Investigation of Therapy Response with Amino Acid Analogue Transport PET Imaging

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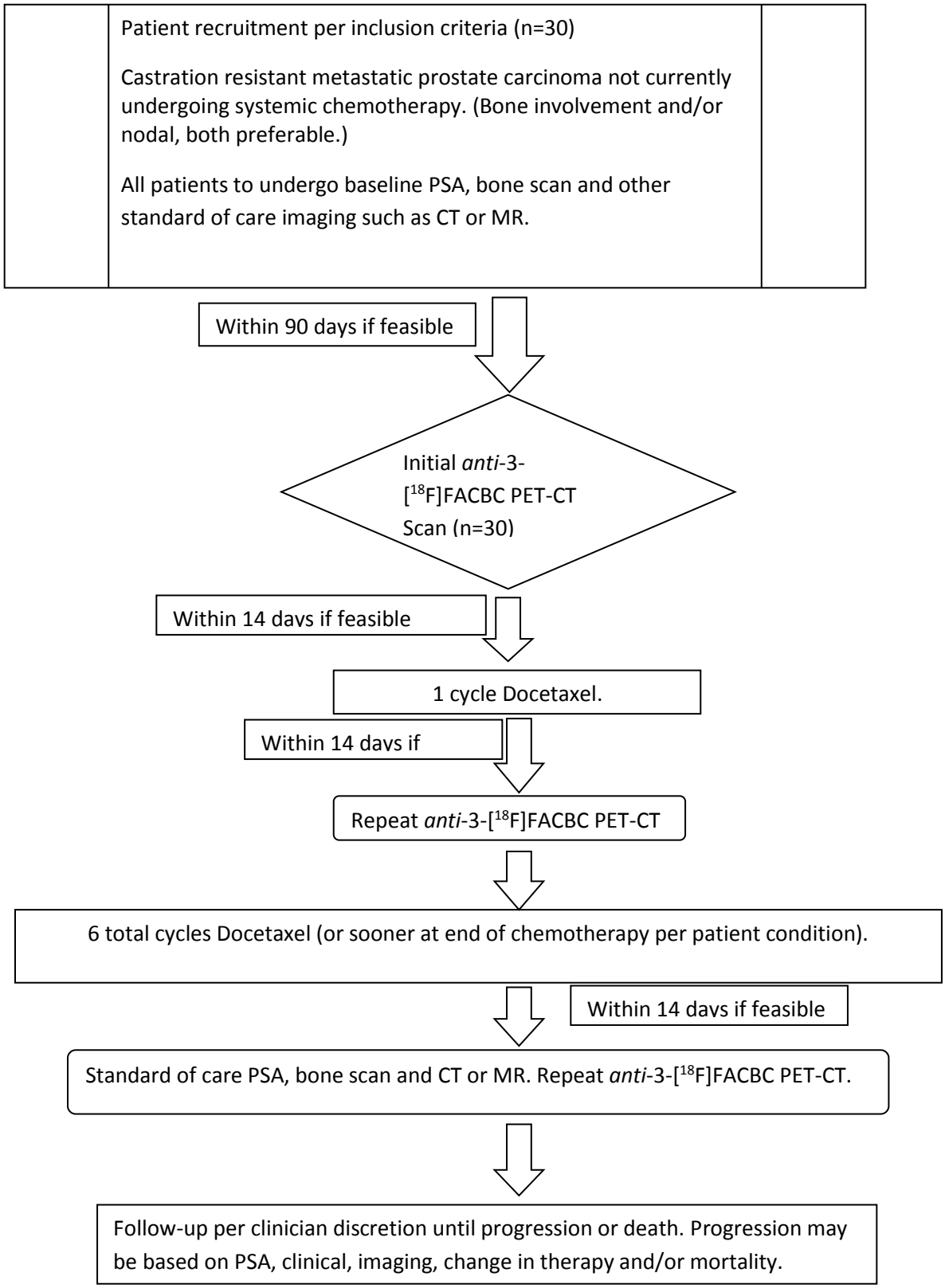
PI: David M Schuster, M.D.
Emory University
Department of Radiology and Imaging Sciences
Emory University School of Medicine, Atlanta, GA
Phone: (404) 712-4859
email: dschust@emory.edu

Schema:


Patient recruitment per inclusion criteria (n=30)
Castration resistant metastatic prostate carcinoma not currently undergoing systemic chemotherapy. (Bone involvement and/or nodal, both preferable.)
All patients to undergo baseline PSA, bone scan and other standard of care imaging such as CT or MR.
Within 90 days if feasible
Initial anti-3-[18F]FACBC PET-CT Scan (n=30)
Within 14 davs if feasible
1 cycle Docetaxel.
Within 14 davs if
Repeat anti-3-[18F]FACBC PET-CT
6 total cycles Docetaxel (or sooner at end of chemotherapy per patient condition).
Within 14 davs if feasible
Standard of care PSA, bone scan and CT or MR. Repeat anti-3-[18F]FACBC PET-CT.
Follow-up per clinician discretion until progression or death. Progression may be based on PSA, clinical, imaging, change in therapy and/or mortality.

