Four-Timepoint **M**ulti-tracer **P**ET imaging to characterize metastatic pr**O**state Cancer heterogeneity **(4TMPO)**

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Sponsor : CHU de Québec-Université Laval (CHUQc-UL)

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SIGNATURES

Sponsor's Principal Investigator Signature Page

Protocol Title: **Four**-**T**imepoint **M**ulti-tracer **P**ET imaging to characterize metastatic pr**O**state Cancer heterogeneity **(4TMPO)**

Sponsor: CHU de Québec-Université Laval

The sponsor's principal investigators reviewed and approved this version of the protocol.

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Site lead / Qualified Investigator Signature Page

Protocol Title: **Four-T**imepoint **M**ulti-tracer **P**ET imaging to characterize metastatic pr**O**state Cancer heterogeneity **(4TMPO)**

Confidentiality agreement

This protocol contains confidential information resulting from the collective work **of Dr. Frédéric Pouliot, Dr. Jean-Mathieu Beauregard and Prof. Brigitte Guérin** except as may be otherwise agreed to in writing. By signing this protocol, I agree that neither I nor the staff under my supervision will disclose it to other parties (except where required by applicable law) nor use it for unauthorized purposes. In the event of a breach of confidentiality, the principal investigators and the **CHU de Québec-Université Laval** should be promptly notified. I also commit my team and myself to respecting the procedures detailed in this protocol.

Site Investigator's Commitment
As the site investigator at _____(name the centre)___, my research team and I will conduct the clinical trial in a manner consistent with good clinical practices (GCP). I also commit my team and myself to respecting the procedures detailed in this protocol. In the event of termination of the clinical trial, I will immediately inform the clinical trial participants and the research ethics committee of the cessation and the reasons for it and will notify them in writing of potential health risks, if applicable;

Site Qualified Investigator

Dr. _____

Address : _____
_____
_____

_____ _____

Name Date

Document history

Version	Effective Date	Section Affected	Summary of Change	Authors
1.0	Oct 25, 2024	Entire document	Original Protocol	All investigators
1.1	Feb 28, 2025	6.4	Typo	All investigators

List of Abbreviations

3TMPO	Triple-Tracer strategy against Metastatic PrOstate cancer
4TMPO	**Four**-**T**imepoint **M**ulti-tracer **P**ET imaging to characterize metastatic pr**O**state Cancer heterogeneity
^{18}F	Flurine-18
^{68}Ga	Gallium-68
ADT	Androgen deprivation therapy
AE	Adverse event
ARPI	Androgen receptor pathway inhibitor
BS	Bone scan
CCC	Central coordination centre
cfDNA	Cell-free DNA
CIMS	Centre d'Imagerie Moléculaire de Sherbrooke
CMR	Complete molecular response
CRPC	Castration-resistant prostate cancer
CT	Computerized tomography
FDG	Fludeoxyglucose
GBq	Gigabecquerel
GCP	Good Clinical Practices
HMSA	N-hydroxy-N-methylsuccinimide acid
HRR	homologous recombination repair
IHC	Immunohistochemistry
IIH	Intrapatient intermetastasis heterogeneity
IIHR	Intrapatient intermetastasis heterogenous response
IP	Investigational product
LDH	Lactate dehydrogenase
mCRPC	Metastatic castration-resistant prostate cancer
mHSPC	metastatic hormone-sensitive prostate cancer
MOHCCN	Marathon of hope cancer centres network
MRI	Magnetic resonance imaging
NE	Neuroendocrine
NED	Neuroendocrine dedifferentiation
PARPi	PARP inhibitors
PCa	Prostate cancer
PET	Positron emission tomography
PMD	Progressive molecular disease
PMR	Partial molecular response
PSA	Prostate specific antigene
PSMA	Prostate-specific membrane antigen
RLT	Radioligand therapy
SAE	Serious adverse events
SMD	Stable molecular disease
SOC	Standard of care
SOP	Standard operative procedures

SUVmax	Standard uptake value max
SUVR	Standard uptake value ratio
TEMPO team	ThEranostics against Metastatic PrOstate cancer team

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1 Protocol Summary

1.1 Study Synopsis

Title	**Four-T**imepoint **M**ulti-tracer **P**ET imaging to characterize metastatic pr**O**state Cancer heterogeneity
Short title	4TMPO
Version	Version 1.0, 2024-10-25
Background and rationale	Prostate cancer (PCa) is the most common solid organ cancer in North American men. Patients becoming refractory to locoregional therapy will inevitably progress to metastatic castration-resistant prostate cancer (mCRPC). As treatment options, patients frequently undergo multiple lines of systemic therapies targeting non-redundant cancer pathways. Cancer phenotype changes (phenotypic plasticity) secondary to successive drug exposure bring novel challenges in monitoring cancer treatment resistance, defining progression and deciding the best therapy. Of these challenges, the intrapatient intermetastasis heterogeneity (IIH) cancer evolution is an example of phenotypic plasticity, which impacts the current clinical practice in oncology. Moreover, when taking under consideration that different molecular imaging tracers alone or in combination can target specific features or phenotypes (e.g. adenocarcinoma vs neuroendocrine tumors), we believe that the sequential use of molecular imaging can overcome the challenges raised by IIH for precision oncology.
Primary objectives	1. Determine the change in prevalence of IIH of mCRPC undergoing consecutive lines of systemic therapy. 2. Determine intrapatient intermetastasis therapeutic heterogeneous response (IIHR) in mCRPC patients.
Secondary objectives	1. Evaluate the impact of systemic treatment change on progressing and non-progressing FDG or PSMA PET lesions under last treatment. 2. Correlate histopathology of biopsies to imaging phenotypes.
Exploratory objectives	1. Link radiomic, and genomic features of single-timepoint and sequential multitracer PET imaging with clinical outcomes. 2. Characterize the biological features of FDG positive/DOTATATE any/PSMA negative lesions (poor prognosis) vs PSMA positive/FDG any/DOTATATE negative lesions.
Primary endpoints	1. Percentage of patients that show heterogeneous FDG and PSMA tracer uptakes between at least two metastases at baseline and at each progression following consecutive lines of study systemic therapies. 2. Percentage of patients that show opposite FDG and/or PSMA tracer uptake and/or ratio responses between two metastases after first and second study systemic therapy.
Secondary endpoints	1.1 Percentage of metastases per patient that will show a significant increase or decrease of FDG or PSMA tracer uptake or ratio after subsequent treatment change for progression.

	1.2 Percentage of metastasis per patient that will change their status from responding to non-responding or non-responding to responding after study treatment change. 2. Associate histopathology and immunohistochemistry (IHC) with double tracer imaging phenotypes of biopsied patients.
Exploratory endpoints	1. Molecular imaging parameters (including DOTATATE data) and genomics that will predict radiographic or clinical failure to systemic therapy from data at baseline, 3 months and at progression. 2. Molecular characterization of aggressive and less aggressive cancer by fresh frozen analysis (omics) and organoid cultures harvested from corresponding lesions.
Study design	Multicentre observational longitudinal study
Number of subjects	45
Sample size consideration	From the 45 participants enrolled, we postulate that ≥30 (66%) will complete baseline to Progression 1 imaging timepoints. For primary objective 1, using the McNemar test with a Type I error rate (α) of 0.05 and a power of 80%, 30 participants will be sufficient to detect a 25 percentage point difference in the proportion of IIH between 2-lines of therapy. Therefore, 30 participants is sufficient to reach statistical significance.
Planned Study Period	Q1 2025 to Q2 2029
Study population	Patients with metastatic castration-resistant prostate cancer, with at least three metastases on reference imaging.
Inclusion criteria	1. Assign male at birth, any gender ≥ 18 years old; 2. Histologically or cytologically proven adenocarcinoma of the prostate; 3. Metastatic disease documented by at least 3 metastatic active lesions*, ** on whole body bone scan and/or measurable soft tissue on CT-scan (lymph nodes and visceral lesions); 4. CRPC & post-androgen receptor pathway inhibitor (ARPI) defined by progression under continuous castration (measured serum testosterone ≤50 ng/dL [1.73 nM]) AND an ARPI (darolutamide, apalutamide, enzalutamide or abiraterone acetate); 5. Eligible for taxane chemotherapy or PSMA-radioligand therapy (before imaging); 6-Able and willing to provide signed informed consent and to comply with protocol requirements. *Metastatic lesions on imaging are defined either: ≥ 10 mm on CT scan or caliper (for lymph nodes, see below), ≥ 20 mm on chest X-ray, lymph node

