Winship Protocol: X

Improved staging of lobular breast cancer with novel amino acid metabolic and tumor neovasculature receptor imaging

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1.1 Synopsis

Invasive lobular breast cancer (ILC) accounts for approximately 10-15% of breast malignancies [1]. ILC has a distinct histology and discohesive growth which makes it difficult to visualize with current imaging modalities such as mammography, ultrasound and breast and whole body MR. *Suboptimal imaging adversely impacts accurate staging upon which therapeutic decisions are based [2]*. The metastatic pattern of ILC also differs from that of invasive ductal carcinoma (IDC) with a tendency to involve the gastrointestinal tract, genitourinary tract, peritoneum, retroperitoneum and leptomeninges. While fluorodeoxyglucose (FDG) PET has been shown to clinically upstage patients with locally advanced IDC, this is not so with ILC, which has been described as "initially indolent but slowly progressive". **There are currently no accurate imaging techniques for staging ILC. Our goal is to address an unmet public health need by improved staging of ILC, specifically detection of metastatic disease.**

Amino acid transporters are upregulated in breast cancer cells [3]. We and others have successfully imaged breast cancer with fluciclovine (^{18}F) PET, an FDA approved synthetic amino acid radiotracer originally developed at Emory for imaging of cerebral glioma and prostate cancer. In exploratory studies conducted at Emory and Memorial Sloan Kettering in 39 women with breast cancer (13 patients with ILC), fluciclovine PET demonstrated promising results in detection of ILC in primary tumors and locoregional metastases [4-6]. Distant disease was not studied. Post-hoc RNA sequencing of the Emory cohort reveals that numerous molecular signaling pathways correlate with fluciclovine uptake including PI3K/**Akt signalling**, which mediates a range of biological endpoints necessary for cancer growth including cell survival, apoptosis, autophagy, cell cycle progression and angiogenesis.

Since the pro-angiogenic factor VEGF contributes to **Akt activation** to improve oxygenation and nutrient supply to areas of poor vascularization, attention has turned to neoangiogenesis as an imaging biomarker for breast cancer. A different PET radiotracer in clinical trials for prostate cancer, prostate specific membrane antigen (PSMA) may also detect breast cancer due to the upregulation of PSMA receptors in tumor angiogenesis. *Prostate-specific* is actually a misnomer as PSMA receptors are upregulated in tumor neovasculature in many cancers including breast cancer. Evolving data suggests PSMA radiotracers may also have utility in breast cancer, including ILC [7-11].

Our preliminary data support a key scientific premise that metabolic imaging with fluciclovine (^{18}F) PET has shown promise in the detection of ILC. There is also emerging data that tumor neovasculature imaging with PSMA PET may improve detection of ILC. **We hypothesize that amino acid transport metabolic imaging with fluciclovine (^{18}F) PET will improve staging of ILC, particularly for distant metastases, compared to conventional imaging. We also hypothesize that receptor directed PSMA imaging of tumor associated neovasculature in ILC will reveal unique information to complement metabolic interrogation with fluciclovine PET. Improved staging will facilitate more appropriate management decisions.**

To test these hypotheses with the highest scientific rigor, we propose an early phase feasibility trial with fluciclovine and PSMA PET strategies centered on detection of metastasis in patients with advanced ILC using histology as the gold standard. As an exploratory aim, we will also correlate the occurrence of circulating tumor DNA (ctDNA) directed against ESR1 and PI3K with presence of metastasis and tumor burden. We will explore if a multiparametric strategy with ctDNA and imaging will help define which patients should undergo molecular imaging and also identify those who may forego biopsy if distant lesions are detected on imaging. We expect this pilot trial will generate sufficient preliminary data to determine feasibility for a definitive NIH sponsored trial developing more accurate staging techniques to help modify current practice for imaging of ILC.

Specific Aim 1: Improve detection of metastasis with fluciclovine and PSMA PET versus best standard of care conventional imaging, as confirmed with histology. We will image 20 patients with ILC who have either: a) clinical or imaging suspicion of metastatic disease; or b) proven metastatic disease but in whom there is suspicion of an even greater tumor burden that could change therapy approach. Abnormal foci which correlate to anatomic lesions previously unsuspected on conventional imaging will undergo optional biopsy as clinically indicated in the safest manner possible (if not already done) to determine the verified detection rate of metastatic disease.