Precis/Abstract:

Anti-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-3-[^{18}F]FACBC) is a synthetic amino acid analog which has demonstrated promise for the staging and restaging of prostate carcinoma. Yet no work to date has been accomplished in the ability of this radiotracer to monitor response to therapy. Yet, the ability to monitor response to therapy with imaging may prove to be important as a surrogate endpoint in treatment effectiveness and the ability to switch to other forms of therapy if the first therapy is ineffective. We will investigate the ability of *anti*-3-[^{18}F]FACBC PET to monitor response to therapy of skeletal and soft tissue lesions in castration resistant metastatic prostate carcinoma after 1 and 6 cycles (or sooner at end of chemotherapy per patient condition) of Docetaxel/Prednisone chemotherapy, and correlate imaging findings with tumor and imaging biomarkers such as PSA, bone scan and other conventional imaging such as CT or MR. We will also correlate *anti*-3-[^{18}F]FACBC PET after 1 and 6 cycles of chemotherapy with response to therapy at 1 year (or earlier if progression).

A. Introduction and Background:

As more therapeutic options for the treatment of castration resistant prostate carcinoma are made available, monitoring response to therapy with biomarkers may play an increasingly important role (1). Typical median survival for this disease is 18 months with a 50% response rate by PSA criteria to chemotherapy. Monitoring therapeutic response has been traditionally been accomplished via serum biomarkers such as prostate specific antigen (PSA) and imaging biomarkers such as bone scanning for skeletal disease and anatomic imaging such as computed tomography (CT) for nodal disease. While bone scanning with Tc-99mMDP is highly sensitive for the detection of skeletal metastasis, the technique is prone to false positive findings. In addition, bone scanning may be subject to a "flare" phenomenon, whereby there is increased radiotracer uptake secondary to lesion healing despite clinical improvement, making the disease appear worse, when in actuality there is metabolic and clinical improvement (2, 3). Response on bone scan also may be subjective though there have been recent efforts to semi-quantify disease burden (4, 5).

Modern chemotherapy regimens for castration resistant prostate cancer include docetaxel and cabazitaxel. Docetaxel given at a dose of 75 mg/m2 every 3 weeks is considered a standard first line chemotherapy regimen, while cabazitaxel is reserved for patients who are either resistant to docetaxel or relapse/progress after docetaxel treatment. Overall response rates in the range of 50% have been reported with de novo standard docetaxel therapy. The regimen is generally well tolerated with a minority of patients developing grade 3-4 adverse events including myelosuppression and fatigue being most common.

While CT or magnetic resonance imaging (MR) may demonstrate exquisite anatomic detail, the differentiation of benign from malignant nodal disease by size criteria alone is problematic. Both CT and MR have demonstrated limitations in the evaluation of metastatic disease from prostate carcinoma in particular (6). Change in the size of a soft tissue lesion may be uncoupled from metabolic response; that is, a lesion can appear to be getting smaller, yet still be metabolically active and visa-versa. In addition, while criteria exist for the quantification of representative soft tissue lesions, bone lesions are more difficult to quantify in a similar manner (7). Thus, metabolic imaging biomarkers for the treatment of prostate carcinoma have been investigated.

^{18}F Fluorodeoxyglucose (FDG) positron emission tomography (PET) has been proven to be very useful in monitoring response to therapy for a wide variety of disease processes. Yet, since FDG monitors only glucose metabolism it may reveal only a limited amount of information compared with the entire gamut of tumor metabolism (8, 9). The utility of FDG PET in therapy response assessment for prostate carcinoma has not been widely researched. In early work, change in FDG uptake had been correlated with response to hormonal therapy but not with chemotherapy (10, 11). In more recent studies, Jadvar has reported in preliminary work that FDG uptake decreases with successful response to both hormonal and chemotherapy and declines concordantly with PSA (12). Yet, in general, FDG PET has been demonstrated to have low utility for the staging and restaging of prostate carcinoma, especially in the detection of osseous metastases (13-15). In addition, other FDA approved and non-FDA approved PET radiotracers such as ^{11}C-choline, ^{18}F-fluorocholine, and ^{11}C-acetate have been utilized mostly outside of the US for the staging and restaging of prostate carcinoma, yet only preliminary work has been completed on monitoring therapy response (16-28).

Amino acids are involved in many aspects of human nutrition including the synthesis of proteins. Amino acid metabolism has been demonstrated to be upregulated in many tumors including prostate carcinoma (29). A number of amino acid radiotracers have been utilized to study prostate carcinoma and other tumors including naturally occurring L-[^{11}C]methionine, L-3-[^{123}I]iodo-alpha-methyl tyrosine (IMT), and L-[1-^{11}C]5-hydroxytryptophan (5-HTP). There has been little work exploring the role of amino acid radiotracers for evaluating response to therapy, most of which has been done with brain tumors (15, 30, 31). Nunez has reported on differences in uptake patterns in prostate carcinoma between ^{11}C-Methionine and FDG PET, and noted these could be exploited in monitoring response to therapy, but no formal study has been completed with amino acid imaging to monitor therapy response in prostate carcinoma (32).

Anti-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-3-[^{18}F]FACBC) is a synthetic amino acid analog (Fig. 1) which has demonstrated promise for the staging and restaging of prostate carcinoma (33). In our recent papers *anti*-3-[^{18}F]FACBC had high accuracy in differentiating prostatic from extraprostatic recurrent prostate carcinoma (34, 35) (Fig 2). The uptake of *anti*-3-[^{18}F]FACBC is likely mediated through a combination of amino acid transport proteins and the radiotracer is not metabolized (36-39). Normal biodistribution of *anti*-3-[^{18}F]FACBC includes relatively intense uptake in the liver and pancreas and little renal excretion or brain uptake compared with ^{18}F-FDG (40).