	≥ 10 mm or having grown by ≥ 5 mm from baseline CT, any metastasis described on bone scan counts as a lesion. Of note: A bone lesion that has been treated by radiation is excluded from the lesions counted in the criterion of ≥ 3 lesions. **The reference imaging (scan with 3 metastases) confirming eligibility must be done either: 1) after biochemical progression on treatment OR 2) ≥ 90 days after last treatment has begun if imaging was performed while patient was still responding (to avoid disappearance of metastasis due to response).
Exclusion criteria	1. Another non-cutaneous malignancy or melanoma diagnosed in the past 5 years; 2. Currently under a randomized controlled trial with unknown allocation; 3-Any disease or condition limiting the patient's capacity to execute the study procedures, based on the investigators' opinion;
Tracers	^{68}Ga-PSMA or ^{18}F-PSMA, ^{18}F-FDG, ^{68}Ga-DOTATATE tracers (intravenous injection)
Visits	-Baseline visit (V1): PET with FDG tracer (standard of care (SOC)), PET with PSMA tracer, PET with DOTATATE tracer (OCREOTATE); -3 months visit (V2): PET with FDG tracer (SOC), PET with PSMA tracer; -Progression 1 visit (V3): PET with FDG tracer (SOC), PET with PSMA tracer, PET with DOTATATE tracer and metastasis biopsy; -Progression 2 visit (V4): PET with FDG (SOC), PET with PSMA, PET with DOTATATE (optional) and biopsy. Blood samples will be collected for biobanking purposes and quality of life questionnaires will be completed at each visit (optional).
Concomitant medication	No restriction
Statistical analysis	**Primary objective 1:** In the 3TMPO study, we have shown that patients with 0, 1 and 2 lines of mCRPC systemic therapy, had at least 1 FDG+/PSMA- lesion (a surrogate IIH) in 13, 35 and 75 %, respectively (average difference in prevalence between lines=31%). For primary objective 1, using the McNemar test with a Type I error rate (α) of 0.05 and a power of 80%, 30 patients will be sufficient to detect a 25-percentage point difference in the proportion of IIH between 2-lines of therapy. Therefore, 30 patients are sufficient to reach statistical significance. **Primary objective 2**: From our previous retrospective study using a single FDG tracer and a new one, 30-40% of PCa patients had opposite metastases PET responses to systemic therapy. For primary co-objective 2 , based on 30 patients that will complete **Baseline** and **Progression 1** timepoints, the calculated IC95 error margin is 16.4 percentage points if we estimate that 30% of patients will show metastases with opposite responses to therapy.

Study Design Overview:

This is a multi-center, open-label, single-arm, non-randomized study using a multi-timepoint triple tracer molecular imaging to characterize intra-patient cancer phenotypes between metastases and to monitor phenotypic plasticity non-invasively. This will be done using ^{18}F-FDG, ^{68}Ga-PSMA (or any other PSMA tracer) and ^{68}Ga-DOTATATE in participants progressing with CRPC and showing at least 3 metastases after conventional imaging. Any PSMA tracer could be used but the first PSMA-PET scan determines the PSMA radiotracer that will be used throughout the study, in order to make the analysis of the radiographic evolution of metastases more accurate (e.g. ^{68}Ga-PSMA-617 or ^{18}F-DCF-PyL or ^{68}Ga-PSMA-11). The reference imaging must have been performed either: 1) after biochemical progression on treatment OR 2) at least 90 days after last treatment has begun if imaging was performed while patient was still responding (to avoid disappearance of metastasis due to treatment response). These imaging will include: 1) bone scan or ^{18}F-Na-PET/CT AND either chest/abdomen CT or chest CT and abdomen MRI OR 2) ^{18}F-FDG-PET/CT. This study is planned to be conducted in up to 4 sites in Canada. Eligible participants (see Section for Eligibility Criteria) will be enrolled in a non-randomized, sequential manner, with competitive enrollment between study sites. A total of 45 participants will be accrued. Enrolled participants will undergo ^{18}F-FDG, ^{68}Ga- or ^{18}F-PSMA and ^{68}Ga-DOTATATE-PET/CT scans. After initial triple tracer PET molecular imaging, the participants will be treated with cabazitaxel or docetaxel or ARPI (as standard of care or as an investigational agent) or PSMA-radioligand therapy (as standard of care or as an investigational agent). Three (3) months after initiation of systemic therapy double-tracer (PSMA & FDG) PET/CT imaging will be performed. Participants will continue their therapy and once progression will occur (V3, Progression 1), triple tracer will be repeated and a biopsy of a progressive lesion will be executed. Patients will be treated with a mandatory second line of approved mCRPC systemic therapies: PSMA-RLT therapy (Pluvicto ^{177}Lu-PSMA-617) or Olaparib (PARPi) if they are found with homologous recombination repair (HRR) mutation (a predictive biomarker for PARPi). If not HRR mutated nor eligible to PSMA-RLT, a second line of approved mCRPC systemic therapy or research protocol will be offered. At Progression 2 (V4) (after treatment change following Progression 1), double-tracer ^{68}Ga-PSMA and ^{18}F-FDG PET/CT imaging will be repeated (DOTATATE scan will be optional). The patient will have another biopsy of a progressing lesion and an optional second biopsy of a non-progressing site offered.

The investigational PET/CT scans will be sent to the central imaging core lab for review (CHUQc-UL).

Protocol :

Triple tracer imaging will be performed using local PET/CT scanners with low-dose CT for attenuation correction and anatomic localization. Whole body PET/CT scans (mid-thigh to skull vertex) will be obtained 60 ±10 minutes following the intravenous administration of ^{18}F-FDG, PSMA tracer or ^{68}Ga-DOTATATE injection. After voiding, a whole-body PET/CT will be acquired from the mid-thigh through the vertex of the skull (approximately 6 to 8 bed positions). All PET data should be reconstructed iteratively with correction for attenuation, scatter, random coincidences and dead time followed by a post-reconstruction filtering. All PET/CT and subsequent conventional imaging scans following injections will be collected and evaluated by

a central imaging core laboratory. Central imaging core lab independent readers will be blinded to all clinical data, including any histopathology results.

Baseline visit: Participants will sequentially undergo a ^{18}F-FDG, a ^{68}Ga- or ^{18}F-PSMA and a ^{68}Ga-DOTATATE-PET/CT scan after which they will receive systemic treatment. All participants will have ECOG status and vital signs (SOC) performed. Blood samples will also be collected for biobanking purpose (optional) and laboratories.

3 Months Visit: Three months after initiation of systemic drug therapy, double-tracer (PSMA and FDG) PET/CT scans will be performed. Blood samples will also be collected for biobanking (optional) and mandated laboratories. If a clinical progression is observed during this visit based on primary physician's opinion, this becomes the Progression 1 Visit.

Progression 1 Visit: At the first progression, triple tracers PET/CT scans will be repeated. All participants will have ECOG status and vital signs (SOC) performed. Blood samples will also be collected for biobanking (optional) and laboratories. CT-guided bone or soft tissue biopsy of a progressive lesion will be performed. An optional biopsy of a non-progressive lesion could also be performed.

Progression 2 Visit: At the Progression 2, after treatment change following Progression 1 Visit, double-tracer ^{18}F-FDG and PSMA PET/CT imaging will be repeated, with an ^{68}Ga-DOTATATE-PET/CT (optional). Blood samples will also be collected for biobanking (optional) and laboratories. The participant will have another biopsy of a progressing lesion and a second biopsy of a non-progressing site offered (optional).

Validated questionnaires (EQ5D, BPI, FACT-P) will be self-administered at each visit by sending the forms to participants by mail or electronically through REDCap or at patient's hospital visits.

As SOC follow-up, all participants will undergo blood work every 3-4 months for up to five years after accrual. Conventional imaging will be performed at least every 3 to 6 months as SOC. The research team will collect data from these follow-ups and patient status every year post-enrollment (survival, best response to treatment).

1.2 State of knowledge

INTRAPATIENT INTERMETASTASIS HETEROGENEITY. Metastatic cancer treatment options have exploded in the last decade and, nowadays, patients frequently undergo multiple lines of systemic therapies targeting non-redundant cancer pathways. While these therapies have translated into better survival, cancer phenotype changes (phenotypic plasticity) secondary to successive drug exposure bring novel challenges in monitoring cancer treatment resistance, defining progression and deciding the best therapy. Of these challenges, the intrapatient intermetastasis heterogeneous (IIH) cancer evolution is an example of phenotypic plasticity, which impacts the current clinical practice in oncology. Heterogeneity means several cancer phenotypes are found within a single tumor lesion (primary or metastasis) and IIH implies that, in a single patient, several phenotypes/genotypes are found, but in different metastases, each potentially harboring differential drug sensitivity[1]. While we are learning more about IIH, little is known on how it develops and how to manage it.

RATIONALE Because of IIH and plasticity, precision medicine and treatment strategies must take into consideration heterogeneous cancer spread and should not rely solely on data derived from liquid or single lesion tissue biopsies. To fulfill these needs, molecular imaging using multiple imaging agents offers the ability to characterize intra-patient cancer phenotypes among metastases and to detect gross phenotypic plasticity non-invasively and in real-time under selective treatment pressure. Moreover, when taking under consideration that different molecular imaging tracers alone or in combination can target specific features or phenotypes (e.g. adenocarcinoma vs neuroendocrine (NE) tumors), we believe that the sequential use of molecular imaging can overcome the challenges raised by IIH for precision oncology.

