Original Article

The Triple-Tracer strategy against Metastatic PrOstate cancer (3TMPO) study protocol

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Objective

To determine the prevalence of intra-patient inter-metastatic heterogeneity based on positron emission tomography (PET)/computed tomography (CT) in patients with metastatic castration-resistant prostate cancer (mCRPC) and to determine the prevalence of neuroendocrine disease in these patients and their eligibility for radioligand therapies (RLTs).

Patients and Methods

This multicentre observational prospective clinical study will include 100 patients with mCRPC from five Canadian academic centres. Patients with radiological or biochemical progression and harbouring at least three metastases by conventional imaging will be accrued. Intra-patient inter-metastatic heterogeneity will be determined with triple-tracer imaging using fluorine-18 fluorodeoxyglucose (^{18}F-FDG), gallium-68-(^{68}Ga)-prostate-specific membrane antigen (PSMA)-617 and ^{68}Ga-DOTATATE, which are a glucose analogue, a PSMA receptor ligand and a somatostatin receptor ligand, respectively. The ^{68}Ga-PSMA-617 and ^{18}F-FDG PET/CT scans will be performed first. If at least one PSMA-negative/FDG-positive lesion is observed, an additional PET/CT scan with ^{68}Ga-DOTATATE will be performed. The tracer uptake of individual lesions will be assessed for each PET tracer and patients with lesions presenting discordant uptake profiles will be considered as having inter-metastatic heterogeneous disease and may be offered a biopsy.

Expected Results

The proposed triple-tracer approach will allow whole-body mCRPC characterisation, investigating the inter-metastatic heterogeneity in order to better understand the phenotypic plasticity of prostate cancer, including the neuroendocrine transdifferentiation that occurs during mCRPC progression. Based on ^{68}Ga-PSMA-617 or ^{68}Ga-DOTATATE PET positivity, the potential eligibility of patients for PSMA and DOTATATE-based RLT will be assessed. Non-invasive whole-body determination of mCRPC heterogeneity and transdifferentiation is highly innovative and might establish the basis for new therapeutic strategies. Comparison of molecular imaging findings with biopsies will also link metastasis biology to radiomic features.

Conclusion

This study will add novel, biologically relevant dimensions to molecular imaging: the non-invasive detection of inter-metastatic heterogeneity and transdifferentiation to neuroendocrine prostate cancer by using a multi-tracer PET/CT strategy to further personalise the care of patients with mCRPC.

Introduction

Patients with prostate cancer (PCa) presenting with metastases or failing locoregional therapy usually receive androgen-deprivation therapy, but the disease will inevitably become castration resistant [1,2]. Castration-resistant prostate cancer (CRPC) can evolve either as a classical adenocarcinoma, an androgen receptor (AR)-independent carcinoma or as a neuroendocrine CRPC, the latter occurring in 10–20% of cases [3,4]. Neuroendocrine CRPC is an aggressive subset of castration-resistant tumours that exhibit neuroendocrine differentiation (NED) pathological features on biopsy, including somatostatin receptor, chromogranin, and synaptophysin expression [5,6]. These tumours do not secrete PSA, a biomarker expressed in almost all adenocarcinomas. As a result, neuroendocrine CRPC needs to be confirmed by biopsy and is often recognised late, after PCa radiographic progression without a concomitant PSA rise. At this state, patients often show a decreased performance status limiting the administration of effective chemotherapy [3,7]. Therefore, a better understanding of the dynamics of NED from adenocarcinoma through lines of therapies is needed, as well as non-invasive tools to recognise it.