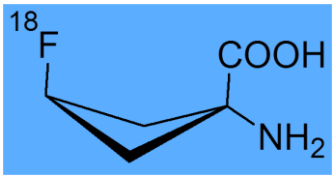


Figure 1: Molecular configuration of *anti*-3-[^{18}F]FACBC

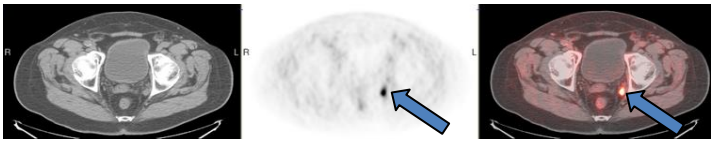


Figure 2: *anti*-3-[^{18}F]FACBC PET-CT top row arrows demonstrates small malignant obturator lymph node

Yet, kinetic studies have demonstrated rapid wash-in of the *anti*-3-[^{18}F]FACBC PET radiotracer into prostate malignancy with variable but generally slower washout (Fig 2). The explanation for this phenomenon is likely secondary to the complex interplay of amino acid transporters (AAT) involved with natural and synthetic amino acid transport. In addition, many of the AAT's demonstrate 1:1 stoichiometry; that is, an amino acid is exchanged out from the cell for one to be brought in.

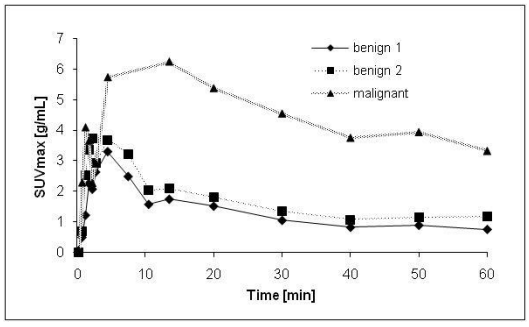


Figure 3: Time activity curve of FACBC wash-in and wash-out in benign versus malignant lymph nodes

In exploratory published data examining test-retest reproducibility of *anti*-3-[^{18}F]FACBC PET, there seems to be general reproducibility of background structures and selected lesions on the order of magnitude of ^{18}F-FDG PET (41). Though a formal test retest study needs to be undertaken, we believe that it is reasonable to utilize *anti*-3-[^{18}F]FACBC to examine therapy response in a similar manner to the commonly utilized ^{18}F-FDG biomarker. In addition, *anti*-3-[^{18}F]FACBC has been demonstrated to be more sensitive than MR for evaluating early tumor response to TMZ therapy in gliomas in a rat model (42). No study to date has been performed on humans or for prostate carcinoma.

Our hypothesis is that amino acid imaging with *anti*-3-[^{18}F]FACBC may be useful in monitoring therapy response for prostate carcinoma and will corollate with ultimate response to chemotherapy including PSA levels and clinical response. The overarching goal of this project is to investigate therapeutic monitoring of chemotherapy in castration resistant prostate carcinoma with *anti*-3-[^{18}F]FACBC in prostate carcinoma to determine if *anti*-3-[^{18}F]FACBC amino acid imaging can serve as an accurate and efficient imaging biomarker.

B. Objectives

Accurate determination of imaging response to chemotherapy in castration resistant prostate carcinoma may be important in understanding if a particular radiotracer may be translated to the clinic and under what conditions it may be useful.

We will explore if amino acid imaging with this radiotracer is responsive to therapy changes of tumors and to establish a framework to understand if *anti*-3-[^{18}F]FACBC may be useful to monitor therapeutic effect. We will commence with a synthetic amino acid analogue radiotracer *anti*-3-[^{18}F]FACBC and prostate carcinoma – study conditions in which we have the greatest amount of experience. Clinical trials with *anti*-3-[^{18}F]FACBC for prostate carcinoma in multiple centers has successfully studied over 900 patients with no untoward effects. Future studies may be directed at other synthetic amino acid radiotracers and other tumors to determine if these findings are generalizable to other amino acid imaging.

The aims of this study is to determine if there is a change in radiotracer uptake in response to systemic chemotherapy in patients with known prostate carcinoma. We will also investigate if *anti*-3-[^{18}F]FACBC uptake after 1 cycle will predict clinical and imaging response after 6 cycles of chemotherapy, if *anti*-3-[^{18}F]FACBC uptake after 6 cycles correlates with clinical and imaging response after 6 cycles of chemotherapy, and if *anti*-3-[^{18}F]FACBC uptake at 1 and/or 6 cycles of chemotherapy will predict clinical and imaging response at one year (or earlier if progression).

Specific Aims

Specific Aim 1. To determine if uptake on *anti*-3-[^{18}F]FACBC PET-CT in metastatic lesions with castration resistant prostate carcinoma is affected by chemotherapy.

Specific Aim 2. To determine if response on *anti*-3-[^{18}F]FACBC PET scan after 1 and 6 cycles of chemotherapy (or sooner at end of chemotherapy per patient condition) correlates with clinical and imaging response after these cycles of chemotherapy as measured by standard parameters including PSA and routine objective measurements.

