CANCER RESEARCH HORIZONS

PCF-SELECT

Specific **E**valuation in **L**iquid biopsies of **E**stablished Prostate **C**ancer **T**argets

Our mission

We are a cancer-focused diagnostic and drug discovery, development and commercialisation organisation that fast-tracks scientific breakthroughs for patient benefit.

INNOVATION ENGINE

A powerful innovation engine, built to complement and realise the full potential of CRUK's network of exceptional investigators and cancer centres, and ~$500M annual R&D spend.

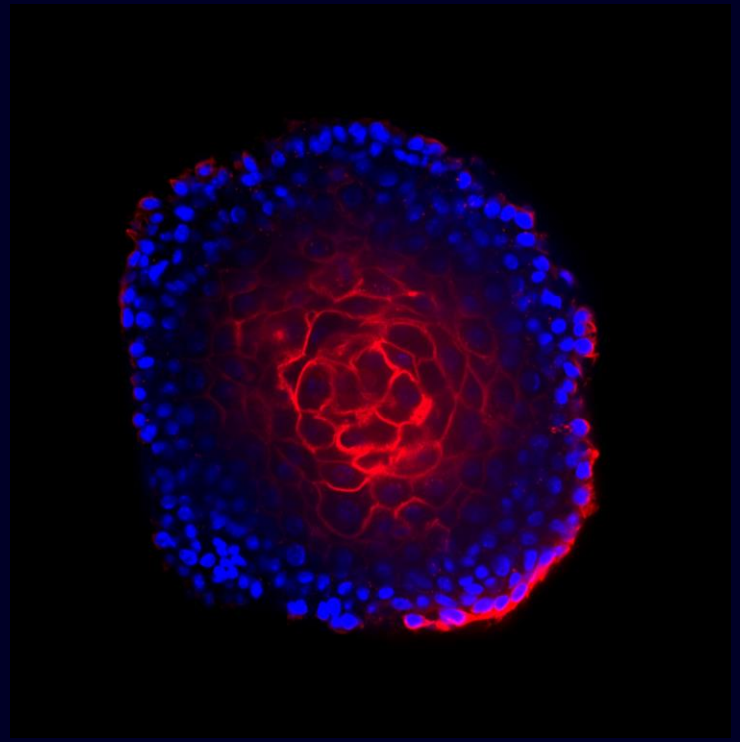
CANCER RESEARCH HORIZONS

Licensing Opportunity

CANCER RESEARCH HORIZONS

PCF-SELECT is a comprehensive liquid biopsy assay for treatment selection in metastatic castrate-resistant prostate cancer (mCRPC). This bespoke prostate cancer assay consists of a targeted sequencing panel and tailored computational method for sensitive detection of allele-specific copy number changes in cfDNA, offering the full potential of whole genome sequencing (WGS) in a targeted panel at low cost.

We are currently exploring potential licensing interest in PCF-SELECT.