Recent studies have clearly established that metastatic CRPC (mCRPC) may evolve as an intra-patient polyclonal disease based on genomic analyses of intra-patient metastatic tissues [8]. Our group and others have shown that as many as 40% of patients exhibit evidence of polyclonal PCa metastases [5,9,10]. This can manifest, in the same patient, as mixed metabolic response to systemic therapies, as well as discordant tracer uptake or histopathology (adenocarcinoma or NED) among different metastases [5,9,10]. However, the true prevalence of intra-patient inter-metastatic heterogeneity or NED at different lines of mCRPC therapy is unknown. To answer this question, multi-tracer molecular imaging offers a unique way to determine PCa intra-patient inter-metastatic heterogeneity or NED prevalence. Indeed, new specific positron emission tomography (PET) tracers have the ability to non-invasively discriminate adenocarcinoma by targeting the prostate-specific membrane antigen (PSMA, e.g. ^{68}Ga-PSMA-617) or neuroendocrine CRPC via the somatostatin receptor (^{68}Ga-DOTATATE) [11,12], offering new tools for visualising intra-patient inter-metastatic heterogeneity and NED. While PSMA-PET/CT is highly sensitive for detecting metastasis in patients with mCRPC, even at low PSA levels [13], this tracer is of little use in NED because PSMA expression is suppressed [5,13]. Furthermore, uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) tends to be low in low-grade/indolent PCa, but progressively increases as PCa cells become more rapidly proliferative, such as in mCRPC with adenocarcinoma or NED [14]. Therefore, ^{18}F-FDG, which is readily available, could be helpful in combination with ^{68}Ga-PSMA-617, to pre-screen the transition toward intra-patient inter-metastatic heterogeneity and NED before the ^{68}Ga-DOTATATE scan. In addition, because both of these ^{68}Ga PET tracers have a therapeutic counterpart (e.g. ^{177}Lu-PSMA-617 and ^{177}Lu-DOTATATE also known as [a.k.a.] theranostics), they enable the non-invasive selection of patients for radioligand therapy (RLT) and potentially predict the outcome, therefore addressing another unmet need in mCRPC care. For all the reasons described above, the Triple-Tracer strategy against Metastatic PrOstate cancer (3TMPO) study was designed to determine, through triple-tracer molecular imaging, the prevalence of intra-patient and inter-metastatic heterogeneity in patients with mCRPC.

Patients and Methods

The 3TMPO Protocol Overview

The 3TMPO imaging study is a multicentre prospective observational cross-sectional study, in which 100 patients will be enrolled in five tertiary hospital centres (Centre Intégré Universitaire de Santé et de Services Sociaux de l'Estrie [CIUSSSE]-Centre Hospitalier Universitaire de Sherbrooke [CHUS], Centre Hospitalier de l'Université (CHU) de Québec – Université Laval [CHUQc-UL], Centre Hospitalier de l'Université de Montréal [CHUM], Centre Intégré Universitaire de Santé et de Services Sociaux [CIUSSS]-Centre-Ouest-de-l'Île-de-Montréal [COMTL], and McGill University Health Centre [MUHC]) in the province of Quebec, Canada, over a 2-year period. The study's main objective is to determine the prevalence of intra-patient inter-metastatic heterogeneity in men with progressive mCRPC using triple-tracer PET/CT imaging. Patients will undergo sequential PSMA-PET/CT and FDG-PET/CT. If a lesion is PSMA-negative/FDG-positive, as determined by central reading, a DOTATATE-PET/CT will be performed, as well as an optional biopsy of one such discordant lesion. The study

schema is shown in Fig. 1. The primary, secondary and tertiary endpoints are listed in Table 1.