Specific Aim 3. To determine if response on *anti*-3-[^{18}F]FACBC after 1 and 6 cycles of chemotherapy (or sooner at end of chemotherapy per patient condition) correlates with clinical and imaging response to therapy at one-year or earlier if progression as measured by standard parameters including routine objective measurements, PSA progression, clinical progression, and/or mortality.

Sub-aim 1. To determine if uptake on the baseline *anti*-3-[^{18}F]FACBC PET-CT correlates with response to chemotherapy after 6 cycles and at one year or earlier if progression as measured by standard parameters.

Rationale. As has been outlined in Section A of the proposal, response of amino-acid imaging PET with the synthetic amino acid analogue radiotracer *anti*-3-[^{18}F]FACBC has not been established, yet has profound implications in translating a promising radiotracer from experimental conditions to the clinic. The specific hypothesis in this proposal is that there may be a better correlation of response with amino acid PET utilizing *anti*-3-[^{18}F]FACBC than MDP bone scanning and with anatomic imaging modalities such as CT or MR. The most comprehensive manner to assess our hypothesis is to undertake a prospective trial examining treatment response with each patient serving as his own control.

We propose assembling a dedicated multidisciplinary team of experts in amino acid radiotracer synthesis and imaging as well as medical and radiation oncology, urology, and biostatistics. Outcomes will be validated by direct comparison of patient serving as their own respective controls. We will examine the data for comparisons between groups with multivariate analysis among other statistical tools.

C. Study design and methods

Experimental design. We will undertake a study with 30 patients who have castration resistant metastatic primary or recurrent prostate carcinoma with skeletal metastases and/or nodal involvement. The patients will serve as their own control. We will perform a baseline *anti*-3-[^{18}F]FACBC PET-CT of the whole body as per the detailed protocol below. All patients will also undergo conventional staging including ^{99m}Tc MDP bone scanning and CT or MR within 90 days if feasible which are standard of care at our institution. Note that on a case by case basis, an individual scan may be accepted outside this window based on the best clinical and scientific judgement of the PI. This study will not interfere with standard patient evaluation or delay therapy. Other imaging may also be obtained clinically.

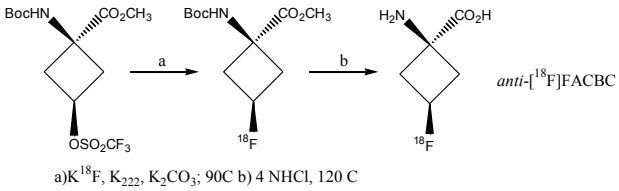
Subsequently, all 30 patients will undergo a standard chemotherapeutic regime Docetaxel/Prednisone for 6 cycles or more as tolerated. Patients will then undergo a repeat *anti*-3-[^{18}F]FACBC PET-CT after 1 and 6 cycles (or sooner at end of chemotherapy per patient condition) and also repeat conventional imaging including ^{99m}Tc MDP bone scanning and CT or MR after 6 cycles (or sooner at end of chemotherapy per patient condition). By the termination of the study, we will then record the response (or lack thereof) on *anti*-3-[^{18}F]FACBC PET-CT (Specific Aim 1) and correlate that response with response per standard clinical criteria including bone scan uptake for skeletal lesions, CT or MR for soft tissue and skeletal lesions, PSA progression or regression, and other clinical parameters (Specific Aim 2) such as declining performance status and time to change to another therapy.

The patients will be also followed up in the context of this study (per clinical routine) for up to one year post-therapy to determine if changes on previously acquired *anti*-3-[^{18}F]FACBC PET-CT predicted ultimate response including evaluable lesions on repeat conventional imaging including ^{99m}Tc MDP bone scanning and CT or MR, as well as PSA progression, clinical progression, and mortality (Specific Aim 3).

C.1 Anti-[18F]FACBC radiolabeling

Methods. We will prepare *anti*-3-[^{18}F]FACBC via the GE FastLab cassette system. Alternatively, production may also be accomplished by the automated synthesis developed by J. McConathy and M.M. Goodman (85) as outlined in Figure 4. The automated radiosynthesis of *anti*-3-[^{18}F]FACBC will be carried out in a chemical process control unit (CPCU) with a computer interface. The two-step reaction sequence will involve incorporation of no-carrier-added potassium [^{18}F]fluoride into a protected triflate precursor and deprotection using aqueous hydrochloric acid. The crude reaction mix will be passed in series through ion-retardation resin, an alumina-N SepPak®, an HLB cartridge and a 0.22 μm sterile filter, and the resulting aqueous solution will be collected in a dose vial. The radiochemical purity of the product will be determined by TLC. Additional chemical solvent purity will be measured by Gas Chromatography (GC). The total time for synthesis of *anti*-3-[^{18}F]FACBC after delivery of 18[F]fluoride will be ~70 minutes, and the average decay-corrected yield of *anti*-3-[^{18}F]FACBC will be 24 ± 4 % (n = 40 runs, average ± standard error) in over 99% radiochemical purity. This procedure will provide 140-200 mCi of *anti*-3-[^{18}F]FACBC at the end of synthesis. We have prepared greater than 150 batch productions for tumor imaging in volunteer subjects. Either methodology will be conducted under FDA IND auspices (IND 72437).