PROSTATE CANCER MOLECULAR IMAGING AND IIH. Prostate cancer (PCa) is the third cause of cancer mortality in Canadian born as male[1]. PCa patients with metastases receive androgen deprivation therapy which is the backbone therapy, but the disease will inevitably become castration resistant and progress[2,3]. In advanced prostate cancer, ADT can be intensified with Androgen Receptor Pathway Inhibitor **(ARPI)** or Docetaxel chemotherapy or PARP-Inhibitors **(PARPI)** or ARPI+Docetaxel or ARPI+PARPI (see EAU guidelines https://uroweb.org/guidelines/prostate-cancer). All these intensified regimens improve recurrence-free survival and have been Health Canada approved but at a cost of patient's and/or financial's toxicities while there is no good biomarker to decide who should benefit from treatment intensification. Castration-resistant prostate cancer (CRPC), a lethal disease, can evolve either as a classical adenocarcinoma (an androgen receptor-responsive carcinoma), an androgen-indifferent carcinoma or as a NE CRPC, the latter occurring in approximately 10% of cases[4,5]. NE CRPC is an aggressive subset of castration-resistant tumors that exhibit NE differentiation pathological features on biopsy, including somatostatin receptor, chromogranin and synaptophysin expression[6,7]. These tumors do not secrete the prostate-specific antigen (PSA), a biomarker expressed in almost all primary adenocarcinoma. Recent studies have clearly established that metastatic CRPC **(mCRPC)** may evolve as an intrapatient heterogeneous disease based on genomic analyses of intrapatient metastatic tissues[8,9]. Our group and others have found that, using positron emission tomography **(PET)** imaging that as many as 40% of metastatic PCa patients exhibit evidence of IIH[7,8,10]. This can manifest, in the same patient, as discordant tracer uptake or histopathology (adenocarcinoma and NE) among different metastases as well as heterogeneous response to systemic therapies [7,8,10]. However, the true prevalence of IIH or NED at different lines

of mCRPC therapy was unknown before our recent 3TMPO study (Fig.1). In 3TMPO, we have used a whole-body approach using multi-tracer molecular imaging to track PCa IIH[11]. This was possible because new specific positron emission tomography (PET) tracers have the ability to non-invasively image PCa biology by targeting either the prostate-specific membrane antigen (**PSMA**; e.g., ^{68}Ga-PSMA-617, adenocarcinoma), increased glucose metabolism (^{18}F-FDG, almost all histologies) or the somatostatin receptor expression (^{68}Ga-DOTATATE, NE phenotype), offering new tools for visualizing IIH and dedifferentiated-CRPC (*Fig. 1A*). PSMA-PET/CT is highly sensitive for detecting metastasis in adeno-CRPC patients, even at low PSA levels[12], but PSMA tracer is of little use in NE-CRPC because PSMA expression is suppressed[7,12]. Furthermore, ^{18}F-fluorodeoxyglucose (^{18}F-FDG) tumor uptake significantly increases in mCRPC[13] and FDG-PET/CT can image both adenocarcinoma-CRPC and NE-CRPC (Fig.1B). Of note, PET is quantitative and measures the Standard Uptake Value (SUV), which reflects the tracer uptake in each metastasis. This allows cancer resistance tracking without metastasis size increase or appearance of new metastasis. Also, note that tracers ratio in each metastasis can reveal different histologies and be an equivalent to "in vivo immunohistochemistry" using antibodies to characterize tumors (Fig.1C)[11].

1.3 Clinical data from the 3TMPO study

Our 3TMPO trial was designed to determine the prevalence of IIH in mCRPC patients at any line of therapy (Fig. 1)[11]. To our knowledge, 3TMPO described for the first time a whole-body IIH prevalence in any cancer using predetermined imaging criteria from a prospective trial. 3TMPO was a prospective multicenter PET-imaging trial that has accrued 100 mCRPC patients showing at least 3 metastases on conventional imaging and with evidence of biochemical or radiographic progression, at least 3 months after initiation of the last systemic therapy. ^{68}Ga-PSMA-617 and ^{18}F-FDG PET/CT scans were performed within 10 days and analyzed quantitatively (Fig.1A). A third scan with ^{68}Ga-DOTATATE was done when a PSMA-/FDG+ lesion (as a surrogate of NE dedifferentiation) was found. For all tracers, positivity of a lesion was defined as its SUVpeak being 1.5 times higher than the SUVmean of the liver. The primary objectives were: 1) To determine the prevalence of IIH in mCRPC patients, defined as the percentage of patients having different PET tracer imaging phenotypes between at least two metastases; 2) To determine the proportion of mCRPC patients candidate for PSMA-radioligand therapy (RLT). The 3TMPO has completed accrual, imaging and follow-up (*Fig.1B and C*). In summary, we have shown that, based on FDG- and PSMA-imaging, the prevalence of IIH was 82.7% and that 45.9 % of patients showed ≥ 1 FDG+/PSMA- lesion (*Fig. 1B*). Based on dual FDG/PSMA tracers, 7 patient's phenotypes of tracer combination were found (*Fig. 1C and D*). Moreover, of the 37 patients who underwent ^{68}Ga-DOTATATE PET, six (13.6%) had significant DOTATATE uptake which is a novel finding for PCa. In this FDG/PSMA/DOTATATE-imaged subgroup, 5 new different combinations of imaging phenotypes were observed between metastases of individual patients (*Fig. 1D and E*). The patient imaged in *Fig.1C* represents the *"phenotype 12"* seen in *D*. Other top key findings of 3TMPO are: 1) IIH prevalence increases with the number of line of treatment; 2) <u>OS is shorter in patients showing imaging phenotypes such as ≥ 1 FDG+/PSMA- lesion, ≥ 1 DOTATATE+ lesion or IIH (Fig.1F, G, H).</u>

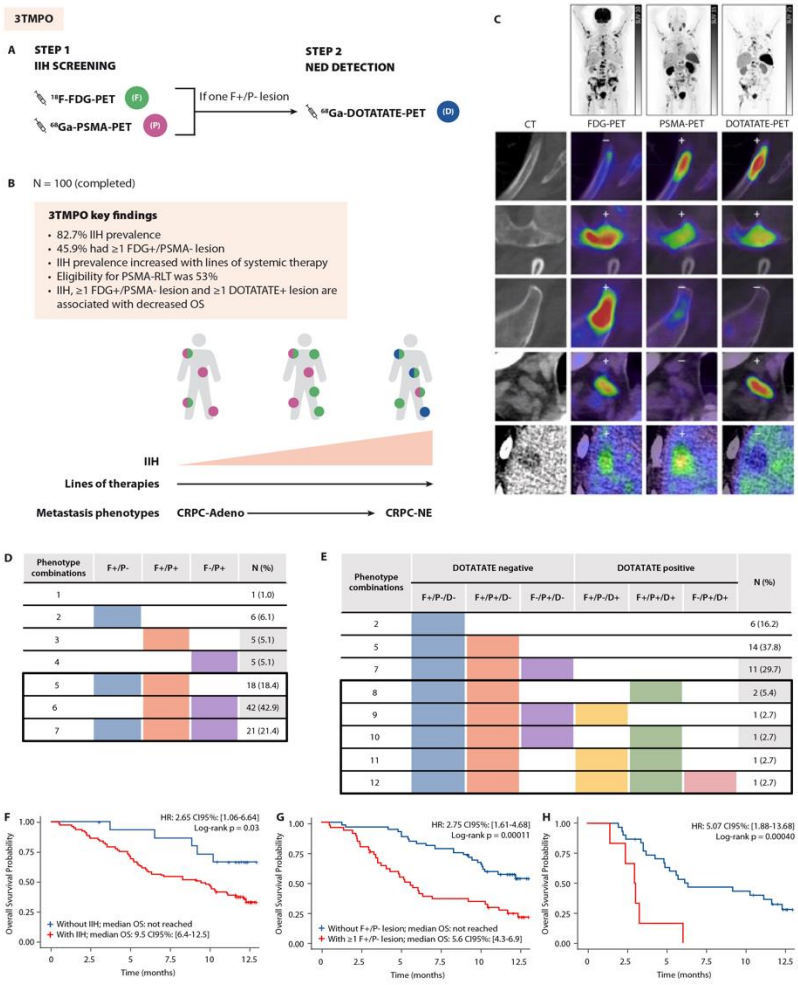


Fig.1 3TMPO study design and result summary that led to 4TMPO

2 STUDY DESIGN, RESEARCH HYPOTHESIS AND OBJECTIVES

2.1 Study Design

The **4TMPO** (**Four**-**T**imepoint **M**ulti-tracer **P**ET imaging to characterize metastatic pr**O**state Cancer heterogeneity) imaging project was designed according to the STROBE guidelines. It is a multicentre observational cross-sectional study, in which 45 participants will be enrolled in up to four tertiary hospital centres (CIUSSSE-CHUS, CHUQc-UL, CHUM, CUSM) in the province of Québec, (completed over 48-month). Data collection is prospective.