This study is registered on clinicaltrials.gov (NCT04000776). Ethical approval has been obtained from the Research Ethics Board of the CIUSSSE-CHUS. Local institutional approval has been obtained in each of the five participating centres. Health Canada has provided a 'No-objection letter' for the use of the investigational ^{68}Ga-radiopharmaceuticals in this study. This study will be conducted in accordance with Canadian regulations (Health Canada Division 5; the Canadian Nuclear Safety Commission regulations for radiation safety), with the ethical principles stated in the Declaration of Helsinki and with the principles of the International Council for Harmonisation-Good Clinical Practice. The enrolment started on 20 January 2020 but was interrupted because of the coronavirus disease 2019 pandemic until 1 July 2020. The last site was activated in July 2021. At the time of preparing this manuscript, more than a half of the enrolment was completed.
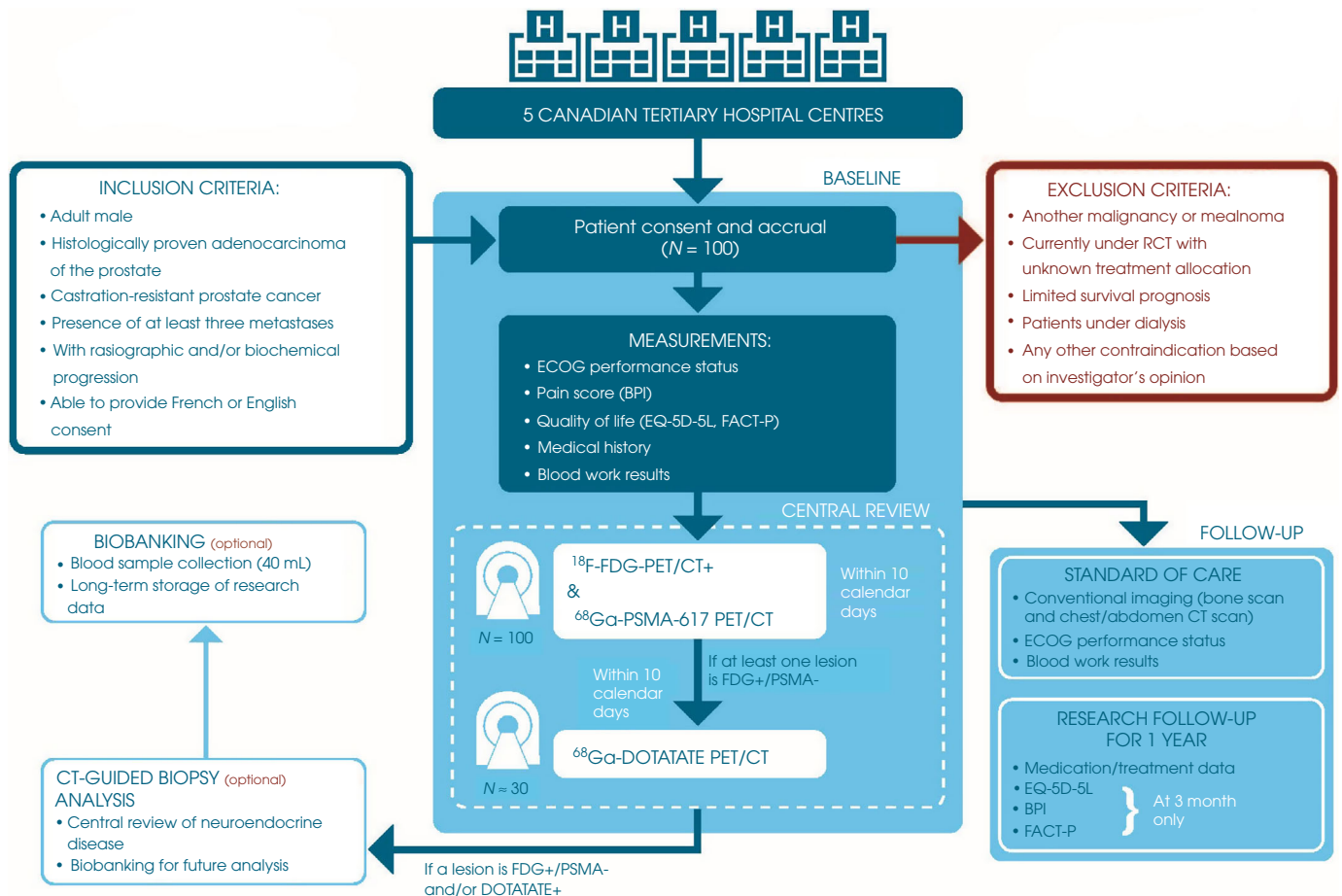
Patients

Urologists, radiation oncologists and medical oncologists at each participating centre will screen patients with mCRPC for eligibility. Eligibility criteria are presented in Table 2. To be eligible, patients will need to show biochemically or radiographically progressive CRPC and at least three metastases based on conventional imaging as validated by a 3TMPO local investigator. All patients participating in the study will provide written informed consent prior to any study procedures.

Initial Visit

Sociodemographic data, medical history, blood work results, previous and current treatment/medication will be collected from the patient's medical record and during an interview. The research team will assist the participant in completing validated patient-related outcome EuroQoL five Dimensions

Fig. 1 Schematic representation of the 3TMPO study to determine the prevalence of intra-patient inter-metastatic heterogeneity, NED, and patient eligibility for future RLT. The 3TMPO study will take place in five Canadian tertiary hospital centres. Patients with progressive mCRPC will first undergo two PET/CT scans with ^{18}F-FDG and ^{68}Ga-PSMA-617. Those with at least one FDG+/PSMA− lesion will undergo a third PET/CT scan with ^{68}Ga-DOTATATE to assess for heterogeneity. All patients will be followed up for 1 year. RCT, randomised controlled trial.

