Establishing a Biomarker Discovery Platform to Track Progression to Treatment-Resistant Neuroendocrine Prostate Cancer

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BACKGROUND & CLINICAL RATIONALE

PROSTATE CANCER: A PERSISTENT CLINICAL CHALLENGE

1 in 8 — North American men diagnosed over lifetime

30% — of CRPC cases progress to NEPC

<1yr — Median NEPC patient survival

Prostate cancer (PC) is the most common malignancy in North American men. Despite initial responsiveness to androgen- deprivation therapy (ADT), up to 30% of castration-resistant cases develop **neuroendocrine prostate cancer (NEPC)**: a treatment-resistant phenotype with severely limited therapeutic options and a median survival of less than one year.

FIGURE 1: ANATOMY OF THE PROSTATE & TUMOUR MICROENVIRONMENT

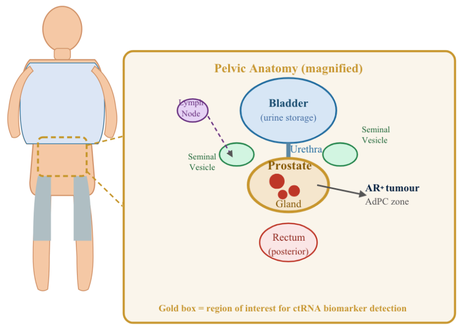


Figure 1. *Male pelvic anatomy showing the prostate gland (gold oval, right). MagniGed cross-section (right) shows the prostate surrounding the urethra, adjacent seminal vesicles, posterior rectum, and regional lymph nodes. Tumour cells (red) in the prostate express AR in early AdPC, with possible lymphatic spread upon progression. The prostate is the primary site of ctRNA biomarker release into biofluids.*

FIGURE 2: DISEASE PROGRESSION & TREATMENT TIMELINE

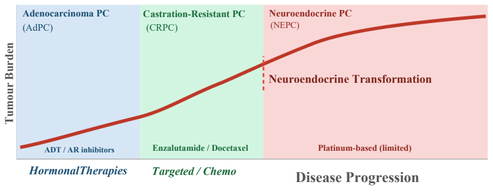
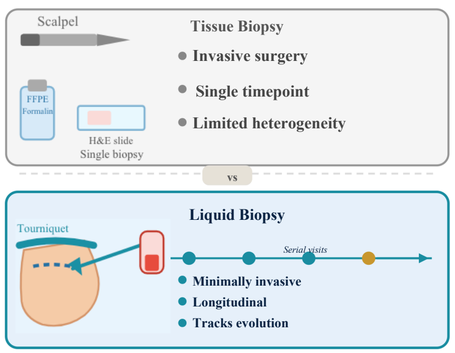


Figure 2. *Disease progression model showing tumour burden across three stages. Neuroendocrine transformation marks a critical juncture point where treatment options become severely limited.*

LIQUID BIOPSY VS TISSUE BIOPSY: CLINICAL COMPARISON



Liquid biopsy enables minimally invasive, longitudinal monitoring of tumour dynamics, capturing disease evolution more effectively than single- site tissue biopsy.

SCIENTIFIC RATIONALE, METHODS & 2026 PROGRESS

STUDY OBJECTIVE

To develop **a multi-biofluid circulating tumour RNA (ctRNA) liquid biopsy platform** capable of detecting early neuroendocrine prostate cancer patients, enabling non-invasive molecular monitoring of disease progression andguiding therapeutic decision-making in real time. Current tissue biopsy is invasice, spatially limited, and unable to capture tumour evolution over time. A ctRNA platform addresses these gaps by enabling serial, non-invasive sampling from blood, saliva, and urine during routine oncology visits at Windsor Regional Hospital. Analysis of samples will build a **biobanked repository of cell cycle signatures associated with disease progression**, enabling retrospective risk modelling in presenting patients.