2.2 Research Hypothesis

We hypothesize that *at least* two pathways of resistance could occur:

1-the whole-body resistance is seen when resistant cells appear concomitantly in several metastasis after predictable genotype/phenotype plastic changes under systemic treatment pressure. In this scenario, such predictable changes would be driven by baseline genotype (Fig.2 *scenario 1*).

2-In the oligoresistance/oligoprogression model, resistance would originate in a single tumor metastasis, randomly (Fig.2 *scenario 2*). This "clone" would locally progress (see pink dot at 3 months and at Progression 1) and then spreads to the other metastasis or form new resistant metastasis.

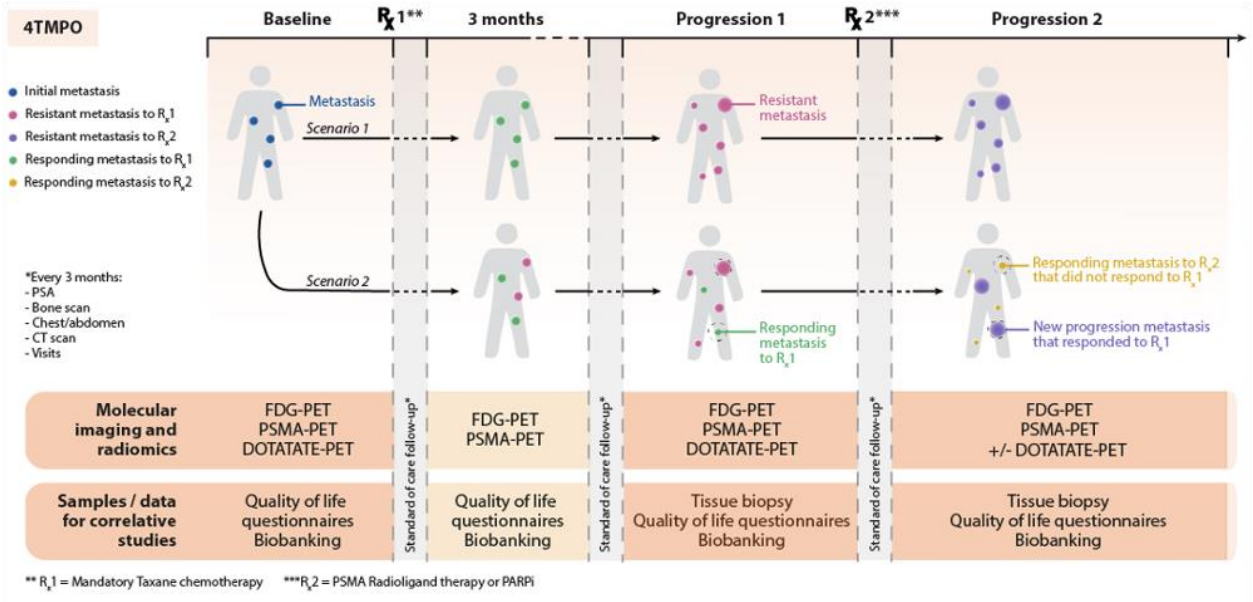


Fig.2 Summary of the 4TMPO study hypothesis, methodology and expected results.

2.3 Objectives

Primary Objectives

1. Determine the change in prevalence of IIH of mCRPC undergoing consecutive lines of systemic therapy.
2. Determine intrapatient intermetastasis therapeutic heterogeneous response (IIHR) heterogeneity in mCRPC patients.

Secondary Objectives

1. Evaluate the impact of systemic treatment change on progressing and non-progressing FDG or PSMA PET lesions under last treatment.
2. Correlate histopathology of biopsies to imaging phenotypes.

Exploratory objectives

1. Link radiomic, and genomic features of single-timepoint and sequential multitracer PET imaging with clinical outcomes.
2. Characterize the biological features of FDG positive/DOTATATE any PSMA negative lesions (poor prognosis) vs PSMA positive/FDG any/DOTATATE negative lesions.

3 METHODOLOGY

3.1 Population

INCLUSION CRITERIA:

1. Assign male at birth, any gender ≥ 18 years old;

2. Histologically or cytologically proven adenocarcinoma of the prostate;

3. Metastatic disease documented by at least 3 metastatic active lesions[*, **] on whole body bone scan and/or measurable soft tissue on CT-scan (lymph nodes and visceral lesions);

4. CRPC & post-androgen receptor pathway inhibitor (ARPI) defined by progression under continuous castration (measured serum testosterone ≤50 ng/dL [1.73 nM]) AND an ARPI (darolutamide, apalutamide, enzalutamide or abiraterone acetate);

5. Eligible for taxane chemotherapy or PSMA-radioligand therapy (before imaging);

6-Able and willing to provide signed informed consent and to comply with protocol requirements.

*Metastatic lesions on imaging are defined, either: ≥ 10 mm on CT scan or caliper (for lymph nodes, see below), ≥ 20 mm on chest X-ray, lymph node ≥ 10 mm or having grown by ≥ 5 mm from baseline CT, any metastasis described on bone scan counts as a lesion. Of note: A bone lesion that has been treated by radiation is excluded from the lesions counted in the criterion of ≥ 3 lesions.

**The reference imaging (scan with 3 metastases) confirming eligibility must be done either: 1) after biochemical progression on treatment OR 2) ≥ 90 days after last treatment has begun if imaging was performed while patient was still responding (to avoid disappearance of metastasis due to response).

EXCLUSION CRITERIA: Patients meeting any of the following exclusion criteria are not eligible for this study:

1. Another non-cutaneous malignancy or melanoma diagnosed in the past 5 years;

2. Currently under a randomized controlled trial with unknown allocation;

3. Any disease or condition limiting the patient's capacity to execute the study procedures, based on the investigators' opinion;

3.2 Study endpoints

Primary:

1. Percentage of patients that show heterogeneous FDG and PSMA tracer uptakes between at least two metastases at baseline and at each progression following consecutive lines[1] of study systemic therapies.
2. Percentage of patients that show opposite FDG and/or PSMA tracer uptake and/or ratio responses between two metastases after first and second study systemic therapy.

Secondary:

1.1 Percentage of metastasis per patient that will show a significant increase or decrease of FDG or PSMA tracer uptake or ratio after subsequent treatment change for progression.

1.2 Percentage of metastasis per patient that will change their status from responding to non-responding or non-responding to responding after study treatment change.

2. Associate histopathology and immunohistochemistry (IHC) with double tracer imaging phenotypes of biopsied patients.

Exploratory:

1. Molecular imaging parameters (including DOTATATE data) and genomics that will predict radiographic or clinical failure to systemic therapy from data at baseline, 3 months and at progression.
2. Molecular characterization of aggressive and less aggressive cancer by fresh frozen analysis (omics) and organoid cultures harvested from corresponding lesions.

[1]Consecutive lines of systemic therapy are life-prolonging agent lines that will be administered after baseline molecular imaging during the study (first study line) or after first progression and subsequent molecular imaging (second study line).

3.3 Definitions for endpoints and outcomes

1-POSITIVE LESION: ^{18}F-FDG, PSMA and ^{68}Ga-DOTATATE lesions uptake will be defined as positive if greater than that of the liver. Using quantitative imaging methods, standardized uptake value ratio (SUVR, i.e. the ratio between lesion uptake (SUV_{peak}) over liver uptake (SUV_{mean})) will be obtained for each lesion with each tracer. For a given tracer, lesion positivity is defined as an SUVR equal or superior to 1.5. Therefore, negative lesions are those < 1.5 x SUVR.

$$\text{SUVR} = \frac{\text{Tumor SUVpeak}}{\text{Liver SUVmean}}$$

2-INTRAPATIENT INTERMETASTASIS HETEROGENEITY (IIH): a patient will be defined as having IIH PCa disease when we will detect: (1) Both [PSMA-positive/FDG-positive or negative] and [PSMA-negative/FDG-positive] lesions OR; (2) Both [FDG-positive/PSMA-positive or negative] and [FDG-negative/PSMA-positive] lesions OR; (3) In patients with only PSMA-negative/FDG-positive lesions undergoing DOTATATE-PET, both DOTATATE-positive and DOTATATE-negative lesions, that were positive on FDG and/or PSMA-PET.

3-METASTASIS IMAGING PHENOTYPE: for each metastasis, double or triple-tracer positivity status will provide a positivity combination status (ex: FDG positive/PSMA negative/DOTATATE positive vs FDG negative/PSMA positive/DOTATATE negative). Each potential combination will generate a metastasis imaging phenotype assigned to each metastasis.

4-PATIENT IMAGING PHENOTYPE: A combination of metastases imaging phenotypes is defined by any single or combination of metastasis phenotype based on double or triple tracer imaging, found in a single patient.