Table 1 3TMPO study endpoints.

Primary endpoint
1. Prevalence of intra-patient inter-metastatic heterogeneity defined as at least two lesions with discordant FDG/PSMA/DOTATATE multi-tracer imaging phenotypes
Secondary endpoints
1. Proportion of patients with mCRPC with neuroendocrine features defined as having at least one DOTATATE-positive lesion or histopathological features of neuroendocrine differentiation
2. Proportion of patients with mCRPC eligible for PSMA-RLT, defined as having at least one PSMA-positive lesion, and no PSMA-negative and FDG-positive lesion
3. Proportion of patients with mCRPC eligible for DOTATATE-RLT, defined as having at least one DOTATATE-positive lesion, and no DOTATATE-negative and FDG-positive lesion
Tertiary endpoints (exploratory)
1. Associations between ^{18}F-FDG and ^{68}Ga-PSMA PET/CT features and time to radiographic progression of mCRPC and survival
2. Comparison between PET/CT and biopsy results
3. Associations between ^{18}F-FDG and ^{68}Ga-PSMA PET/CT features and patient-reported outcomes at baseline, at 3 months or with the change of those outcomes between the two time points

Table 2 Inclusion and exclusion criteria.

Inclusion criteria
1. Male aged ≥18 years
2. Histologically or cytologically confirmed adenocarcinoma of the prostate with or without neuroendocrine carcinoma features at initial diagnosis
3. CRPC defined by progression under continuous castration (measured serum testosterone ≤50 ng/dL [1.73 nM]) anytime while on androgen-deprivation therapy
4. Metastatic disease documented by at least three metastatic active lesions on whole body bone scan* and/or measurable soft tissue on CT-scan.† Metastatic lesions on imaging are defined by Response Evaluation Criteria In Solid Tumors (RECIST) 1.1
5. Evidence of disease progression (biochemical or radiographic) on prior therapy or watchful waiting
6. Able and willing to provide signed informed consent in French or English and to comply with protocol requirements
Exclusion criteria
1. Another non-cutaneous malignancy or melanoma diagnosed in the past 5 years
2. Currently under a randomised controlled trial with unknown allocation
3. Limited survival prognosis (ECOG PS ≥3)
4. Patients under dialysis
5. Any disease or condition limiting the patient's capacity to execute the study procedures, based on the investigators' opinion

*A bone lesion that has been treated with site-directed radiation therapy is excluded from the target lesion count to fulfil the criteria of at least three metastatic lesions on conventional imaging. †The reference conventional imaging (confirming the presence of at least three active metastases) must be done either: (i) after biochemical progression on treatment or (ii) at least 90 days after last treatment has begun if imaging was performed while the patient was still responding (to avoid disappearance of metastasis due to treatment response).

five Levels (EQ-5D-5L), Brief Pain Inventory (BPI), and Functional Assessment of Cancer Therapy-Prostate (FACT-P) questionnaires. Conventional blood biomarkers (PSA, lactate dehydrogenase, complete blood count, alkaline phosphatase [ALP]) will be collected and a baseline Eastern Cooperative Oncology Group Performance Status (ECOG PS) determined.

Radiopharmaceuticals

The ^{18}F-FDG will be obtained directly by the participating sites from their usual provider. ^{18}F-FDG is a standard, approved, non-investigational PET imaging agent. Effective dose is estimated at 1.9×10^{-2} mSv/MBq [15]. Gallium-68 (^{68}Ga, half-life 67.83 min) is a well-established PET radionuclide mainly produced via a ^{68}Ga/^{68}Ge generator. At the Centre d'Imagerie Moléculaire de Sherbrooke (CIMS), enriched Zinc-68 (^{68}Zn) pressed targets are used to produce ^{68}Ga by cyclotron [16], which is used to prepare large amounts of ^{68}Ga-PSMA-617 and ^{68}Ga-DOTATATE (~20–25 GBq) with high apparent molar activity (425–520 GBq/μmole).