Figure 4. Synthesis of anti-FACBC



C.2 PET-CT imaging protocol

PET-CT images will be acquired on a GE Discovery 690 (16 Slice) or other PET-CT scanner. All studies will use measured attenuation correction (routinely acquired through the initial CT portion of the scan). Dead time, detector efficiency and scatter corrections will be applied using the routines supplied by the manufacturer. The resulting images will be quantitatively calibrated and have 6 mm isotropic resolution.

All subjects will initially be required to fast for at least four hours to normalize their neutral amino acid levels. Non-strict fasting is not an absolute contraindication and may be overridden on a case by case basis since it is unknown if fasting affects FACBC uptake positively or negatively. The study will typically take place in the afternoon. Within one hour prior to scanning, the patient will drink 450 ml of oral contrast as possible to maximize conspicuity of abdomen and pelvic structures. IV contrast will not be used. Prior to placement in the tomographic gantry, an intravenous catheter will be placed for injection of tracer. The subject will be placed in the tomograph gantry for completion of the CT scan. *anti*-3-[^{18}F]FACBC (10 mCi) will be injected into an antecubital vein in a bolus infusion. At 4 minutes post-injection), consecutive 2 minutes per frame acquisitions (z axis FOV is: 15cm with 3 cm overlap per table position) will be obtained starting from the proximal thighs and extending superiorly for 7 table positions which typically reaches at least the skull base. This will immediately be repeated to obtain dual time point (early and delayed) data. All efforts will be made to always study the patient on the primary and same scanner unless there is a technical scanner failure.

Summary of PET-CT Scanning Procedure

1) The patient will be placed in the tomographic gantry for a CT scan of the skull to proximal thighs (80-120 mA) to be utilized for anatomic imaging and correction of emission data (approximately 1 minute).
2) The patient will then receive a bolus of *anti*-3-[^{18}F]FACBC injected IV
3) The dosage will be approximately 10.0 mCi (370 MBq).
4) At 4 minutes, a 2 minute per bed position PET acquisition will start at the proximal thighs and extend for 7 table positions typically to the skull base or higher.
5) This will be repeated to achieve dual time point imaging.
6) The entire study including injection of radiotracer should take approximately 60 minutes or less.
7) The study will be repeated within 14 days after the first cycle and 14 days after completion of the 6th cycle of chemotherapy as below, allowing for patient condition (or sooner at end of chemotherapy per patient condition). To be clear, a cycle starts from the time of chemotherapy administration on day one, and lasts for 3 weeks until the next cycle (or until cessation of chemotherapy). Therefore, the ideal time to scan the patient in followup is within a few days before the next cycle commences. Yet, this is not always logistically possible. Therefore, subsequent scanning may occur within 14 days after the first cycle, and could therefore take place within the next cycle.

C.3 Chemotherapy

Chemotherapy will consist of docetaxel 75 mg/m2 administered intravenously every 21 days with appropriate pre-medications (steroids and anti-emetics) administered to prevent acute adverse effects. Growth factor support will be provided per clinical need. If the neutrophil and platelet counts are adequate, chemotherapy will be repeated for a total of 6 cycles after which the patient will be treated at his physician's discretion. Prednisone 5 mg can be administered orally twice daily along with docetaxel chemotherapy throughout the study per physician's discretion. In patients who experienced either febrile neutropenia, neutrophil count <500 cells/mm3 for more than one week, severe or cumulative adverse event or severe peripheral neuropathy during docetaxel therapy, the dose of docetaxel may be reduced from 75 to 60 mg/m2. If the subject continues to experience these reactions at 60 mg/m2, the docetaxel treatment may be discontinued per physician's discretion. In addition, a cycle of therapy may be delayed to allow for recovery of adverse event if indicated per physician's discretion. CBC, CMP and PSA are done before each dose of chemotherapy. Patients may or may not be on anti-hormonal therapies including but not limited to LHRH agonists, antiandrogens, Abiraterone or Enzalutamide.

C.4 Routine Laboratory and Imaging Biomarkers

As per clinical routine, all patients will undergo routine PSA and CT or MR as well as bone scanning at baseline (within 90 days before the beginning of therapy if feasible), after 6 cycles of chemotherapy (or sooner at end of chemotherapy per patient condition), and at 1 year (or earlier if progression). This is considered a clinical standard and will not be funded from the project budget. These studies will be performed with the standard Emory protocols on file. The only study-specific event is the FACBC scan.

C.5 Image Analysis of *anti*-3-[^{18}F]FACBC PET-CT

Methods: The methods of image analysis to be used for the *anti*-3-[^{18}F]FACBC PET-CT are as follows:
1) Images will be reconstructed with iterative technique and hardware fused (PET to CT) on a GE AW or MimVista or similar workstation which enables SUV (mean, maximum) and total lesion activity as well as standard bidimensional size measurements of lesions. Whenever possible we will use 3 dimensional

