# Plasma cell-free DNA (cfDNA) profiling of PTEN-PI3K-AKT pathway aberrations in two multi-institutional independent metastatic castration-resistant prostate cancer (mCRPC) cohorts

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## INTRODUCTION

- Tumor tissue from mCRPC harbors frequent copy number variations (CNVs) in the PTEN-PI3K-AKT pathway, but identifying them in plasma cfDNA has proven challenging, especially *PTEN* copy number loss.
- With emerging data supporting Akt inhibition in PTEN-deficient mCRPC, cfDNA assays that robustly characterize PTEN-PI3K-AKT pathway aberrations are urgently needed.

## AIM

Using a validated cfDNA liquid biopsy assay in two independent mCRPC cohorts totalling 231 patients, our aims were to:

1. Characterize the mutational landscape of PTEN-PI3K-AKT pathway aberrations, with an emphasis on robust detection of *PTEN* loss.
2. Correlate genomic aberrations with longitudinal clinical outcomes.

## METHODS

Cohort description and targeted cfDNA sequencing

- Pre-treatment plasma samples were collected from mCRPC patients (pts) across two cohorts in Australia (AU; n=78) and USA (n=153).
- Plasma PTEN-PI3K-AKT pathway aberrations were characterized using the CLIA-certified Predicine targeted panel-based NGS cfDNA assay.[1,2]
- We included *AR* aberrations recognizing reciprocal PI3K regulation.[3,4]

Outcomes and analysis

- Kaplan-Meier curves and multivariable Cox proportional-hazards models assessed associations between PTEN-PI3K-AKT and AR pathway aberrations and overall survival.

## RESULTS

Patient cohort

- Median follow-up was 28.0 and 80.7 months in AU and US cohorts, respectively; patient characteristics for each cohort shown in **Table 1**.
- In the AU cohort, 49 pts (63%) commenced AR pathway inhibitors (ARPI; abiraterone or enzalutamide) and 29 pts (37%) commenced taxane chemotherapy (docetaxel or cabazitaxel).

***References***: *1. Fettke H et al. Eur Urol 2020;78:173-80. 2. Kohli M et al. EbioMedicine 2020;54:102728. 3. Carver BS et al. Cancer Cell 2011;19:575-86. 4. Mulholland DJ et al. Cancer Cell 2011;19:792-804.*

| Table 1: Patient characteristics | AU cohort n = 78 | US cohort n = 153 |
|---|---|---|
| **Age** | | |
| Median [interquartile range] | 72 (63-78) | 72 (66-77) |
| **Gleason score** | | |
| ≤ 7 | 19 (24) | 64 (42) |
| ≥ 8 | 30 (50) | 75 (49) |
| No biopsy / unknown | 20 (26) | 14 (9) |
| **Local treatment type, N (%)** | | |
| Radical prostatectomy | 22 (28) | 62 (41) |
| RT +/- adjuvant ADT | 13 (16) | 38 (25) |
| None or metastases at dx | 40 (52) | 53 (34) |
| Primary ADT | 3 (4) | |
| **Prior treatment, N (%)** | | |
| Prior chemotherapy only | 12 (21) | 41 (27) |
| Prior ARPI only | 26 (46) | 110 (72) |
| Prior chemotherapy and ARPI | 18 (32) | 102 (66) |
| **Baseline biochemistry, N(%)** | | |
| PSA (ng/ml) | 46 (0.5-2720) | 20 (5.0-98) |
| Hb (g/ml) | 122 (113-131) | 202 (176-249) |
| ALP (U/L) | 151 (94-346) | 98 (73-155) |

ARPI, androgen receptor pathway inhibitor; ADT, androgen deprivation therapy; ALP, alkaline phosphatase; Hb, haemoglobin; RT, radiotherapy.

Detection and clinical associations of PTEN-PI3K-AKT pathway aberrations

- ***PTEN* loss was observed in 37%** (85/231) of pts (**Fig 1)** and was *independently associated* with OS in AU and US cohorts (**Fig 2** & **Table 2**).
- ***PIK3CA* gain was observed in 17%** (39/231) of pts (**Fig 1**) and was *independently associated* with poor survival in the AU but not the US cohort (**Table 2**).
- In a subset of AU samples with additional plasma (n=46), **low-pass WGS confirmed panel *PTEN* loss in 90% (28/31), and PIK3CA gain in 84% (16/19)**, with high correlation for absolute copy number between methods (R=0.85 and R=0.80, respectively).
- Of 146 *PTEN*-neutral pts, **31 (21%) had alternate PTEN-PI3K-AKT aberrations** (**Fig 1**).