Both ^{68}Ga-PSMA-617 and ^{68}Ga-DOTATATE are sterile and pyrogen-free diagnostic agents for intravenous administration, constituted in 21 mL solution with 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 specifications for ^{68}Ga-PSMA-617 and ^{68}Ga-DOTATATE are based on the new monograph of the European Pharmacopoeia (3109) in preparation for accelerator-produced ^{68}Ga chloride [17]. The ^{68}Ga-radiopharmaceuticals will be delivered to the participating centres on the day of patient's PET imaging scan for use within the expiration time of 5 h after the end of synthesis.

The injected dose will be 2.2 MBq/kg with a maximum of 300 MBq or 370 MBq for ^{68}Ga-PSMA-617 and ^{68}Ga-DOTATATE, respectively.

PET/CT Procedure

Each PET camera used in the study will be approved by the Imaging Core Laboratory (Lead: J.M.B.). The acquisition and reconstruction parameters will be standardised and validated using an International Electrotechnical Commission (IEC) National Electrical Manufacturers Association (NEMA) phantom modified in-house and set-up with six spheres (4.0, 4.9, 6.2, 7.8, 9.8, 12.4 mm). Daily quality assessments of the PET/CT cameras will be performed by the sites according to the manufacturer's recommendations.

Patients will undergo both ^{18}F-FDG and ^{68}Ga-PSMA-617 whole-body PET/CTs in any order, with at least 18 h but no more than 10 days between each scan. Both scans will be reviewed centrally within 2 days by the Imaging Core Laboratory, which will confirm the patient's eligibility for a biopsy and for the ^{68}Ga-DOTATATE PET/CT scan, i.e. whether there is at least one metastatic lesion that is PSMA-negative and FDG-positive (the criteria for positivity for each

tracer is described in the Outcomes section). If mandated, the ^{68}Ga-DOTATATE PET/CT will be performed within 10 calendar days of the last PET/CT scan and the findings thereof may influence the lesion targeted for a biopsy, e.g. if there is a DOTATATE-avid lesion that is suspicious for NED.

For each PET/CT, the patient will be measured and weighed before the imaging. For the FDG-PET/CT only, capillary glycaemia will be measured, and the use of insulin will be permitted as per local practice. An intravenous catheter will be inserted in a peripheral vein. The tracer will be injected (^{18}F-FDG: as per local practice; ^{68}Ga-PSMA-617: 2.2 MBq/kg, maximum 300 MBq; ^{68}Ga-DOTATATE: 2.2 MBq/kg, maximum 370 MBq) and an uptake period of 60 ± 5 min will be observed. The PET acquisition will be performed from vertex to proximal thighs and accompanied by a low-dose CT without contrast. The use of diuretic and additional acquisition coverage will be left at the discretion of local investigators.

Biopsy

Based on the patient's willingness and clinical indication for a biopsy, imaging-guided biopsy of an FDG-positive/PSMA-negative or DOTATATE-positive lesion will be performed by an interventional radiologist. Whenever possible, a piece of the biopsy or digitalised slides of the biopsy will be provided for research purposes. If the biopsy is not clinically indicated, a biopsy could be collected after consent for research purposes only.

Histopathology

All biopsy samples, whether on glass slides or digitalised, will be centrally reviewed by the study Pathology Core Lead (D.T.) at the end of the study to validate the nature of the tissue in a standardised manner. For research purposes, at the end of the study, depending on the quantity and quality of the residual tissues, sub-analyses of the biopsies will be performed: (i) immunohistochemistry staining with PSA, somatostatin receptor 2, NKX3.1, p501s or PSMA to validate the prostatic origin of the lesions; (ii) staining with synaptophysin, chromogranin or cluster of differentiation (CD)56 to validate the presence or absence of NED when relevant; (iii) other diagnostic grade immunohistochemistry will be done to validate the presence of ARs or the differentiation of cells (lineage-specific keratins [7, 20, 5/6], AR) [18–20].

Follow-Up

Patients will be followed as per standard of care practice and will undergo a bone scan and chest/abdomen/pelvic CT, ECOG PS determination, and blood work including PSA at a maximum of 4-month intervals. Other data such as medication history and PCa progression-free and overall survival will be collected for up to 1 year after accrual. Moreover, we will collect patient-reported outcomes (with EQ-5D-5L, BPI, and FACT-P instruments) at 3 months after accrual.