PET-Edge conformational regions of interest (ROI) to encompass the entire structure under question such as a lymph node or prostate bed.
2) Visual inspection of the PET-CT images in separate sessions by a board certified nuclear medicine physicians/nuclear radiologist will take place with calculation of SUVmax, SUVmean, total lesion activity of 5 representative index lesions each for bone and lymph nodes (10 total). If 5 lesions each are not definable, all demonstrable lesions up to 5 will be utilized. Lesions chosen will be independent but may coincide with index lesions on conventional imaging. In addition, we will derive similar measurements of background structures from key organs which we have identified from prior studies with this radiotracer including bladder, liver, pancreas, bone marrow (L3), and blood pool (aorta at arch). Tumor to background values will also be calculated. All measurements will be made at both time points to calculate retention indices.
3) The same measurements of the lesions and background structures will be undertaken at baseline and post-therapy scan. We will utilize the following parameters to follow response to therapy: SUVmax and total lesion activity of most intense lesion each of bone and node, sum and mean SUVmax of up to 5 index lesions for each of bone and node, total lesion activity of most intense lesion each of bone and node, and sum and mean total lesion activity of the 5 index lesions for each of bone and node. Percent change will be calculated before and after therapy. Comparison of data for differences will be undertaken using standard statistical analyses (see below).
4) In addition, the measurements above will be supplemented by the following from RECIST 1.1 Criteria (43): Negative *anti*-3-[^{18}F]FACBC PET at baseline, with a positive *anti*-3-[^{18}F]FACBC-PET at follow-up is a sign of PD based on a new lesion.

C.6 Image Analysis of Abdominopelvic CT or MR

A standard clinical report will have been generated per routine. The results of this report will be recorded. In addition, one of the investigators will record bi-dimensional measurements of up to 5 index lymph nodes as applicable, identified on the study. These will be used to calculate RECIST 1.1 response criteria briefly excerpted below (43, 44). Note that this assumes ideal conditions which may vary in individual patients.

Specifically, measurable lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10 mm by CT or MR scan (assuming slice thickness no greater than 5 mm). To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness is recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed. Non-measurable lesions are all other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with 10 to <15 mm short axis), as well as truly non-measurable lesions. Up to five target lesions as appropriate will be measured. Target lesions will be selected on the basis of their size (lesions with the longest diameter) and be representative of all involved organs, as well as their suitability for reproducible repeated measurements. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters, which will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease. Since mostly lymph nodes are to be included in the sum, only the short axis will contribute. If 5 index nodes cannot be recorded, non-target lesions will be identified to increase the total index lesions to 5 and followed as 'present', 'absent' or in

rare cases, 'unequivocal progression'. If the patient does not have nodal disease, lymph nodes will not be used as index lesions.

The response criteria will be as follows: Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of diameters. Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this may include the baseline sum). The sum must also demonstrate an absolute increase of at least 5 mm. Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Since lymph nodes will be the target lesions, the sum of lesions will never be zero even if CR criteria are met since a normal lymph node is defined as having a short axis of <10mm. If the node becomes too small to measure, a default value of 5mm will be assigned.

If non-target lesions are utilized, a qualitative assessment with the following criteria will be applied. Complete Response (CR): Disappearance of all non-target lesions and normalization of tumour marker level. All lymph nodes must be non-pathological in size (<10mm short axis). Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits. Progressive Disease (PD): Unequivocal of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression). If there is no true soft tissue lesion, no soft tissue index lesion is used.

C.7 Image Analysis of Bone Scan

As noted above, bone scanning will be obtained per routine with a standard clinical report. For description of up to 5 index lesions, we will utilize standard nomenclature of none, mild (similar to surrounding bone), moderate (greater than surrounding bone and similar to characteristic degenerative uptake), and intense (greater than characteristic degenerative uptake), supplemented or supplanted by anatomic measurement when appropriate. Standard of truth will be characteristic metastatic appearance on bone scan and/or CT.

In addition, if sufficient funds are secured, we may process these planar bone scan images in order to obtain a quantitative index of bone involvement using the Bone Scan Index (BSI) which has been previously validated (4, 5, 45). This parameter will be utilized to compare response to therapy on all the bone scans and will be accomplished via automated measurements with EXINI-Bone software (EXINI Diagnostics AB; Scheelevägen, Sweden), an FDA approved clinical tool.

C.8 Comparison to "Truth"

For response criteria after 1 and 6 cycles (or sooner at end of chemotherapy per patient condition), patients will serve as their own controls.

1) Change in *anti*-3-[^{18}F]FACBC uptake between baseline and post cycle 1, as well as between baseline and post 6 cycles (or sooner at end of chemotherapy per patient condition) will be correlated with imaging response criteria including change in bone lesion conspicuity, and RECIST 1.1 nodal indices post 6 cycles (or sooner at end of chemotherapy per patient condition) and 1 year or earlier if progression. This will be completed both qualitatively as above and using standardized response criteria.

2) Change in *anti*-3-[^{18}F]FACBC uptake will be correlated with clinical response criteria post 6 cycles (or sooner at end of chemotherapy per patient condition) and one year or earlier including PSA values (progression or regression), clinical progression, and mortality.
3) Initial uptake on *anti*-3-[^{18}F]FACBC will be correlated with response post 6 cycles (or sooner at end of chemotherapy per patient condition) and 1 year or earlier using standardized qualitative and quantitative criteria including bone scan, RECIST 1.1 nodal indices, and PSA values as well as clinical progression and mortality.
4) The *anti*-3-[^{18}F]FACBC quantitative indices post 1 cycle and post 6 cycles (or sooner at end of chemotherapy per patient condition) will be correlated to each other with linear regression and other analyses.

C.9 Followup

This will be done as part of routine standard of care without special visits required of the patient. At one year (or earlier if progression), ultimate response to therapy using standard criteria including repeat CT or MR, bone scan and PSA will be obtained as well as mortality. Response on *anti*-3-[^{18}F]FACBC PET-CT will also be correlated to ultimate response at 1 year (or earlier if progression) using these parameters as above. Accrual goals will be 2 first year, 4 second year, and then 8 each subsequent 3 years.