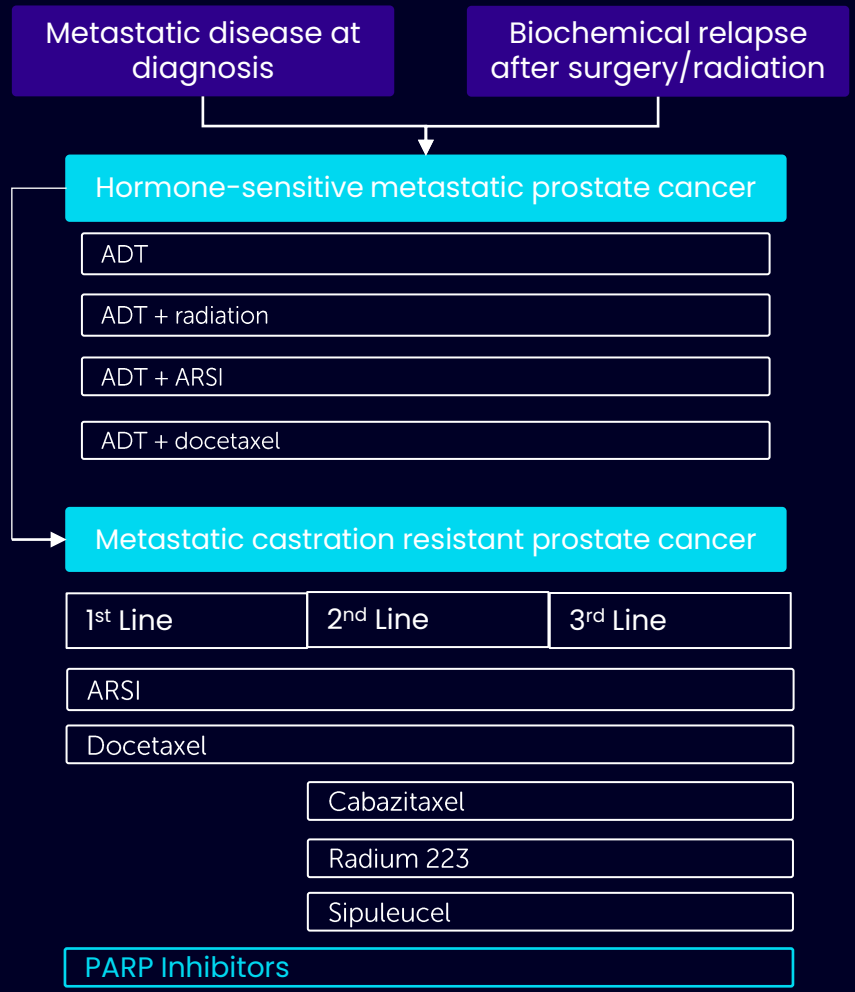


Prostate cancer showing LY6D (red), which is an indication that they are castration-resistance, and DAPI (blue) - Dr Esther Baena, Lead of Prostate Oncobiology Laboratory, Cancer Research UK Manchester Institute

Targeted therapies offer more options for mCRPC treatment

CANCER RESEARCH HORIZONS

- Prostate cancer is the most common cancer in men and 2nd most common cancer overall in the UK, and the 5th leading cause of cancer-related death in men worldwide
- Metastatic castrate-resistant prostate cancer (mCRPC) is a particular challenge clinically
- Poly (ADP-ribose) polymerase inhibitors (PARPi) have increased treatment options for mCRPC patients with alterations in DNA repair genes (e.g. BRCA)
- Personalising therapy to individual patients based on genomic biomarker testing can therefore be used to inform treatment selection and ultimately improve care



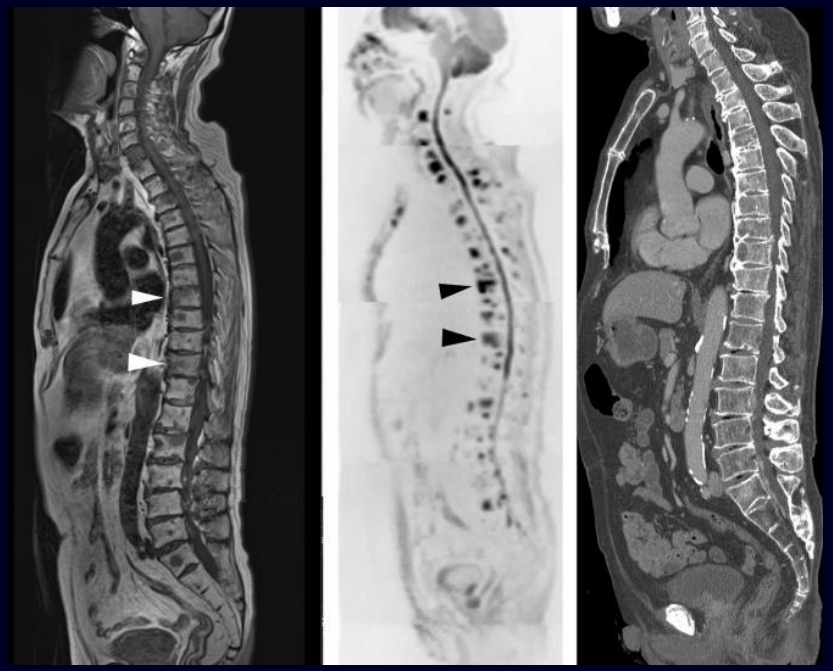
Targeted therapies may be used earlier as testing is adopted into the pathway and on the outcome of key trials

Use of tissue biopsies for genomic biomarker testing has limitations

CANCER RESEARCH HORIZONS

Sampling solid biopsies from prostate cancer patients for biomarker analysis is invasive, costly and can fail to retrieve clinically relevant information and/or tissue amenable for NGS based analysis.

1. Despite imaging-guidance, biopsy of osteoblastic bone metastases can be technically challenging. Because of these challenges, clinicians frequently use archived primary tumour tissue from prostate biopsy or prostatectomy that is years to decades old.
2. Even in high-volume academic centres that have tissue acquisition protocols in place, the viable tumour tissue yield from bone biopsies for genomic assays is relatively low.
3. Moreover there are challenges with tumour heterogeneity, as a single sample biopsy point may not represent other metastatic sites.