Optional Biobanking Protocol

Participants will be asked if they wish to participate in a parallel study with an independent approval and informed consent form, approved by the Research Ethics Board of the CHUQc-UL. This biobank will collect and store samples of ~40 mL of blood to allow additional analysis for research purposes. The biobank will also store and provide restricted access to medical, research and imaging data from these participants under the supervision of the Biobank Core Lead (F.P.) after appropriate ethics approval.

Outcomes

Study Endpoints

The primary endpoint of this study is the prevalence of intra-patient inter-metastatic heterogeneity, defined by imaging, in patients with progressing mCRPC. Heterogeneity will be defined by more than one lesion phenotype on dual- or triple-tracer PET in a single patient, i.e. the presence of (i) both [PSMA-positive/FDG any] and [PSMA-negative/FDG-positive] lesions or; (ii) both [FDG-positive/PSMA any] and [FDG-negative/PSMA-positive] lesions or; (iii) in patients with only PSMA-negative/FDG-positive lesions undergoing DOTATATE-PET, both DOTATATE-positive and DOTATATE-negative/FDG-positive lesions. Using quantitative imaging methods, the standardised uptake value ratio (SUVR, i.e. the ratio between peak lesion uptake [SUV_{peak}] and mean liver uptake [SUV_{mean}]) will be obtained for each lesion with each tracer. For a given tracer, lesion positivity is defined as a SUVR ≥ 1.5, corresponding to an uptake that is clearly above that of the liver [21].

The secondary endpoint is the presence of NED features, defined by at least one DOTATATE-positive lesion (regardless of heterogeneity) or histological confirmation with synaptophysin, chromogranin or CD56 staining.

The additional secondary endpoint of the study is the percentage of progressing mCRPC patients who would be eligible for either PSMA- or DOTATATE-RLT (a.k.a. peptide receptor radionuclide therapy, PRRT).

A patient will be defined as eligible for PSMA-RLT if he shows at least one lesion that is PSMA-positive, but no lesion that is PSMA-negative and FDG-positive. A patient eligible for DOTATATE-RLT (PRRT) will be defined as having at least one measurable lesion that is DOTATATE-positive, but no lesion that is DOTATATE-negative and FDG-positive. All

the imaging endpoints will be centrally reviewed and determined by the Imaging Core Laboratory (Lead: J.M.B.) at the end of the study.

Other exploratory endpoints are patient-reported outcomes (pain score [defined by the BPI questionnaire], physical function [using EQ-5D-5L instrument], disease associated symptoms [using FACT-P instrument]), radiographic progression within 12 months (increased volume of a lesion or appearance of a new lesion), histological results from biopsies and other PET-tracer uptake derived parameters (such as SUV_{peak}, maximum SUV (SUV_{max}), SUV_{mean}, sum of SUV_{max}).

Adverse Events (AEs)

Adverse events are defined as any new untoward medical event (symptoms, side-effect or reaction) in a study participant after research interventions (^{68}Ga-tracer injection or biopsy for research purposes only). This study will not be monitored by a data and safety monitoring board because it is considered a minimal-risk study as the investigational products are positron-emitting radiopharmaceuticals that are injected at sub-pharmacological doses. Furthermore, peer-reviewed literature shows that these and related tracers have been used in humans in numerous clinical trials and that they were not associated with any serious AEs. Low frequency of low-grade AEs potentially related to generator or cyclotron produced ^{68}Ga-tracers was observed during our previous studies. Pursuant to the precautionary principle, an independent monitoring committee will review any serious unexpected suspected AEs in *ad hoc* meetings and all other serious AEs will be reviewed once a year.

Any AE-related data will be collected by questioning the patient after each PET scan or self-reported by participants within 48 h following PET scans. After a biopsy collected for research purposes only, at 7 days post-intervention, research staff will collect relevant data in the patient's medical chart and will report any AE in the study form accordingly. AEs will be reported electronically within 5 calendar days. If an AE is considered as possibly related to the study, unexpected and serious, it will be reported rapidly to the external independent monitoring committee and reported to Health Canada and the Research Ethics Board within 15 days, or 7 days if life-threatening.

Sample Size and Statistical Analyses

Considering an expected prevalence of intra-patient and inter-metastatic heterogeneity of 30% [9,10] as a primary outcome, 81 men are needed to obtain a ±10-percentage point precision with a 95% CI. To be sure to reach the proposed precision (±10 percentage points), regardless of the obtained heterogeneity prevalence, and to account for a small proportion (~3%) of inability to evaluate the primary outcome, a total of 100 men will be recruited (97 participants would be needed for a prevalence of 50% for which the CI would be the largest).