5-METASTASIS TRACER UPTAKE RESPONSE CRITERIA: The individual metastasis SUVR will be calculated. Inspired by PERCIST criteria, a 30% change in SUVR will be considered as a clinically significant partial molecular response (PMR) or progressive molecular disease (PMD). The apparition of a new lesion that fulfills positivity criteria will define progressive molecular disease (PMD)[16]. A lesion that fulfilled at baseline the positive criteria described above (SVR > 1.5) and that becomes negative (SVR ≤ 1.5) under treatment will be defined as complete molecular response (CMR). A lesion is defined as stable (stable molecular disease: SMD) when it does not fulfill the criteria for either PMR, PMD nor CMR. In case of discordance between tracer changes, truth standard will be determined by FDG uptake first, followed by PSMA uptake (if FDG neg) followed by DOTATATE if both FDG and PSMA neg at previous imaging series.

6-INTRAPATIENT INTERMETASTASIS HETEROGENEOUS RESPONSE (IIHR): A patient's response will be considered to have an IHHR when, in the same compartment (bone or soft tissue): (1) at least one lesion progressed (PMD or new lesion) while another presented either SMD or PMR or CMR; or (2) at least one lesion responded (CMR or PMR) while another had an increasing SUVmax. Indeed, for a patient to meet the heterogeneity criteria, one of his metastases had to show an increase, and one a decrease in FDG uptake between the two scans. Moreover, the difference in FDG uptake changes between the two divergent lesions had to be greater than 30%.

7- RESPONSE OR PROGRESSION CRITERIA: criteria of response or progression will be determined by the treating physician who will be asked to follow the Prostate Cancer Working Group 3 definitons. Criteria of response (for study data entry) will be according to PCWG3 criteria.

3.4 Run-in Phase

The first four (4) enrolled participants will constitute the run-in phase (representing 10% of the sample size). During this phase, the 4TMPO team will validate site compliance with the study protocol, conformity to the eligibility criteria and long-term feasibility of the current protocol. If major issues are encountered or expected, the protocol and/or the corresponding documents will be amended and resubmitted to regulatory agencies, if applicable. The study protocol will be prepared for publication following the run-in phase.

3.5 Screening and Enrolment

Screening and identification of potential participants will be overseen by clinical or research teams (following the Director of Professional Services' authorization to access medical charts). First contact will be done by clinical staff (treating physicians, urologists, outpatient clinic nurses) to explain their eligibility for the 4TMPO study. They may refer to the specific research team member to better describe the study, to explain the informed consent procedure and to solicit participation in the study.

Each site must enroll at least three participants to secure participation from the site and to increase external validity of the results for the province of Québec mCRPC population.

3.6 Participant Workflow

A) Eligible participants diagnosed with at least three metastases after reference imaging will be approached by the research team who will present the study.

B) After obtaining signed consent *and during each of the imaging series 4 timepoints* (Fig. 2a), clinicopathological data (medical history, previous and current treatment/medication, symptomatology), ECOG performance status and laboratories (including PSA) will be collected. The research team will assist the participant in completing validated standard metastatic PCa quality of life questionnaires (EQ5DL, Brief inventory pain (BPI) and Functional Assessment of Cancer Therapy– Prostate (FACT-P)[11,20]. Blood samples will be collected by a nurse and will be stored according to the biobank framework (see section below).

C) Patients will sequentially undergo ^{18}F-FDG, ^{68}Ga- or ^{18}F-PSMA and ^{68}Ga-DOTATATE whole-body PET/CT. The order of these sequential scans will depend on the availability of the radiotracers. However, it will be important that the consecutive scans will be performed at least 18 hours apart (due to ^{18}F half-life) but no later than 10 days (to avoid next line of treatment delay). After FDG, PSMA and DOTATATE PET scans, technologists will complete the corresponding electronic case report file (eCRF) on REDCap and upload the denominated images on the PACS platform within 24 hours following the last scan.

D) Then, patients will be treated with taxane-based chemotherapy (cabazitaxel, docetaxel) or ARPI (as standard of care or as an investigational agent) or PSMA-radioligand therapy (as standard of care or as an investigational agent) which are life prolonging agents (or investigated to be life prolonging agents) after ARPI resistance in mCRPC systemic therapies.

E) Three (3) months after initiation of systemic therapy double-tracer (^{68}Ga-PSMA and ^{18}F-FDG) PET/CT imaging will be performed. If progression occurs at 3 months, patients will follow protocol as in G.

F) Between progressions, patients will be followed as per SOC with serial conventional imaging (bone scan and Chest-abdomen CT) and labs including PSA.

G) At **Progression 1** (biochemical or radiological) determined by the Prostate Cancer Working Group 3 criteria[21] triple-tracer PET/CT imaging will be repeated (see C). Central reads of PET will be performed by one nuclear medicine specialist co-applicant (JMB or ER) and reported according to definitions in Section 3.3. Central nuclear medicine reads will be done <2 days after the last PET of the timepoint and REDCap CRF entry performed. An automated message will be sent to the treating physicians and coordinating team who will, in collaboration with nuclear medicine specialist and interventional radiologists, organize biopsy of a progressing lesion (PMD) as determined by the metastasis tracer uptake response criteria in Section 3.3 An optional second biopsy site of non-progressing lesion that is positive on any PET will be offered to patients.

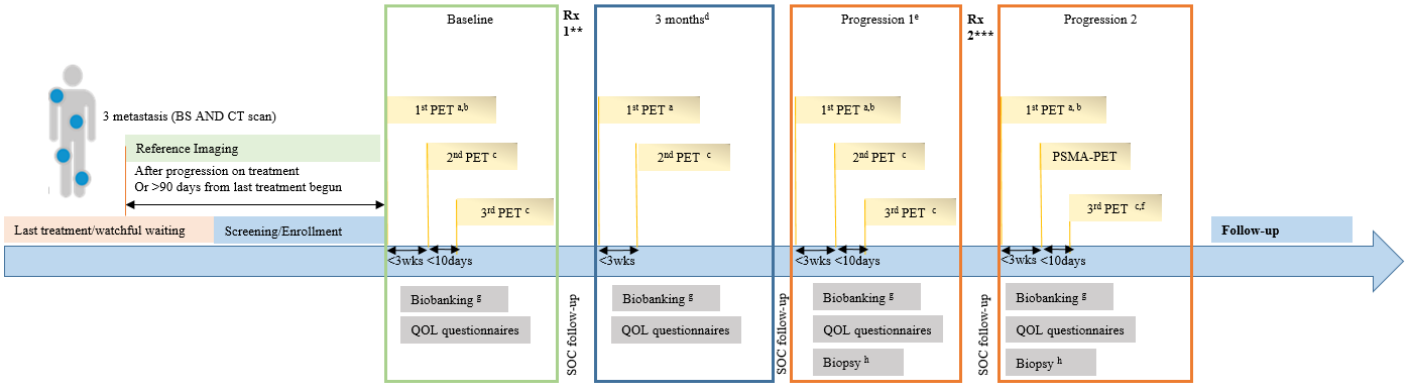
H) Patients will be treated with a mandatory second line of approved mCRPC systemic therapies: PSMA-RLT therapy (Pluvicto ^{177}Lu-PSMA-617) or Olaparib (PARPi) if they are found with HRR mutation (a predictive biomarker for PARPi). If not HRR mutated nor eligible to PSMA-RLT, a second line of approved mCRPC systemic therapy or research protocol will be offered.

I) At **Progression 2** after treatment change following **Progression 1** (Fig. 2a) double-tracer ^{68}Ga-PSMA and ^{18}F-FDG PET/CT imaging will be repeated (DOTATATE scan will be optional for logistics and costs purposes). The patient will have another biopsy of a progressing lesion (see in G) and an optional second biopsy of a non-progressing site offered.

J) As SOC follow-up, all patients will undergo ECOG performance status and blood work every 3-4 months for up to 5 years after accrual (or as determined by the medical team). Conventional imaging will be performed at least every 6 months as per SOC. The research team will collect data from these follow-ups and patient status every year post-enrollment (survival, best response to treatment).

4TMPO flowchart (Fig.2a)

At each visit two PETs scan will be performed: one ^{18}F-FDG PET and one PSMA-PET that will remain the same throughout the study (i.e ^{68}Ga-PSMA-617 or ^{18}F-DCF-PyL or ^{68}Ga-PSMA-11). When applicable, one ^{68}Ga-DOTATATE-PET will also be done.