MOLECULAR DRIVERS OF NEPC: SPY1/CDK2 AXIS & CELL CYCLE REGULATION

Spy1 (SPDYA) → **CDK2** activated ⊣ **↓p21 ↓p27** → **NEPC** phenotype

The Porter Lab has identified elevated **Spy1 (SPDYA)** and **Cyclin A1 (CCNA1)** as candidate cell-cycle drivers of NEPC. The hypothesis, currently under active investigation, proposes that Spy1-mediated CDK2 activation leads to: **upregulation** of CCNA1 driving S-phase entry; **downregulation** of CDK inhibitors p21 (CDKN1A) and p27 (CDKN1B), removing G1/S checkpoint brakes; and subsequent **checkpoint evasion**, allowing cells to bypass DNA damage surveillance and proliferate unchecked. This enables neuroendocrine lineage switching. Key biomarker targets: **SRRM4** (NE splicing factor, upregulated), **SYP** (synaptophysin, upregulated), **REST** (transcriptional repressor, lost in NEPC), **CCNA1** (upregulated), **SPDYA** (Spy1, upregulated).

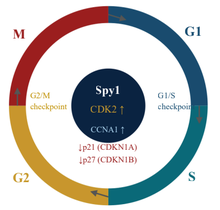


Figure 3 (right). *Cell cycle schematic with Spy1/CDK2 as central mediators promoting unregulated G1/S & G2/M progression and reduced prognosis (Bakht, unpublished).*

PROJECT TIMELINE & MILESTONES

- **2024** — Grant & Ethics: WE-SPARK IDG, REB approved
- **2025** — Platform Setup: Protocols set, REDCap live
- **Early 2026** — Collection n=9: Multi-biofluid, RNA QC done
- **Now** — RNA Analysis: Uof Windsor, ctRNA quantified
- **Mid 2026** — Sequencing: WTS of cell cycle signatures

Figure 4. *Study timeline from REB approval (2024) through active clinical sample collection (n=9), RNA analysis currently underway at the University of Windsor, and whole transcriptome sequencing of cell cycle signatures planned for mid-2026. Gold dot = current phase; navy dots = completed milestones.*

LIQUID BIOPSY MULTI-BIOFLUID COLLECTION STRATEGY

1 Patient Identification
- Dr. Kanjeekal identifies eligible patients
- Samavia Ahmad contacts & onboards
- Verbal consent obtained; appointment scheduled
- Patients fast 30 min before collection

2 Consent & Arrival
- Patient arrives at WRH Cancer Centre
- Guided to blood lab & exam room
- Written consent signed; LOI & consent copies provided
- REDCap chart completed (DOB, stage, therapy, HCN)

3 Sample Collection
- **Blood:** 2 STRECK tubes by phlebotomist
- **Urine:** sterile container; transferred within 15 min to preservation tube
- **Saliva:** 2 mL pipetted into 2 tubes with preservative
- All tubes labelled: e.g. NEPC_B_1, NEPC_U_1, NEPC_S_1

4 Transport
- Blood & urine in biohazard bag to Porter Lab (U of Windsor)
- Saliva transported on ice by WE-SPARK fresh tissue associate
- Chain of custody maintained throughout

5 Processing at Porter Lab
- ctRNA extraction: QIAamp Circulating Nucleic Acid Kit
- RIN & concentration assessed: High Sensitivity Fragment Analyzer (Agilent)
- Samples stored at −80°C

6 Data & Sequencing
- De-identified data entered into REDCap
- Samples biobanked; WTS planned (facility TBD)
- Analysis: DESeq2, ssGSEA, cell cycle signature characterization

Blood — 2 STRECK tubes | Saliva — 2 mL, 2 tubes | Urine — sterile, <15 min transfer

POST-SEQUENCING ANALYSIS PIPELINE

Raw ctRNA Sequencing Data — WTS reads per sample → **Gene Expression Quantification** — DESeq2 normalisation → **Clustering & Pattern Detection** — ssGSEA clustering → **Pathway Analysis** (AdPC vs NEPC) — Cell cycle signature characterization

Transforms raw sequencing data into biologically meaningful patterns of disease progression.