Demographic and baseline characteristics will be summarised for the entire cohort using statistics for continuous or for categorical variables following the nature of the variable analysed.

For the primary and secondary outcomes (prevalence of intra-patient inter-metastatic PCa heterogeneity, prevalence of NED and patient's eligibility for PSMA or DOTATATE RLT), the percentage of participants will be presented along with the 95% CI based on the Clopper–Pearson exact binomial definition.

As for tertiary explorative outcomes: (i) multivariate Cox models, considering potential confounding variables including line of therapy, type of therapy, baseline ECOG PS, PSA and ALP, will be used to assess the association between intra-patient inter-metastatic heterogeneity and time to radiographic failure of systemic therapies and to test whether ^{18}F-FDG and ^{68}Ga-PSMA-617 PET-derived measurements, alone or in combination, are independent prognostic markers for time to radiographic progression. (ii) Fisher's exact test will be used to compare the presence of neuroendocrine disease suspected by PET tracer (PSMA-negative/FDG-positive and DOTATATE-positive metastases) and the biopsy results along with qualitative analyses of biopsy characteristics. (iii) Finally, linear or logistic regression models (according to the nature of the outcome of interest) considering same potential confounding variables as in first tertiary outcome will be used to evaluate the association between patient-level PET uptakes-derived parameters at baseline (including heterogeneity status and possible combinations of parameters) with patient-reported outcomes (such as pain, physical function and disease-associated symptoms) at baseline, at 3 months or for changes in outcomes (deltas). A two-sided $P \leq 0.05$ will be considered to indicate statistical significance. All statistical analyses will be performed using the Statistical Analysis System (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA) or R (R Foundation for Statistical Computing, Vienna, Austria).

Study Management

The study will be supervised by the Executive Committee led by the three principal investigators (B.G., F.P., J.M.B.). At each participating centre, a site lead/qualified investigator is designated to oversee the study locally (F.P., P.R., W.K., F.S., S.P.). The principal investigators, along with the qualified investigators, the biostatistician (F.C.L.), the Pathology Core Laboratory (D.T.), the patient partners and the project leader (A.T.) will constitute the Steering Committee that will

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provide guidance and direction for the overall study. An external independent monitoring committee comprising a nuclear medicine physician (K.V.K.), a urologist oncologist (J.B.L.) and a trialist (F.D.) will be formed to assess serious AEs and provide recommendations to the study team through the Executive Committee. The Unité de Recherche Clinique et Épidémiologique (URCE, an academic research hub of the Centre de Recherche du CHUS) will centrally coordinate and internally monitor study quality.

Discussion

The therapeutic landscape of metastatic PCa and/or CRPC is evolving rapidly. The number of effective systemic agents is growing, which translates into better survival, but the choice of best agents at each line of therapy remains complex. After androgen deprivation and AR axis-targeting therapy, a number of systemic treatments have been shown to delay progression. These are docetaxel [22], cabazitaxel [23], olaparib [24], ipatasertib [25], radium-223 [26] or PSMA-radioligands [27]. Ipatasertib, olaparib and PSMA-radioligands indications are driven by response predictive biomarkers such as phosphatase and tensin homologue (PTEN)-loss, DNA-repair gene defects or PSMA tracer uptake within the tumours, respectively. However, most of these predictive biomarkers rely on tissue sampling, which might not reflect the whole tumour genotype nor the dedifferentiated adenocarcinoma/NED disease.

