45 (ctDNA ≥ 3%) patients immediately prior to treatment with Lutetium-PSMA. Patients were stratified based on tertile cutoffs of predicted expression of PSMA.

CONCLUSIONS

- This retrospective study profiled plasma based epigenomic profiles of 45 patients with mCRPC who were treated with 177Lu-PSMA-617 based on PSMA PET evaluation.
- Evaluating PSMA levels from blood is crucial for optimizing patient selection and treatment efficacy in the growing landscape of PSMA and other surface receptor-targeted therapies. The current analysis suggests that comprehensive epigenomic cfDNA profiling provides an accurate surrogate of tumor PSMA expression as well as response to Lutetium-PSMA in men with mCRPC.
- Pending ongoing validation in a larger cohort, these results highlight the potential of epigenomic cfDNA profiling as a real-time, non-invasive readout of multiple tumor drug targets expression as well as predictive biomarkers of therapeutic response to enable precision medicine in prostate cancer.