FIGURE 6: EXPECTED CTRNA TRANSCRIPTOMIC SIGNATURES (ADPC VS NEPC)

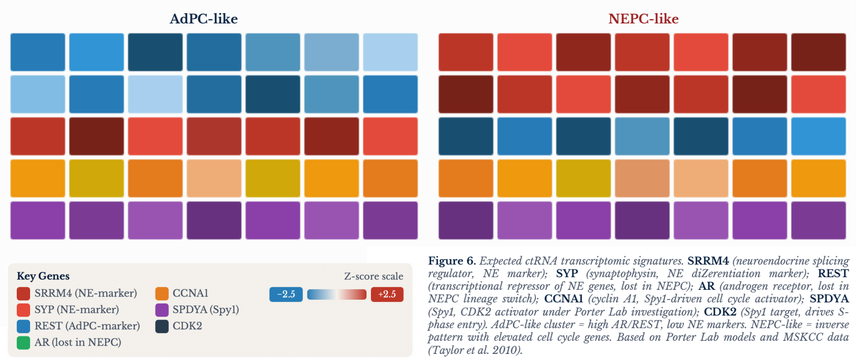


Figure 6. *Expected ctRNA transcriptomic signatures.* **SRRM4** *(neuroendocrine splicing regulator, NE marker);* **SYP** *(synaptophysin, NE diZerentiation marker);* **REST** *(transcriptional repressor of NE genes, lost in NEPC);* **AR** *(androgen receptor, lost in NEPC lineage switch);* **CCNA1** *(cyclin A1, Spy1-driven cell cycle activator);* **SPDYA** *(Spy1, CDK2 activator under Porter Lab investigation);* **CDK2** *(Spy1 target, drives S-phase entry). AdPC-like cluster = high AR/REST, low NE markers. NEPC-like = inverse pattern with elevated cell cycle genes. Based on Porter Lab models and MSKCC data (Taylor et al. 2010).*

EXPECTED OUTCOMES, IMPACT & FUTURE DIRECTIONS

FIGURE 7: CTRNA YIELD BY DISEASE STAGE (N=9 PATIENTS)

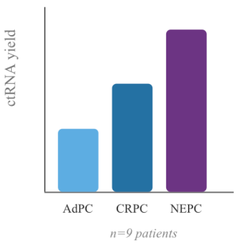
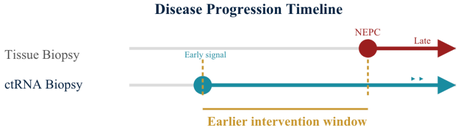


Figure 7. *Expected ctRNA yield trend across disease stages (n=9, preliminary). Adenocarcinoma PC (AdPC) represents the earliest stage with lowest expected yield. As disease progresses to Castration-Resistant PC (CRPC) and Neuroendocrine PC (NEPC), greater tumour burden and increased exosomal release from proliferating cells is expected to correspond with higher ctRNA yield. Data to be updated with Gnal patient-stage annotations post-enrolment.*

PLATFORM READINESS: 2026 STATUS

Ethics & REB	Complete	100%
Patient Recruitment	9/9	100%
Saliva cfRNA QC	9/9	100%
Urine cfRNA QC	9/9	100%
Plasma cfRNA QC	9/9	100%
UWindsor Sequencing		Prep

CLINICAL & TRANSLATIONAL IMPACT



ctRNA biomarkers may enable earlier detection and inform future intervention strategies.

- Detect NEPC transformation early
- Guide treatment decisions
- Improve equity of access
- Reduce invasive biopsies
- Enable real-time monitoring

ETHICS & DATA GOVERNANCE

REB Approval → Patient Consent → De-ID REDCap → Secure Biobank → CanDIG Access

All specimens collected under Windsor Regional Hospital and University of Windsor REB approval. Samples de-identified, stored at −80°C. Sequencing data deposited to **CanDIG** federated network. Gender-neutral consent ensures inclusive enrolment.

FUTURE DIRECTIONS

Research Roadmap

- **Phase 1 (Now)** — 9-patient WTS at the University of Windsor
- **Phase 2 (2026)** — Signature validation + expansion
- **Phase 3 (2026–2027)** — Diagnostic model development
- **Phase 4 (2027+)** — Multi-site clinical trial

Goal: clinical translation

- Complete WTS & transcriptomic analysis at the University of Windsor (Spring 2026)
- Validate NEPC ctRNA biomarker signature across n=9 patients
- Expand cohort to n=15–20 for powered signature discovery
- Correlate ctRNA with clinical outcomes and treatment response
- Develop clinically deployable diagnostic prediction model
- Seek large-scale clinical trial funding for multi-site validation

ACKNOWLEDGMENTS

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