The 3TMPO study aims to provide clinicians with unique knowledge about PCa intra-patient inter-metastatic heterogeneous phenotype prevalence at each line of therapy, as well as the percentage of patient candidacy for DOTATATE or PSMA RLTs. We expect to observe a 30% prevalence of intra-patient inter-metastatic heterogeneity based on ^{68}Ga-PSMA-617/^{18}F-FDG imaging in our recruited population and a 10–20% prevalence of NED, as confirmed by ^{68}Ga-DOTATATE imaging. However, the percentage of DOTATATE-positive patients is difficult to determine because there are only a few reports looking specifically at such a prevalence in the literature with most patients being already diagnosed with neuroendocrine disease [5,28–30]. Based on our secondary analyses, we anticipate that intra-patient inter-metastatic heterogeneity and DOTATATE-positive patients with PCa will be more prevalent among patients who received several lines of mCRPC therapies. We also expect that a majority of patients with mCRPC will be candidates for PSMA RLT. Finally, we expect that the sum of SUV$_{max}$ of ^{18}F-FDG PET/CT and/or intra-patient inter-metastatic heterogeneity will be associated with rapid radiographic progression and deterioration of quality-of-life scores. Another key question to be answered with our study will be the link between FDG/PSMA discordant lesions and histopathology or genomics. If FDG-positive/PSMA-negative lesions are shown to harbour specific genotypes or histologies, early targeted treatments could be contemplated.

The proposed triple-tracer approach will allow whole-body metastatic PCa characterisation, looking at cancer intra-patient inter-metastatic heterogeneity and plasticity in order to better understand how often and when adenocarcinoma and NED occur in mCRPC progression after one, two or more lines of systemic therapies. *In vivo* and whole-body determination of pathological transdifferentiation by state-of-the-art multi-target molecular imaging is highly innovative and might set the basis for new cancer monitoring strategies. Indeed, we are challenging the current dogma that cancer resistance to therapy only equals tumour growth or appearance of new lesions. Instead, we propose that transdifferentiation is an important and early event leading to treatment resistance and, only later, manifests as radiographic progression. Moreover, through our approach, we will not only be able to identify the number of patients deemed eligible for PSMA or DOTATATE RLT, but also determine the number of metastases that could be targeted. Indeed, instead of viewing RLT as only another line of therapy, the proposed approach could unveil how complementary PSMA and DOTATATE RLTs could be integrated, in a highly personalised fashion, to the current mCRPC therapeutic paradigm. These RLTs could target resistant and/or neuroendocrine clones of PCa progression while most of the disease is controlled by approved mCRPC drugs.

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Conflict of Interest

The authors declare having received financial support of Merck Canada Inc. and of the Fonds de Recherche du Québec – Santé, as well as from the Cancer Research Society through the Oncopole organisation for the conduct of this study. The authors also acknowledge the contribution of Hermes Medical Solutions Canada for providing free infrastructure for imaging transfer. Dr Pouliot has received consultant honoraria or grants from Sanofi, Genzyme, Amgen, Astellas, Bayer and Janssen; he is a consultant/ advisory board member of Sanofi, Ferring, Merck, Tersera, Amgen, Abbvie, Bayer, Astellas, Janssen, Genzyme and Eisai. Dr Kassouf received honorarium for Advisory Boards from the following companies: Ferring, Merck, Roche, BMS, EMD Serono/Pfizer, Sesen Bio, Janssen. Dr Probst is an Advisory Board member of Bayer. He has received consultant honoraria (speaking fees) from Bayer, Abbvie, Astellas, AAA, Isologics, Lantheus, Point Biopharma. Dr Anidjar has received grants as principal or co-investigator from AngioDynamics Inc., Aragon Pharmaceuticals, Inc., Janssen Research and Development, Movember Foundation and Canadian Urology Research Consortium. The other authors have no potential conflicts of interest to declare.

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Abbreviations: (m)CRPC, (metastatic) castration-resistant prostate cancer; 3TMPO, Triple-Tracer strategy against Metastatic PrOstate cancer; a.k.a., also known as; AE, adverse events; ALP, alkaline phosphatase; AR, androgen receptor; BPI, Brief Pain Inventory; CHUQc-UL, Centre Hospitalier de l'Université (CHU) de Québec – Université Laval; CHUS, Centre Hospitalier Universitaire de Sherbrooke; CIUSSSE, Centre Intégré Universitaire de Santé et de Services Sociaux de l'Estrie; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EQ-5D-5L, EuroQol-5 dimensions-5 levels; FACT-P, Functional Assessment of Cancer Therapy-Prostate; FDG, fluorodeoxyglucose; mCRPC, metastatic castration-resistant prostate cancer; NED, neuroendocrine differentiation; PCa, prostate cancer; PET, positron emission tomography; PRRT, peptide receptor radionuclide therapy; PSMA, prostate-specific membrane antigen; RLT, radioligand therapy; SUV(max)(mean)(peak)(R), standardised uptake value (maximum) (mean) (peak) (ratio).