Whole body MRI for showing mixed-type/intertrabecular metastases from carcinoma of the prostate, Osaka

Lorente, David, et al. "Castration-resistant prostate cancer tissue acquisition from bone metastases for molecular analyses." Clinical genitourinary cancer 14.6 (2016): 485-493,

Liquid biopsy enables characterisation of metastatic tumours

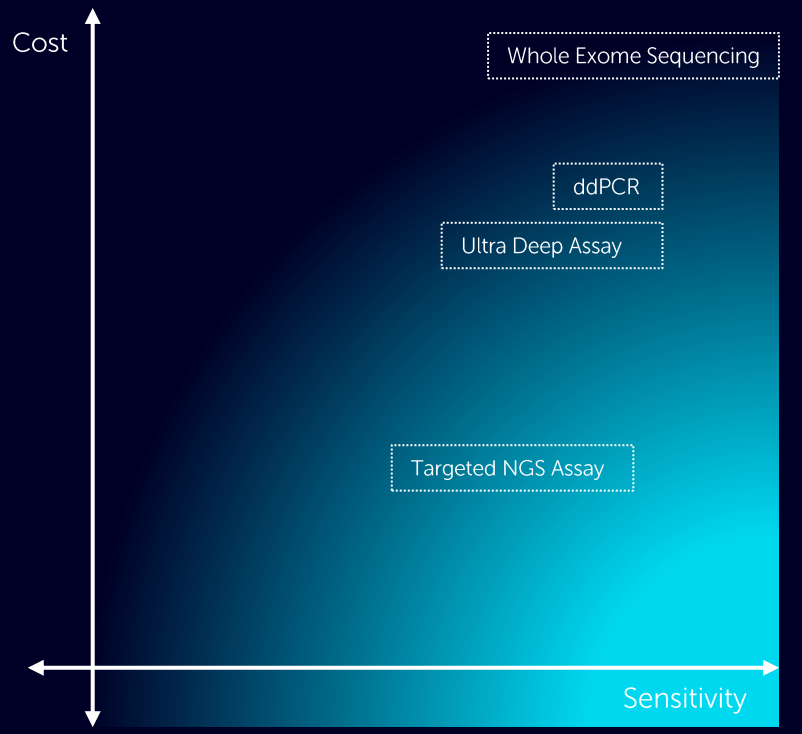
Liquid biopsy offers opportunities to non-invasively characterize molecular features of metastatic tumours in a manner tailored to the patient.

1. Liquid biopsies capture intra-patient tumour heterogeneity by identifying tumour alterations across metastases whose cells have entered the bloodstream.
2. Liquid biopsies also address temporal evolution of alterations, as repeated samples may be obtained over time without major discomfort for patients.
3. ctDNA tumour content increases with disease progression, making assays more sensitive & ideally suited to the metastatic disease setting.

However, there are challenges of utilising the benefits of liquid biopsy in some settings, including mCRPC

Sensitive detection remains a challenge at low cost

- ctDNA assays have biological and technical limitations that impact the ability to accurately stratify patients, e.g. low ctDNA fractions limit the accurate detection of copy number changes
- Whole Exome Sequencing (WES) error rate and sequencing depth are bottlenecks for detection of low-frequency variants
  - WES is usually performed at 100 × for somatic mutation discovery, ~ $500, LoD of 5–10% VAF
  - 5% VAF mutation sensitivity is sufficient for many applications, but higher mutation sensitivity is required to identify sub-clonal drug resistance mutations and perform non-invasive tumour profiling with cfDNA from peripheral blood samples
- Ultra-deep NGS assays and whole genome sequencing (WGS) are currently prohibitively expensive to be used in the SOC or trial setting



Cost / sensitivity trade-off for liquid biopsy panels in prostate cancer

LoD, Limit of Detection; VAF, Variant Allele Frequency; SOC, Standard of Care; NGS, Next Generation Sequencing