**Rx1= Mandatory Taxane chemotherapy ***Rx2= PSMA Radioligand therapy or PARPi

[a] The first PSMA-PET scan determines the PSMA radiotracer that will be used throughout the study, in order to make the analysis of the radiographic evolution of metastases more accurate (e.g. ^{68}Ga-PSMA-617 vs ^{18}F-DCF-PyL vs ^{68}Ga-PSMA-11);
[b] The ^{18}F-FDG-PET scans can be used to evaluate progression as SOC;
[c] The order of the 2nd and 3rd sequential PET scans (PSMA and DOTATATE) will depend on the availability of both radiotracers;
[d] If progression is observed during the 3 months visit, it therefore becomes the Progression 1 visit and no data must be entered in the eCRF for the 3 months visit;
[e] Progression is defined by biochemical or radiological progression leading to a change in treatment;
[f] DOTATATE PET scan is optional;
[g] Biobanking is optional;
h Biopsy of progressive lesion, an optional biopsy of a non-progressive lesion could also be performed

BS: Bone scan; eCRF: electronic Case Report Form; PET: Positron Emission Tomography; QOL: Quality of Life; SOC: Standard of care, wks: weeks;

3.7 Schedule of assessments

Table 1. Schedule assessments

Period	Imaging Procedures				EOS
Visit	Baseline	3 months	Progression 1	Progression 2	Follow-up
Visit number	V1	V2	V3	V4	Once a year
Window (days)	Day 0	+/- 7 Days			
General Study Procedures					
Informed Consent (main and biobank) [1]	X				
Verify Inclusion/Exclusion	X				
Medical History/Demographics	X				
Disease assessments [2]	X				
ECOG Performance Status	X	X	X	X	
Prior/Current tx and Medications	X	X	X	X	
Adverse events	X	X	X	X	
Biological Samples Collection					
Laboratories	X	X	X	X	
PSA [4]	X	X	X	X	
Testosterone [4]	X	X	X	X	
Samples Biobanking [5]	X	X	X	X	
Biopsy			X	X	
Questionnaires					
QOL questionnaires	X	X	X	X	
Imaging [7,8]					
Vital Signs (Blood Pressure, Heart Rate)	SOC [3]	SOC [3]	SOC [3]	SOC [3]	
FDG-tracer administration	SOC	SOC	SOC	SOC	
PSMA-tracer administration	X	X	X	X	
^{68}Ga-DOTATATE administration	X		X	X[9]	
Whole Body PET/CT	X	X	X	X	
Conventional imaging [6]	SOC, At minimum 3 to 6 months intervals				
Data collection from patient medical file					X

1. Must be obtained prior to any study-specific procedures being performed.
2. Disease assessments with conventional imaging (i.e. chest-abdomen CT, bone scan, MRI). These conventional imaging must have been performed within the last 90 days before signing the ICF.
3. To be measured pre-imaging only as SOC.
4. If PSA and testosterone have not been tested within 30 days of visit, a blood draw will be collected prior to dosing.
5. If consent is obtained for blood biobanking, 30 ml of blood is requested at Visit 1 to Visit 4 (serum, plasma, buffy coat, whole blood, RNA, cfDNA).
6. Bone scan + Chest-Abdomen CT
7. Tracers must be scheduled on the day of the visit but administration must be done from the day of the visit + 3 wks or 3wks + 10 days when 3 tracers are targeted
8, AEs will be documented after each PET scan
9. ^{68}Ga-DOTATATE administration is optional

4 STUDY PROCEDURES

4.1 Laboratory Assessments

See Table 2 for the list of required clinical laboratory tests routine, to be performed and refer to schedule of assessments for timing and frequency. All samples for laboratory analysis must be collected, prepared, labeled, and shipped according to local laboratory requirements.

The investigator or sub-investigator must review the laboratory report and document this review.

Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator or sub-investigator who is a qualified physician.

Table 2

Hematology	**Chemistry**
Hematocrit Hemoglobin Platelet count Red blood cell count	Albumin Alkaline phosphatase ALT AST Blood urea nitrogen and creatinine Ca++ Creatinine Glucose Lactate dehydrogenase Magnesium, phosphate Na+, K+, total CO2 (bicarbonate), Cl- Total bilirubin (TBili) Total protein
Other PSA Testosterone	

4.2 ECOG

ECOG performance status assessments will be performed throughout the study according to the schedule of activities.

4.3 Blood Sample for Biomarker Analysis

Study participants will be asked, as part of a second and optional project, using a distinct consent form, to contribute to the biobank. In short, a blood sample will be collected at each timepoint. Sampling material will be provided to the site by the coordinating centre. Biological samples will be sent and managed by the BIOBANK Corelab, chaired by Dr. Frédéric Pouliot at CHUQc-UL.

4.4 Radiotracers for PET imaging

Detailed instructions are provided in the Image acquisition and transfer manual.

4.5 Image analysis

The imaging core-lab will be led by Dr. Beauregard. An imaging biobank will be created along with the REDCap clinical database and tissue biobank and include all PET/CT and conventional imaging studies (including those of each biopsy procedure). PET/CT images will be Quality Controlled and analyzed in real time (<48 h) by the core-lab. Lesions will be segmented semi-automatically using a SUV threshold (1.5x liver SUVmean), discarding foci of physiological uptake and lesions <1cc. Segmented lesions will be matched and labeled across FDG, PSMA and DOTATATE PETs (if negative on one tracer, a VOI will be pasted from a positive PET), and PET metrics (SUVmax, SUVpeak, SUVmean, molecular tumor volume and total lesion activity) will be extracted per lesion and for the whole-body tumor burden.

4.6 Biopsy and histopathology

Patients presenting progressive lesions will be asked to undergo a biopsy of the corresponding lesions for research purposes. Bone or soft-tissue biopsies will be collected by a local interventional radiologist according to the site's SOC under CT or ultrasound guided procedures and sent to the local pathology department for preparation. Two to three 18G needle samples will be collected by targeted lesion: the first will be paraffin embedded for histopathological analysis; the second will be snap frozen for genomics analysis and the third will be sent for organoid culture. Interventional radiologists will be dedicated to the protocol per center to ensure proper site biopsy and patient's flow. In the context (majority of cases) where a biopsy is clinically indicated, the local pathologist will analyze the biopsy (macro description and IHC) before sending the residual biopsy (or the first core) or the digitalized images to the Pathology Corelab.

Histopathology from metastases biopsy will mirror the analyses performed in 3TMPO[11]. Diagnostic will be performed following to the Urinary and Male Genital Tumours WHO Classification of Tumours, 5th Edition, Volume 8 by an experienced genitourinary pathologist (DT). Possible diagnoses will include treatment-related NE PCa, with subdivisions in small cell NE PCa, large cell NE PCa and mixed NE/adenocarcinoma tumors, as well as de novo small cell NE carcinoma and large cell NE carcinoma. The diagnosis will be supported by cyclic immunofluorescence, allowing to perform immunofluorescence targeting androgen receptor, PSMA, PSA, NKX3.1, Ki-67, synaptophysin, chromogranin as well as CD56 on a single slide, allowing the single-cell evaluation of marker co-expression while ensuring all biomarkers can be performed on the expected small biopsies[26]. Histopathology findings will be linked to imaging phenotypes (see Fig.1D-E) below and treatment resistance. To evaluate tumor plasticity during evolution, a representative section of the original diagnostic material will be compared to the metastasis's biopsies. Finally, intralesional heterogeneity within each biopsy core will be analyzed after IHC stainings and then potentially spatial transcriptomics (in future correlative studies).

Biopsy samples, when available for research purposes, will be sent to the Pathology Corelab, for histologic validation and secondary outcomes analyses. Residual samples or digitalized slides from participants who agreed to contribute to the biobank will be stored at the biobank facility (CHUQc-UL) and managed according to the biobank framework. Biobank Corelab is chaired by Dr. Pouliot. The biobank will be submitted independently from this protocol and participants' consent will be collected.

5 STUDY AND DATA MANAGEMENT

5.1 Study Management

The study will be supervised by the Executive Committee led by Frédéric Pouliot (responsible for the sponsoring institution), Prof. Brigitte Guérin and Dr. Jean-Mathieu Beauregard. This committee will provide overall supervision and guidance for the study as well being involved in day-to-day management. It will evaluate any issues occurring (feasibility, safety, etc.) during the course of the study.

The CHUQc-UL will take the lead in terms of study coordination across the three participating centres. It will be involved in: 1) launching pre-study activities (REB, SOPs, personalized study tools, training); 2) study and budget management (agreements, tracer shipment coordination, imaging and biopsy central revision coordination; site fee payments); 3) planning investigators' meetings; 4) securing site initiation visits, monitoring and closeout activities; 5) developing the REDCap database; 6) following-up on serious and non-serious adverse events (SAE, AE); 7) statistical analyses; 8) planning knowledge transfer activities - including patients' engagement.

5.2 Data Management

Clinical and medical data will be collected using a web-based eCRF called REDCap within 5 days of collection. The REDCap database is hosted on certified ISO 27001 secured servers at the CHUQc-UL. The investigator or site designee is responsible to ensure that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with the source. These documents should be appropriately maintained by the study site.

All data from participants who agree to contribute to the biobank will be preserved and associated to corresponding samples in the biobank, according to the biobank framework.

5.3 Imaging management

Denominated conventional (bone scan and CT) and molecular imaging (PET) scans will be imported to a central repository on the PACS platform and transferred to CHUQc-UL for central review. PET scans will be co-registered and all tumour lesions will be segmented to derive uptake parameters. IMAGING PET CORE is chaired by Dr. Jean-Mathieu Beauregard, Nuclear Medicine Clinician-Scientist at the CHUQc-UL and co-PI. His laboratory will provide support in establishing quantitative endpoints for imaging trials and SOP for imaging acquisition and analysis. This will include procedures and methods for image acquisition and reconstruction, image archiving, review, and access, methods for qualitative and quantitative data analyses and centralized, blinded reading.