D. Participant Selection:

Methods: All patients will be recruited from Emory Healthcare Urology, Radiation Oncology, or Medical Oncology clinics per the inclusion and exclusion criteria below. It is estimated that 2-4 patients per month who meet inclusion criteria are seen in the Emory Medical Oncology clinic from which most recruitment will occur.

Inclusion Criteria:

1. Patients must be 18 years of age or older.

2. Primary or recurrent castration resistant prostate carcinoma with skeletal and/or nodal involvement not currently undergoing systemic chemotherapy who are about to commence therapy with docetaxel/prednisone. (Note that systemic hormonal targeted therapy including LHRH agonists (Lupron or Trelstar), other anti-androgens, and/or Abiraterone or Enzalutamide may be in use.)

3. Ability to lie still for PET scanning

4. Patients must be able to provide written informed consent

Exclusion Criteria:

1. Age less than 18.

2. Inability to lie still for PET scanning.

3. Cannot provide written informed consent.

4. Undergoing current chemotherapy for organ confined or systemic disease. **This does not preclude patients who had previously received upfront docetaxel in the hormone sensitive setting.**

Patients will be required to fast in preparation for PET scan. Verbal consent will be obtained from patient prior to the PET scan day if written consent had not been earlier obtained.

Procedures:

Day 1: **PET Scan Day**

- Obtain written informed consent if not earlier obtained.
- Inclusion/exclusion criteria review if not earlier done.
- Medical history and medication review if not earlier done.

Within 14 days after completing first cycle of chemotherapy:

- A 2nd FACBC PET/CT will be performed if feasible per patient condition.

Within 14 days after completing six cycles of chemotherapy (or sooner at end of chemotherapy per patient condition):

- A 3rd FACBC PET/CT scan will be performed if feasible per patient condition.

E. Statistical Analysis.

In the primary analysis, a two–sided paired t-test will be used to compare difference in the maximum SUV between two repeat measurements at each time point during the FACBC PET. A Mixed Model will be used to compare the overall curve of maximum SUV during the multiple time points of FACBC PET between the two repeat measurements before and after the experiment. Similar analysis will be conducted for each of other measurement outcomes of FACBC PET, such as SUV ratio, etc. In the secondary analyses, Spearman's correlation coefficient and Chi-square test will be employed to measure the correlation between the response on *anti*-3-[^{18}F]FACBC PET scan after 1 and 6 cycles of chemotherapy (or sooner at end of chemotherapy per patient condition) and the clinical response after 6 cycles of chemotherapy (or sooner at end of chemotherapy per patient condition) as measured by standard parameters including PSA and routine objective measurements. Similarly, Spearman's correlation coefficient and Chi-square test will be also employed to measure the correlation between the response on *anti*-3-[^{18}F]FACBC PET scan after 1 and 6 cycles of chemotherapy (or sooner at end of chemotherapy per patient condition) and the response to therapy at one-year (or earlier if progression) as measured by standard parameters including routine objective measurements, PSA progression, clinical progression, and mortality. The significance levels will be set at 0.05 for all tests. The SAS statistical package V9.2 or similar (SAS Institute, Inc., Cary, North Carolina) will be used for all data managements and analyses.

Statistical power: A two sided paired t-test was used to calculate power and sample size. From our previous knowledge, the mean maximum SUV was about 6.28 with a standard deviation as of 2.42. The sample size of 30 patients in each arm will achieve a power of at least 80% to detect a difference of about 20% in mean maximum SUV of *anti*-[^{18}F]FACBC PET between two repeat measurements before and after the experiment at the significance level of 0.05.

Interim analyses: There are 2 Interim analyses and 1 final analysis in the study. The first and second interim analysis will be conducted after 10 and 20 patients have been enrolled and measured, respectively. The final analysis will be conducted after all 30 patients have enrolled and completed their measurement. In order to maintain the overall significance level of 0.05 while conducting two interim analyses, the O'Brien & Fleming method is employed to calculate the p-value for each interim analysis and final analysis. According to O'Brien & Fleming boundary, the p-value to be used in first interim analysis, second interim analysis, and final analysis is 0.0005, 0.014, and 0.045, respectively.

F. Adverse Event

An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans whether or not considered drug-related.

A significant shift from baseline which can be attributable to the radiotracer injection and not the patient's medical condition will be considered an unexpected AE. **An event greater than 20 hours post scan will not be considered an AE since the radiotracer has effectively decayed by 20 hours.**

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 (June 14, 2010) will be used as a guide address potential AEs subject to limitations above and medical and scientific judgment as to plausibility of such criteria in a diagnostic radiotracer study.

Adverse Event Reporting

An SAE is defined as any adverse event occurring at any dose that results in any of the following outcomes:
• death
• a life-threatening adverse experience
• inpatient hospitalization or prolongation of existing hospitalization
• a persistent or significant disability/incapacity
• a congenital anomaly/birth defect
Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.
Any serious adverse events (see above) will be communicated by the PI to the Emory IRB using standard adverse event reporting forms.in case of a serious adverse event definitively ascribed to *anti*-[^{18}F]FACBC imaging, Drs. Kucuk, Schuster, Halkar, and Jani will meet formally or communicate via email to investigate the reported adverse event.