Current liquid biopsy tests are not optimal for use in mCRPC

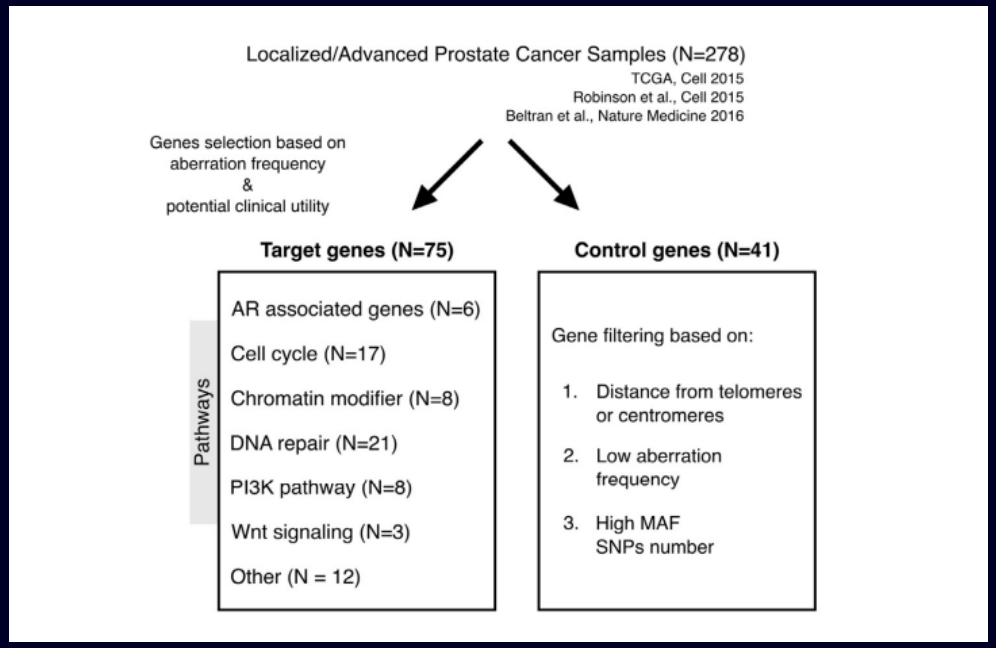
CANCER RESEARCH HORIZONS

- Copy number changes and/or aneuploidy are common features of mCRPC, but are challenging to detect accurately at low ctDNA fractions
  - Many of the genomic alterations enriched in mCRPC involve loss of tumour suppressor genes (such as PTEN, TP53, RB1) rather than activating events.
  - Loss of tumour suppressor genes can be the result of truncating mutations, gene deletions (complete or partial) or complex gene rearrangements
- For mCRPC, there remains a clinical need to better identify patients suitable for targeted therapies through more accurate assessment of copy number
- This would allow for the advantages of liquid biopsy tests to be realised in the mCRPC setting, and for the improved identification of patients suitable for targeted therapies

PCF-SELECT: a bespoke prostate cancer liquid biopsy assay

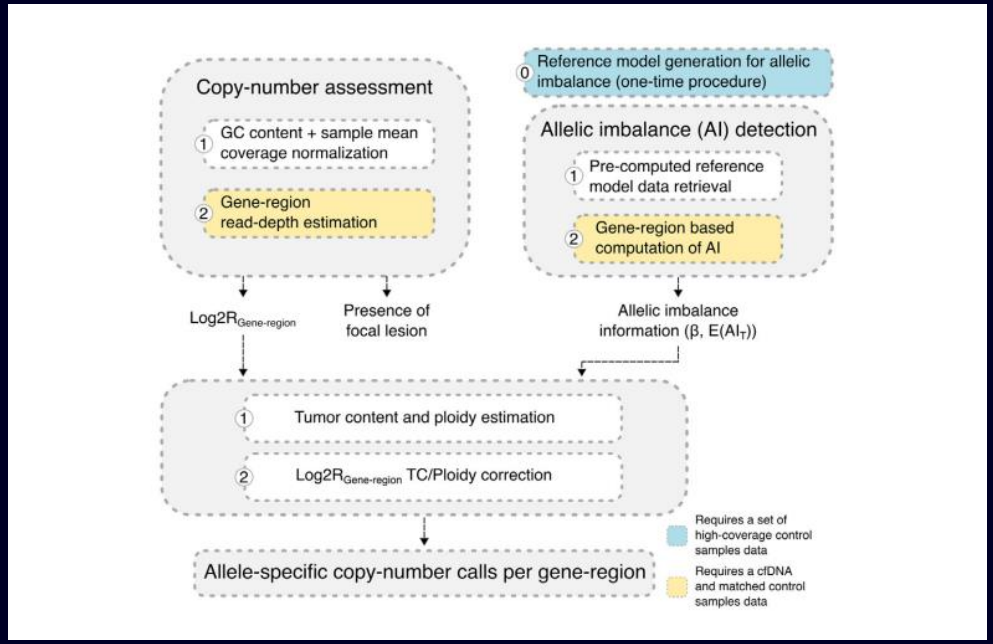
Consortium specifically set up to design an assay to meet these challenges, leading to PCF-SELECT - a targeted sequencing panel and tailored computational method for sensitive detection of allele-specific copy number changes from cfDNA

Custom targeted sequencing panel



Design enriched for high minor allele frequency (MAF) SNPs. Final panel covers 116 gene-regions and spans 3.49 Mb (1.13 Mb on exonic/intronic regions of targeted genes and 2.36 Mb on flanking regions).

Computational method for allele-specific copy number assessment



Computational method takes full advantage of panel design to accurately assess copy number states and increase sensitivity of detecting imbalances in low moderate ctDNA samples

PCF-SELECT offers potential of WGS in a targeted panel, optimised for mCRPC

CANCER RESEARCH HORIZONS

Designed to detect genomic aberrations informative for therapeutic selection, including drugs targeting DNA repair deficiency, immune checkpoints and the PI3K/AKT pathway, both for standard of care treatments and the clinical evaluation of experimental therapies.

Key advantages:

Detects a breadth of genomic alterations at high sensitivity

Covers key mutation alteration types: short variants, rearrangements and copy number changes across 116 recurrently aberrant prostate cancer genes and control genes, very high coverage sequencing (1500-2000x). It is uniquely able to detect mono-allelic deletions at high sensitivity

Computational approach to minimise false positives

Through sequencing of a germline/normal sample the heterozygous SNPs in the patient's regions of interest are determined within the context of the germline model for each informative SNP. False positives rate <0.01%.