5.4 Ethics and Institutional Feasibility

The study will be evaluated and approved by the Comité d'éthique à la recherche du CHUQc-UL, acting as the evaluating REB for all the study's participating sites. The CHUQc-UL will be responsible for obtaining ethics approval and renewal. Sites will be accountable for their institutional feasibility approval, which is mandated to formalize collaboration with radiologists (imaging, biopsy collection) and pathologists (biopsy preparation). Interinstitutional agreements will be implemented to fully execute collaboration between the sites and the coordinating centre and the obligation/responsibilities of each party. A qualified investigator at each site will be provided a restricted access to the central REB Nagano system, to submit the SAE form, if any.

5.5 Clinical Trial Registration

To reduce publication biases and fulfill journal requirements, the study is registered in a public registry such as www.clinicaltrials.gov.

5.6 Codification of Participants, Samples and Data

In order to preserve the confidentiality of participants, each will be provided a unique code. The code includes the project name, followed by the identification number of the site and a unique identification number for the participant.

- Name of the project refers is : 4TMPO
- Identification numbers of the site are allocated as follows:
  1: CHUQc-UL
  2: CIUSSS de l'Estrie-CHUS
  3: CHUM
  4: CUSM
- Identification number of the participant is the consecutive number assigned to the participant in his corresponding centre: 01 for the first participant, 02 for the second participant, etc.

The following example represents the 3rd participant recruited in the CHUQc-UL centre:

Project name	# Site	# participant
4TMPO	1	03

5.7 Incidental Findings

If an incidental finding arises from a research analysis conducted during the study (following pathology, imaging or biochemical analysis), the corresponding site lead will first evaluate the clinical significance of this finding and act on it based on the participant's best interest. Disclosure of incidental findings will rely on the local treating team that has access to the chart, the participant's medical history and images. Since imaging or pathology specimen readings for the study will be batch read at the end of the study, first local reader conclusions will stand as the truth standard for clinical management, unless an incidental finding was completely missed. In this case, the local PI and treating physician will be contacted.

5.8 Adverse Events and Serious Adverse Events

Adverse events (AE) are defined as any new untoward medical event (symptoms, side effect, reaction) in a study participant. Serious adverse events (SAE) are AE that are life threatening, or result in death, hospitalization, invalidity or anomalies. AE will be documented after each PET scan directly from participant interrogation by the research team. Participants will also be encouraged to self-report any new side effects within 48 hours following PET scans, since the radiotracers are completely eliminated from the body after one day (half-life of gallium is 1 hour).

Only low-grade events were reported by previous observational studies using diagnostic radiopharmaceuticals and these were potentially related to ^{68}Ga-tracers that were produced by generator. The ^{68}Ga used for the preparation of both investigational products in our study is

produced from a cyclotron, which provides higher activity and apparent molar activity. Consequently, even less adverse effects are expected in this study.

Therefore, this study will not be monitored by a Data and Safety Monitoring Board because it is considered a minimal-risk study since the investigational products are positron emitting radiopharmaceuticals that are injected at subpharmacological doses and as such, do not solicit any therapeutic/pharmacologic effect. Furthermore, peer-reviewed literature shows that the tracers have been used in humans in numerous clinical trials over a period of at least 5 years and were not associated with any serious adverse events. However, if any SAE occurred during the established period (2 days after PET scans or seven days after biopsy collected for research purpose), the site qualified investigator will report it within 24 hours to the coordination centre. Combining their radiochemistry/tracer production (BG) and medical (JMB, FP) expertise, the Executive Committee will decide whether the event is possibly **related** to the study, **unexpected** and **serious**. If so, they will recommend that the site qualified investigator submit a full SAE report to the REB within 7 days (if life threatening or associated with death) or within 15 calendar days (other case scenarios). All SAE will be followed until a stable situation has been reached. The Executive Committee will investigate each SAE and suggest corrective actions if necessary. In his role as the investigator responsible for the sponsoring institution, Dr Pouliot will report to Health Canada, with a mention of the consensus agreement with the medical principal investigators and the site qualified investigator. Detailed instructions are provided in the study procedures.

5.9 Monitoring of the study

Detailed instructions are provided in the Data and Safety monitoring procedure manual.

5.10 Publications

We will use named authorship (as opposed to group authorship) for the primary 4TMPO results publications. The Executive Committee will collaboratively determine named co-authors based on the study procedures and on the ICMJE Authorship requirements. Subsequent secondary use of the data will be possible following investigators' or collaborators' request and Executive committee approval. Detailed instructions are provided in the study procedures.

6 IMAGING AGENTS

FDG and PET radiotracers will be supplied (produced and distributed) either by the Centre d'Imagerie Moléculaire de Sherbrooke (CIMS) or directly by the study's participating sites as per their standard practice.

The CIMS is a world-class imaging research centre, having two on-site ACSI's cyclotrons for the production of clinical-grade radiopharmaceuticals under Good manufacturing practices guidelines and covering preclinical/clinical imaging. A regulatory affairs agent and a quality control manager are also in place to provide good manufacturing practice and good clinical practice surveillance prior and during the study.

For more details please refer to the Imaging acquisition manual.

6.1 ^{68}Ga-DOTA-cTATE (^{68}Ga-DOTATATE) Injection

Gallium-68 (^{68}Ga) is a well-established PET isotope produced by medical cyclotron bombardment of enriched Zinc-68 (68Zn) targets. Gallium-68 isotope is bound to a somatostatin analogue, DOTA-DOTATATE, or DOTA-TATE, that has high affinity for the somatostatin receptor type 2 (SSTR2) and is used as a PET tracer for imaging of primary and recurrent NE tumours. ^{68}Ga-DOTA-cTATE is sterile and pyrogen-free diagnostic agent for intravenous administration.

Name: Gallium-68 (cyclotron produced)-DOTA-DPhe1, Tyr3-DOTATATE

Abbreviation: ^{68}Ga-DOTA-cTATE

Formulation: sterile and pyrogen-free solution

Administration: Intravenous Injection

The ^{68}Ga-DOTA-cTATE will be provided to the investigational site in the shielded container (vial) on the day of participant's PET imaging study. ^{68}Ga-DOTA-cTATE is a sterile and pyrogen-free diagnostic agent in 21 mL solution constituted of phosphate (0.14 g Na_2HPO_4 and 0.024 g KH_2PO_4), NaCl (100 mg), ascorbic acid (100 mg) and ≤ 9.4 % ethanol. The solution could be injected directly or diluted in saline prior injection. One should not dilute the bulk directly with saline, it could compromise stability for shelf live.

^{68}Ga-DOTA-cTATE will be administered intravenously at activities suitable for the application and the equipment used. The dose range must be between 185-370 MBq (±10%) or 2-4 MBq/kg. Maximum administered dose is 370 MBq. The participant dose must be measured by an appropriate radioactivity calibration system immediately prior to administration. Parenteral drug products such as ^{68}Ga-DOTA-cTATE are inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. The solution to be administered should be clear, colourless, and contain no particulate matter. Expiration date and time reported on the label placed on the lead container should always be verified before the tracer injection.

The site is responsible for ensuring that the appropriate dose is administered to the participant as indicated in Imaging Manual. The ^{68}Ga-DOTA-cTATE injection dosing syringe will be assayed for ^{68}Ga activity just prior to and immediately following the injection.

Dosimetry: Dosimetry of ^{68}Ga-DOTA-TATE and ^{68}Ga-DOTA-cTATE was considered essentially equivalent following results of our preclinical studies (see data in investigator brochure, supplied in appendix to the protocol).

Dose in an adult human male is estimated to be 2.57x10-2 for ^{68}Ga-DOTA-cTATE[27]. For the dose range considered in this study (185-370 MBq), this will yield an effective dose range of 4.8-9.5 mSv. See also data and references in investigator brochure, supplied in appendix to the protocol.

6.2 ^{68}Ga-DOTA-cPSMA617 Injection

Gallium-68 (^{68}Ga), a well-established PET isotope produced by medical cyclotron bombardment of enriched Zinc-68 (^{68}Zn) targets. Gallium-68 isotope is bound to a prostate-specific membrane antigen inhibitor (PSMA-617) that has high affinity for the prostate-specific membrane antigen

(PSMA) and is used as a Positron Emission Tomography (PET) tracer for imaging of primary and recurrent prostate cancer.

Name:^{68}Ga-(2S)-2-[[(1S)-1-carboxy-5-[[(2S)-3-naphthalen-2-yl-2-[[4-[[[2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetrazacyclododec-1-yl]acetyl]amino]methyl]cyclohexanecarbonyl]amino]propanoyl]amino]pentyl] carbamoylamino]pentanedioic acid.