The Investigator will report all Serious Adverse Events (as defined above) occurring in a subject on the day of or within 28 days following the Agent administration to Pharsafer® Associates Ltd ("**Pharsafer**") by telephone (+44 1483 212151), FAX (+44 1483 212178) or e mail (drugsafety@pharsafer.com). Events

should be reported to Pharsafer within 24 hours of the investigator becoming aware of the events occurrence. The Investigator is responsible for informing the ethics committee of serious events occurring during the study in compliance with local regulations.

Any patient death that may be due to the study procedure (i.e. severe radiotracer reaction), unanticipated problem, would be promptly reported to the Emory IRB office. Additionally any patient death not associated with the study procedure or serious unanticipated event(s) (i.e. radiotracer allergy) will be reported to the Emory IRB and FDA upon continuing review. Protocol deviation/non-compliance will be reported according to IRB Policies & Procedures. This radiotracer is studied under IND 72437 and monitoring will be performed per already agreed upon FDA guidance. Over 900 patients in multiple centers have been studied without adverse events.

G. Data and Safety Monitoring Plan (DSMP):

Patients will be monitored by the technologists and study nurse before and after the studies for any adverse events/reactions. They will be given contact phone numbers to call if they experience any problems (i.e. problems with the IV site, any allergic reaction symptoms). They will be followed routinely by their referring oncologist with clinical exams, and the PI will work with the co-investigators and referring physicians to ensure that the patients continue to follow up as scheduled.

The Data and Safety Monitoring Committee (DSMC) of the Winship Cancer Institute will also oversee the conduct of this study (every 6 months or annually – depending on the risk level of the protocol). This committee will review pertinent aspects of study conduct including patient safety, compliance with protocol, data collection and efficacy. The committee will review the charts of 10% of patients enrolled to the study and two of the first 5 patients entered to the study. The Committee reserves the right to conduct additional audits if necessary. The Principal Investigator (PI) or designee is responsible for notifying the DSMC about the accrual of patients when the first 5 have been entered to the study.

It should be kept in mind that this patient cohort typically has a median survival of 18 months and it is of much greater probability that an SAE may occur and be related to the patient's natural disease progression and/or therapy. Only those SAE's related to the radiotracer FACBC itself will be reported as part of this trial as the therapy itself is not considered experimental.

H: Pharmaceutical, biologic, and device information

Radiation Dosimetry. The study will be approved by not only the Emory IRB but also the Radiation Safety Committee. The maximum number of PET studies a patient will receive in one year is 3 *anti*-[^{18}F]FACBC studies.

Depending on the distribution of the radionuclide in the body, the whole body dose may not be the critical factor in single or longitudinal studies. It is also of interest to know which organ receives the highest absorbed dose. This organ is referred to as the critical organ. Often the limit for an individual organ dose is reached before the limit established for the whole-body. The United States Food and Drug Administration in Title 21 CFR Part 361 limits the whole body radiation dose to adult research subjects to less than 3 rem (30 mSv) for a single injection and 5 rem (50 mSv) effective dose equivalent (EDE)

annually. A single organ cannot receive more than 5 rem (50mSv) in a single injection and 15 rem (150 mSv) effective dose equivalent annually. These constraints on dose will limit the maximum number of injections for research subjects. The studies proposed in this application fall within these guidelines.

Whole-body human biodistribution studies of FACBC show that it is retained in liver and pancreas. A 10 mCi (370 MBq) injection of FACBC results in a whole-body effective dose equivalent of 0.52 rem (5.2 mSv) and a critical organ absorbed dose of 1.9 rad (19.3 mGy) to liver (40).

The level of radiation for the PET scan is the same as received in widely used diagnostic studies such as the currently used 18F-FDG and is equal to or less than 80 percent of the amount allowed a radiation worker in a year (5 rem). The calculated whole body exposure to the individual will be less than 0.6 rem for each PET scan (1.15 total). Radiation exposure for the transmission images on the GE690 PET-CT (with which this study would be conducted) is 0.55 rem (5.5 mSv). Thus, total effective dose for a single FACBC PET/CT scan is 1.07 rem (10.7mSv). Therefore, total exposure for the 3 FACBC PET-CT scans proposed in this study would be 1.8 rem (18 mSv). For the CT portion of the SPECT-CT, added radiation exposure would be an additional 1.65 rem (16.5 mSv).

Thus maximum total body exposure would be 3.45 rem (34.5 mSv) per year in this protocol as proposed.

The radiotracer used in this study is governed by FDA IND 72437 for FACBC and is subject to agreed upon safety monitoring by the FDA, a copy of which is on file with the IND holder, Dr. David Schuster.

I. References and appendices

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Required Data Calendar. **(Note that this calendar does not preclude other routine or standard clinical labs.)**

* (or sooner at end of chemotherapy per patient condition)

** (or earlier if progression)

	Screening	Day 1 ≤ 90 days Post screen	Begin chemotherapy (3 weeks/cycle)	≤ 14 days Post cycle 1	≤ 14 days Post cycle 6 *	1 year follow up **+/- 60 days
Informed consent		**X**				
Standard of care medical history & examination	**X**				**X**	**X**
Bone scan	**X**				**X**	**X**
CT or MR	**X**				**X**	**X**
PSA	**X**				**X**	**X**
FACBC PET		**X**		**X**	**X**	
Chemotherapy begins			**X**			