Enriched for informative SNPs

Enriched for high Minor Allele Frequency (MAF) SNPs (N=27,115) to assure the presence of informative SNPs (iSNPs, heterozygous SNPs) in each individual.

Amenable to low input plasma DNA amounts

The assay is amenable to **low input plasma DNA amounts**, **10-20ng,** making it applicable across the 'early' metastatic prostate cancer disease setting where there may be a low tumour content.

Proof of principle demonstrated

Assay performance assessed on plasma samples from 50 advanced PC patients

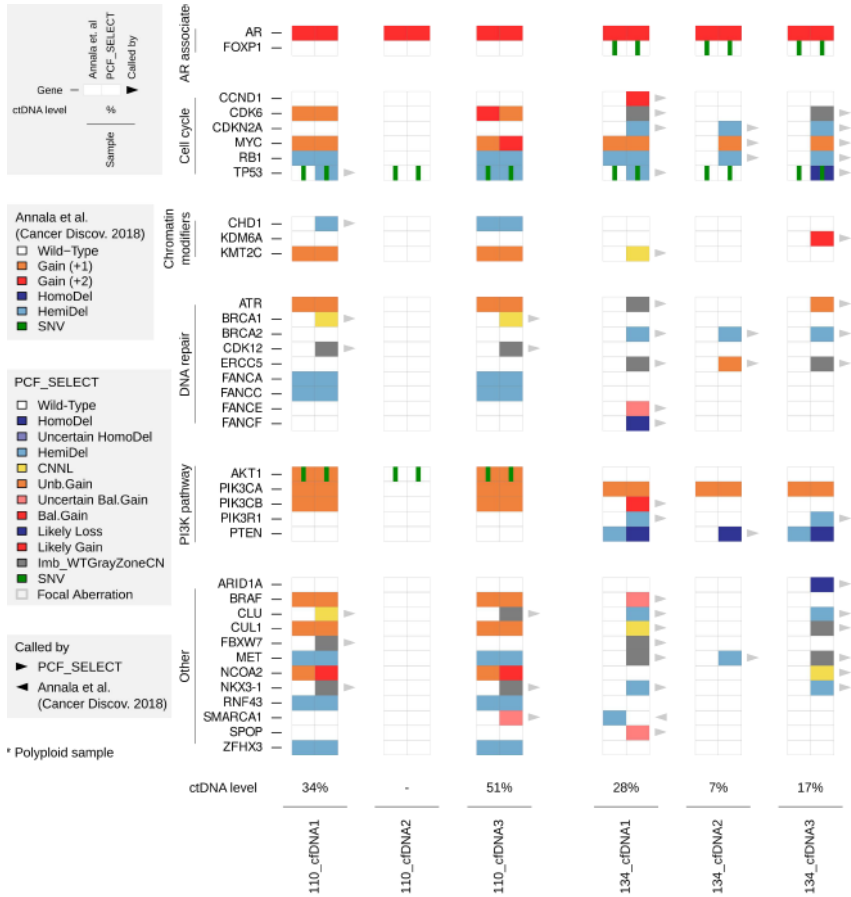
- Low false positive rate
- Confirmed higher number of iSNPs improves detection of allelic imbalance
- Synthetic dilutions use to evaluate performance in detecting allelic imbalance at decreasing ctDNA fractions – able to detect signal imbalance at 5% ctDNA level

Sensitivity assessed through comparison of serial samples versus independent assay

- Serial samples from three patients analysed & compared to results using assay from Annala et al.
- At the first time point, only PCF-SELECT was able to detect hemi-deletions of TP53 and CHD1 from patient 110 which was subsequently confirmed at time point three. PCF-SELECT also showed improved ability to detect known CN changes in low ctDNA level sample (TP-2 of patient 134, 7% ctDNA level)

Allelic imbalance detected in serial plasma samples with decreasing tumour fraction

- PCF-SELECT applied to serially-collected samples from three patients treated with a PARPi