Abbreviation: ^{68}Ga-cPSMA-617

Formulation: sterile and pyrogen-free solution

Administration: Intravenous Injection

The ^{68}Ga-cPSMA-617 will be provided to the investigational site in the shielded container (vial) on the day of participant's PET imaging study. ^{68}Ga-cPSMA-617 is a sterile and pyrogen-free diagnostic agent in 21 mL solution constituted of phosphate (0.14 g Na_2HPO_4 and 0.024 g KH_2PO_4), NaCl (100 mg), ascorbic acid (100 mg) and ≤ 9.4 % ethanol. The solution could be injected directly or diluted in saline prior injection. One should not dilute the bulk directly with saline, it could compromise stability for shelf live.

^{68}Ga-cPSMA-617 will be administered intravenously at activities suitable for the application and the equipment used. The acceptable dose range is 100-300 MBq (±10%) or 1.8-2.2 MBq/kg (maximum dose of 300 MBq). The participant dose must be measured by an appropriate radioactivity calibration system immediately prior to administration. Parenteral drug products such as ^{68}Ga-cPSMA-617 are inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. The solution to be administered should be clear, colourless, and contain no particulate matter. Expiration date and time reported on the label placed on the lead container should always be verified before the tracer injection.

The site is responsible for ensuring that the appropriate dose is administered to the participant as indicated in Imaging Manual. The ^{68}Ga-cPSMA-617 injection dosing syringe will be assayed for ^{68}Ga activity just prior to and immediately following the injection.

Dosimetry: Dose in an adult human male is estimated to be 2.1x10-2 for ^{68}Ga-PSMA-617[28]. For the dose range considered in this study (100-300 MBq), this will yield an effective dose range of 2.1-6.3 mSv. See also data and references in investigator brochure, supplied in appendix to the protocol.

6.3 ^{68}Ga-PSMA-11 Injection

^{68}Ga-PSMA-11 is a standard, approved, non-investigational imaging agent for PET. Each participating centre will administer activity based on their local clinical protocols. Sites are nonetheless encouraged to follow EANM procedure guidelines for tumour imaging[29]. The effective radiation dose resulting from the administration of 259 MBq (7 mCi) is about 4.4 mSv.

6.4 ^{18}F-DCF-PyL Injection

^{18}F-DCF-PyL is a an investigational imaging agent for PET. The final drug product (18F-DCFPyL) is a clear, colorless injectable solution at a strength of 1-156 mCi/mL (37-5772 MBq/mL) 18F-

DCFPyL at end of synthesis. 18F-DCFPyL will be dispensed and filled into a unit-dose syringe, placed into a lead pig and delivered to the clinical site in shipping containers with lead shielding.

Each participating centre will administer activity based on their local clinical protocols. Sites are nonetheless encouraged to follow EANM procedure guidelines for tumour imaging[29].

The site is responsible for ensuring that the appropriate dose is administered to the participant as indicated in the Image acquisition and transfer manual.

6.5 ^{18}F-FDG Injection

The participants will undergo a PET imaging scan with commercially available Fludeoxyglucose F18 (^{18}F-FDG), as an intravenous injection according to the Image acquisition and transfer manual. The ^{18}F-FDG must be provided by a qualified vendor in Canada.

^{18}F-FDG is a sterile and pyrogen-free solution for intravenous injection. The solution is clear, colourless, or slightly yellow and with a pH between 4.5 and 7.5.

Administration: ^{18}F-FDG is a standard, approved, non-investigational imaging agent for PET. Each participating centre will administer activity based on their local clinical protocols. Sites are nonetheless encouraged to follow EANM procedure guidelines for tumour imaging[23]. Effective dose is estimated at 1.9x10-2 mSv/MBq.

The site is responsible for ensuring that the appropriate dose is administered to the participant as indicated in the Image acquisition and transfer manual.

6.6 Packaging and Labeling

The labeling of the shielded containers containing ^{68}Ga-DOTA-cTATE or ^{68}Ga-PSMA-617 or PSMA tracer or ^{18}F-FDG injection will meet local law and regulations and will provide the investigational product name, ^{68}Ga or ^{18}F amount (mCi) at calibration, expiration time and date, lot number, manufacturer name and address, storage conditions, and caution warnings for radioactive material and new/drug/investigational product use.

6.7 Investigational Product (tracers) Accountability

Each investigator is responsible for ensuring that the deliveries of investigational product (IP) and other study materials from Université de Sherbrooke are correctly received, recorded, handled and stored safely and properly in accordance with all applicable regulatory guidelines, and used in accordance with this protocol.

All IP containers (product vials: opened, unopened or empty) must be destroyed on-site after its scheduled use in accordance with site policies. See IP handling procedures for further details on receipt, recording, handling, and accountability procedures. A list of IP and other materials that were destroyed must be prepared and signed by the principal investigator or designee. If there are any discrepancies, an explanation for these should also be provided.

6.8 Registration of Investigational Product Complaints

In the event of an IP complaint (e.g. breakage, leakage, particulate matter, discolouration…), the investigator or recipient of the IP is requested to report the problem to the central coordination team and Dr Pouliot.

Once the complaint is received, it will be recorded and it will be determined whether the complaint is minor or significant. All complaints will be followed up and the appropriate action will be implemented as per CIMS procedures.

7 SAMPLE SIZE CALCULATION AND STATISTICAL ANALYSIS

This is an observational study to understand how PCa progresses during systemic therapy. Therefore, number needed to enrol is driven by the need to describe repeatedly a biological process to reach statistical confidence, such as the **scenario 2** from Fig.2. From the 45 patients enrolled, we postulate that ≥ 30 (66%) will complete **Baseline** to **Progression 1** imaging timepoints including biopsies. **Primary objective 1:** In the 3TMPO study, we have shown that patients with 0, 1 and 2 lines of mCRPC systemic therapy, had at least 1 FDG+/PSMA- lesion (a surrogate IIH) in 13, 35 and 75 %, respectively (average difference in prevalence between lines=31%). For primary objective 1 (Fig.3 and 5A), using the McNemar test with a Type I error rate (α) of 0.05 and a power of 80%, 30 patients will be sufficient to detect a 25-percentage point difference in the proportion of IIH between 2-lines of therapy. Therefore, 30 patients are sufficient to reach statistical significance. **Primary objective 2:** From our previous retrospective study using a single FDG tracer and a new one [10], 30-40% of PCa patients had opposite metastases PET responses to systemic therapy. For primary co-objective 2 (Fig.5B), based on 30 patients that will complete **Baseline** and **Progression 1** timepoints, the calculated IC95 error margin is 16.4 percentage points if we estimate that 30% of patients will show metastases with opposite responses to therapy. Finally, we also expect that 22 (50%) of patients will complete the study 4 timepoints, and that 11 (25%) will progress radiographically at 3-months based on prostate cancer trials in the same space[22–24]. Loss of patients will happen because of study barriers happening in metastatic patient clinical trials (hospitalization, death, tracer delivery/imaging issues, withdrawal etc.). Chi-square and Fisher Exact test will be used to compare proportions between groups, T-test and Mann-Whitney test to compare continuous variables between these groups (using R12 4.3.0). P-value <0.05 will be considered statistically significant.

8 POTENTIAL ISSUES

8.1 Accrual/completion of the study

Our team (PI: FP and JMB) has accrued more than 700 PCa patients in imaging trials with exotic tracers under Health Canada Clinical trial applications over the last ten years. Despite pandemic, we have accrued 100 patients in the 3TMPO and 25 in the PROSTEP-002 (ongoing) study. Our 45 patients' goal is therefore clearly achievable by enrolling at three sites, equipped with PET facilities and infrastructure, across the province of Québec. In addition, we have included the 3TMPO clinicians and nuclear medicine physicians (see authorship of posters and manuscript) as collaborators to facilitate accrual due to their previous engagements.

8.2 Biopsies

Although metastasis biopsies will be of high scientific value to correlate imaging and pathological NED features, patients might decline this routine but non-SOC procedure. We have therefore increased our patient's accrual target to 45. Also, bone biopsies might not retrieve enough tissue for all samples and will be probably underrepresented in the organoid tissue samples. Moreover, we expect a 25 % success for organoid growth from metastases based on previous reports[37,38]. We believe that 10 organoids will be sufficient to answer important biological questions about specific imaging phenotypes of progressing or non progressing lesions.

8.3 Imaging interpretation to define IIH

The dichotomisation of uptake as positive/negative with fixed threshold does not fully exploit the continuous nature of PET metrics and may contribute to increase the apparent prevalence of IIH. On the other hand, the choices of the positivity threshold, of the minimum tumour volume and of maximum number of lesions analysed is guided by both clinical and practical considerations, and intentionally biased towards specificity by excluding the smallest lesions and those with mild uptake. While one could argue that will not characterize the totality of the potential lesions for many participants, doing so would in fact increase the apparent prevalence of IIH by introducing apparent lesions for which the clinical relevance and/or specificity could be questioned. Correlative studies with radiomics and artificial intelligence models combined with biopsy genomics will help to better define IIH (Fig. 1).

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