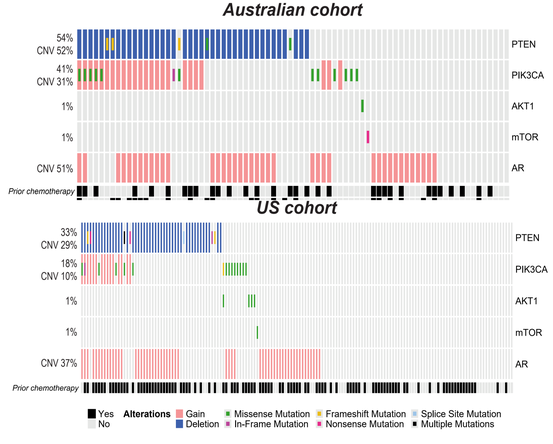


**Fig 1:** Genomic landscape of PTEN-PI3K-AKT and AR pathway aberrations in AU (top) and US (bottom) cohorts.

Cumulative CNVs in PTEN-PI3K-AKT and AR pathways

- Considering PTEN loss, PIK3CA gain and AR gain, cumulative CNVs (0 vs 1 vs ≥2 CNVs) in the PTEN-PI3K-AKT and AR pathways were significantly associated with worse clinical outcomes (Fig 2 & Table 2).

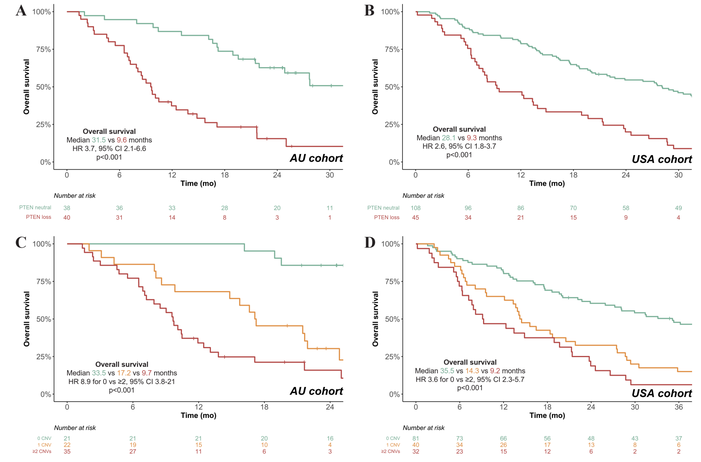


**Figure 2**: Kaplan-Meier analysis of OS according to *PTEN* copy number status (A,B) and cumulative CNVs in PTEN-PI3K-AKT and AR pathways (C,D).

| Table 2: MVA | AU cohort (n = 78) [a] | | | US cohort (n = 153) [b] | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p* | HR | 95% CI | *p* |
| **CNVs** | | | | | | |
| *PTEN* loss | 3.0 | 1.5-5.7 | **0.001** | 1.9 | 1.3-2.9 | **0.002** |
| *PIK3CA* gain | 2.9 | 1.5-5.5 | **0.001** | 1.7 | 0.92-3.0 | 0.09 |
| *AR* gain | 2.2 | 1.2-4.1 | **0.02** | 2.3 | 1.5-3.4 | **<0.001** |
| **Cumulative PTEN-PI3K-AKT and AR pathway CNVs** | | | | | | |
| *0* | REF | - | - | REF | - | - |
| *1* | 6.2 | 2.3-17 | **<0.001** | 1.8 | 1.2-2.8 | **0.006** |
| ≥2 | 9.3 | 3.5-25 | **<0.001** | 3.2 | 2.0-5.3 | **<0.001** |

[a] Covariates in multivariable variable analysis (MVA): ctDNA% ≥2%, prior chemotherapy, prior ARPI, visceral metastases, baseline pain and ECOG PS ≥2. [b] Covariates in MVA: ctDNA% ≥2%, prior chemotherapy, alkaline phosphatase ($log_{10}$).

## CONCLUSION

- PTEN-PI3K-AKT CNVs were readily detected using Predicine cfDNA assay, with prevalence of *PTEN* loss comparable to tissue studies.
- Over one-fifth of *PTEN*-neutral patients had other activating aberrations in the PTEN-PI3K-AKT pathway.
- Plasma cfDNA profiling of PTEN-PI3K-AKT and AR pathway aberrations may identify a poor-risk cohort primed for dual AR/Akt targeted therapy.