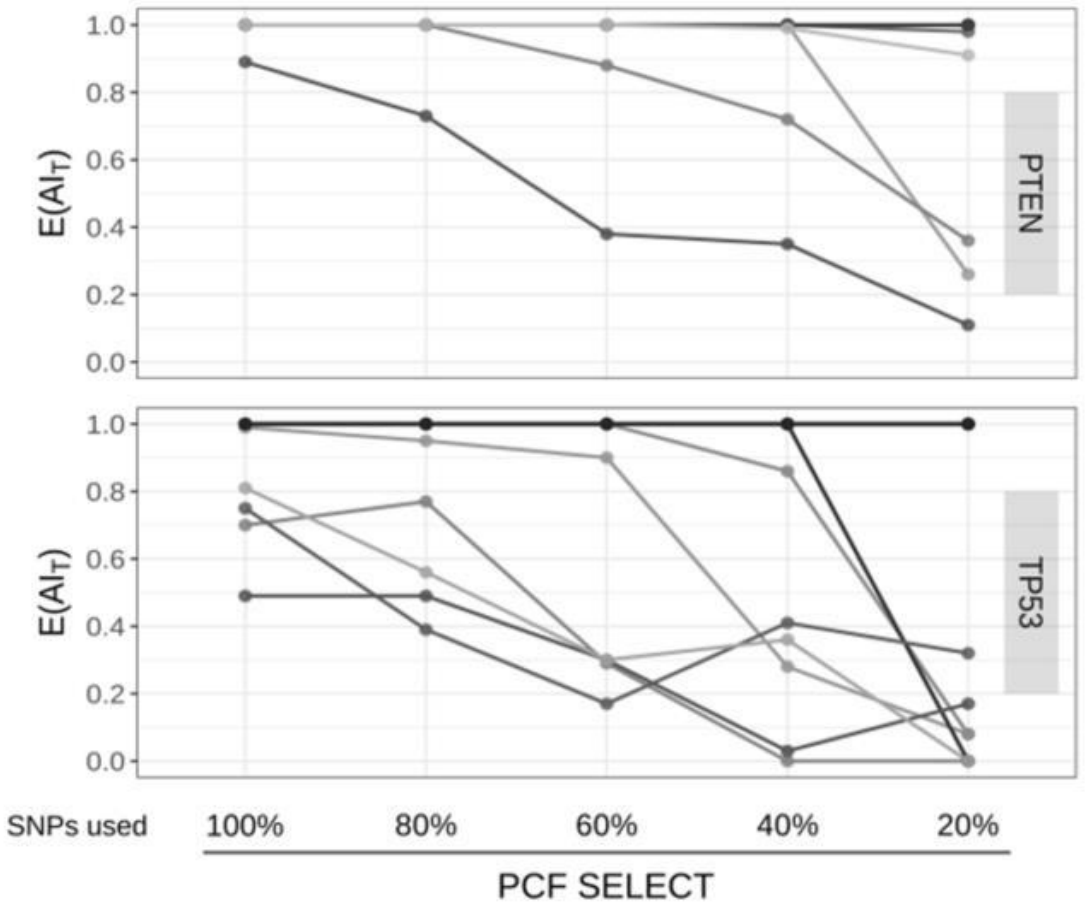
Comparative overview of somatic copy number aberrations (SCNA) calls on three CRPC serial samples from patients using PCF-SELECT and an independent assay from *Annala et al. Cancer Discovery 2018.*

Detection of mono-allelic deletions

Major advance is the novel strategy for detection of allelic imbalance, which enables detection of mono-allelic deletions

Assessment of allelic imbalance calls using high density iSNPs achieves increased accuracy and sensitivity.

The impact of the number of SNPs on allelic imbalance detection - detection performance decreased significantly by lowering the number of SNPs. Moreover, independently of the estimated proportion of tumour reads, a higher number of SNPs led to higher confidence in calling allelic imbalance



Evidence of allelic imbalance (E(AIT), y-axis) for representative gene-regions at varying percentages of iSNPs (WCM cohort, N = 66). Lines colour shade indicates ctDNA level of the sample

Several immediate and longer-term clinical opportunities for PCF-SELECT

CANCER RESEARCH HORIZONS

1

Detection of BRCA mutations

- Tissue often fails and is difficult to get hold of
- Immediate opportunity to better identify patients with metastatic PC eligible for PARP inhibitors

2

Early indication of response

- Understand whether a treatment is working or not
- Separate information to PSA
- Studies ongoing to clinically validate

3

Additional molecular data

- More information on mutations from tumour samples
- Specifically optimised for prostate cancer (not possible from pan-cancer panel)
- One test and multiple ways to analyse the data

4

Risk stratification at diagnosis

- Potential future application – will require further validation to demonstrate impact on survival

Opportunity to develop first and best targeted sequencing panel specifically optimised for mCRPC to identify patients eligible for approved targeted therapies (PARPis), with further potential clinical applications in future.

Developed by world-leading team in plasma DNA analysis & prostate cancer

CANCER RESEARCH HORIZONS

This technology has been developed with significant funding by Cancer Research UK, which has supported a world leading international team with deep expertise in ctDNA diagnostic development and prostate cancer.

The panel was developed from an international collaboration between University College London, Weill Cornell, The University of Trento and The University of British Columbia. The research was led by Professor Gert Attard's Treatment Resistance Group at the UCL Cancer Institute and Dr Francesca Demichelis, world leading experts in ctDNA and prostate cancer.

UCL · UNIVERSITY OF TRENTO - Italy · Weill Cornell Medicine · UBC THE UNIVERSITY OF BRITISH COLUMBIA

Dr Francesca Demichelis
Computational Oncology Laboratory at the Centre for Integrative Biology at the University of Trento

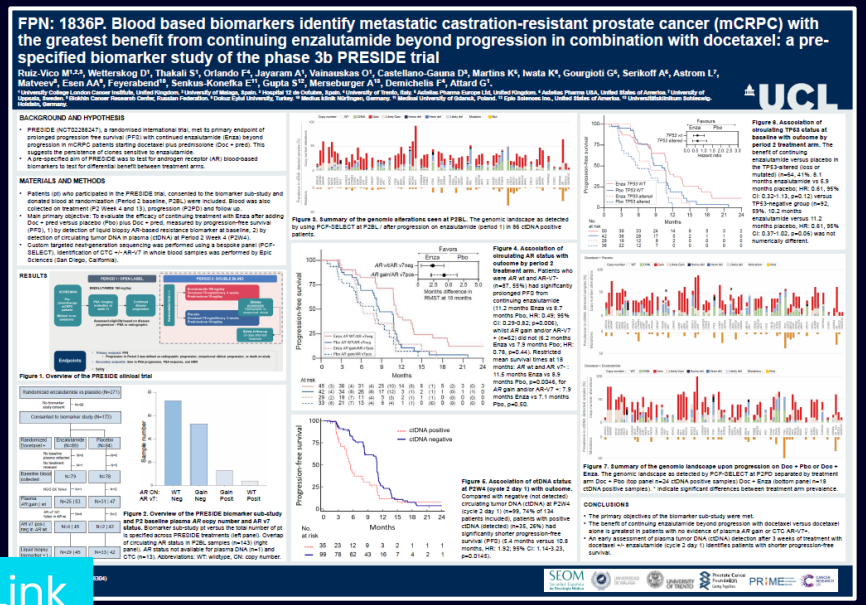
Professor Gert Attard
Treatment Resistance Group at the UCL Cancer Institute

Recent & ongoing validation

CANCER RESEARCH HORIZONS

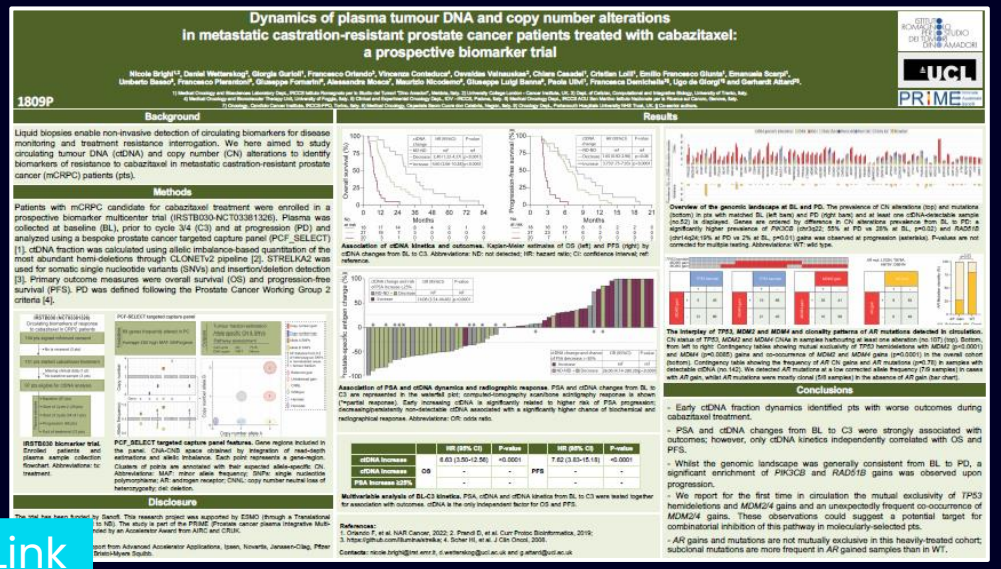
- Approx. 1000 samples have been run through the assay to date and it continues to be tested in multiple ongoing prospective trials
- 2 posters presented at ESMO October 2023

Blood based biomarkers identify metastatic castration-resistant prostate cancer (mCRPC) with the greatest benefit from continuing enzalutamide beyond progression in combination with docetaxel: a prespecified biomarker study of the phase 3b PRESIDE trial (NCT02288247)



Link

Dynamics of plasma tumour DNA and copy number alterations in metastatic castration-resistant prostate cancer patients treated with cabazitaxel: a prospective biomarker trial (IRSTB030-NCT03381326)



Link

Intellectual Property

- An international (PCT) patent application was filed in June 2022 with claims covering the PCF-SELECT methodology (PCT/GB2022/051447) and published on 15 December 2022 as WO2022/258975.
- The International Search Report and Written Opinion of the European Patent Office concluded that the claims are novel and inventive.
- National/regional phase entry (USA & Canada) – December 2023
- National/regional phase entry (Europe) – January 2024

Get in touch

Help us bring forward the day
when all cancers are conquered.

olivia.edwards@cancer.org.uk

Olivia Edwards

Business Development Executive

CANCER
RESEARCH
HORIZONS

Further reading

Published online 27 May 2022

NAR Cancer, 2022, Vol. 4, No. 2 1
https://doi.org/10.1093/narcan/zcac016

Allele-informed copy number evaluation of plasma DNA samples from metastatic prostate cancer patients: the PCF_SELECT consortium assay

Francesco Orlando[1], Alessandro Romanel[1], Blanca Trujillo[2,3], Michael Sigouros[4], Daniel Wetterskog[2], Orsetta Quaini[1], Gianmarco Leone[2,3], Jenny Z. Xiang[5], Anna Wingate[2], Scott Tagawa[6], Anuradha Jayaram[2,3], Mark Linch[2,3], PEACE Consortium†, Mariam Jamal-Hanjani[3,7,8], Charles Swanton[2,3,9], Mark A. Rubin[10], Alexander W. Wyatt[11], Himisha Beltran[4,12], Gerhardt Attard[2,3,*,‡] and Francesca Demichelis[1,4,*,‡]

[1]Department of Cellular, Computational and Integrative Biology, University of Trento, Trento, Italy, [2]UCL Cancer Institute, University College London, London, UK, [3]Department of Medical Oncology, University College London Hospitals, London NW1 2BU, UK, [4]Englander Institute for Precision Medicine, Presbyterian Hospital, Weill Cornell Medicine, NY, USA, [5]The Genomics Resources Core Facility,Department of Microbiology and Immunology, Weill Cornell Medicine. NY, NY, USA, [6]Department of Medicine, Division of Hematology and Medical Oncology, Weill Cornell Medicine. NY, NY, USA, [7]Cancer Metastasis Laboratory, University College London Cancer Institute, London, UK, [8]Cancer Research UK Lung Cancer Centre of Excellence, UCL Cancer Institute, London, UK, [9]The Francis Crick Institute, London NW1 1AT, UK, [10]Department for BioMedical Research and Bern Center of Precision Medicine, University of Bern and Inselspital, Bern, Switzerland, [11]Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada and [12]Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA, USA

Received December 28, 2021; Revised March 25, 2022; Editorial Decision April 14, 2022; Accepted May 05, 2022

ABSTRACT

Sequencing of cell-free DNA (cfDNA) in cancer patients' plasma offers a minimally-invasive solution to detect tumor cell genomic alterations to aid real-time clinical decision-making. The reliability of copy number detection decreases at lower cfDNA tumor fractions, limiting utility at earlier stages of the disease. To test a novel strategy for detection of allelic imbalance, we developed a prostate cancer bespoke assay, PCF_SELECT, that includes an innovative sequencing panel covering ~25 000 high minor allele frequency SNPs and tailored analytical solutions to enable allele-informed evaluation. First, we assessed it on plasma samples from 50 advanced prostate cancer patients. We then confirmed improved detection of genomic alterations in samples with <10% tumor fractions when compared against an independent assay. Finally, we applied PCF_SELECT to serial plasma samples intensively collected from three patients previously characterized as harboring alterations involving DNA repair genes and consequently offered PARP inhibition. We identified more extensive pan-genome allelic imbalance than previously recognized in prostate cancer. We confirmed high sensitivity detection of *BRCA2* allelic imbalance with decreasing tumor fractions resultant from treatment and identified complex *ATM* genomic states that may be incongruent with protein losses. Overall, we present a framework for sensitive detection of allele-specific copy number changes in cfDNA.

INTRODUCTION

Prostate cancer is a leading cause of cancer death among men and in the past few years studies investigating the genomic landscape of metastatic prostate cancer have led to the identification of targetable molecular alterations, emerging resistance mechanisms, and new therapeutic options. Following the approval of poly (ADP-ribose) polymerase inhibitors (PARPi) as a therapeutic option in metastatic castration resistant prostate cancer (mCRPC) patients with

*To whom correspondence should be addressed. Tel: +39 0461 285305; Fax: +39 0461 283937; Email: f.demichelis@unitn.it
Correspondence may also be addressed to Gerhardt Attard. Email: g.attard@ucl.ac.uk
†PEACE consortium members listed in Supplementary Data.
‡The authors wish it to be known that, in their opinion, the last two authors should be regarded as Joint Senior Authors.

Orlando F, Romanel A, Trujillo B, Sigouros M, Wetterskog D, Quaini O, Leone G, Xiang JZ, Wingate A, Tagawa S, Jayaram A, Linch M; PEACE Consortium; Jamal-Hanjani M, Swanton C, Rubin MA, Wyatt AW, Beltran H, Attard G, Demichelis F. **Allele-informed copy number evaluation of plasma DNA samples from metastatic prostate cancer patients: the PCF_SELECT consortium assay**. NAR Cancer. 2022 May 27;4(2):zcac016. doi: 10.1093/narcan/zcac016. [Link]