Specific Aim 2: **Determine concordance and discordance of ILC detection with PSMA versus fluciclovine PET, as confirmed with histology.** We will compare verified detection rates for metastasis between fluciclovine and PSMA PET modalities. This may necessitate a second biopsy of a

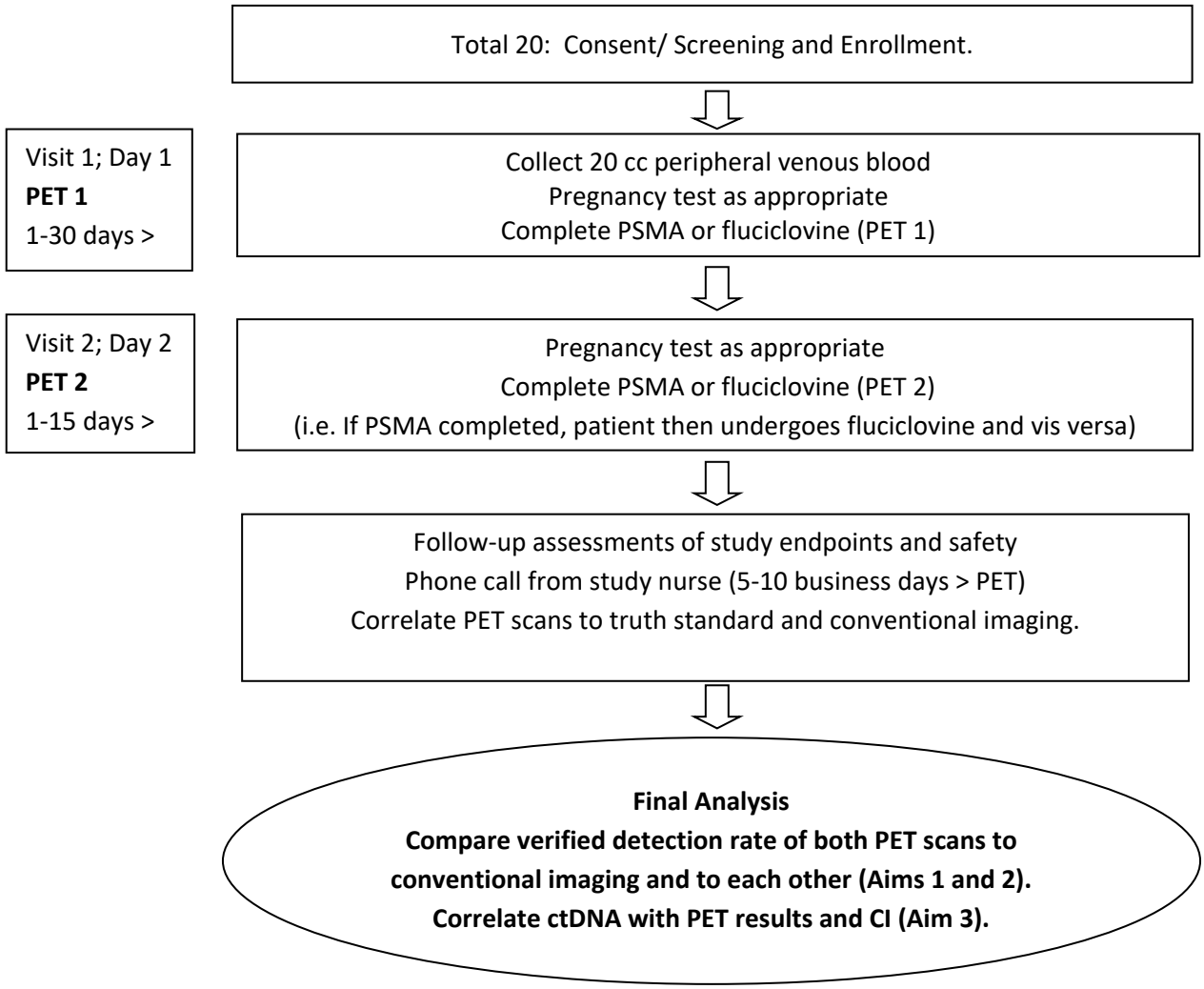
distant lesion discordant between the 2 PET techniques. We will also analyze images for biodistribution in the primary lesion(s) for both radiotracers.

Exploratory Aim 3: Establish the role of molecular detection by ctDNA mutations including PIK3CA, ESR1, HER2, and AKT1 in characterizing the degree of tumor burden as identified by metabolic amino acid transport and/or tumor neovasculature receptor imaging [12]. We will collect peripheral blood for ctDNA in all patients and determine if ctDNA correlates with presence or absence of metastases and tumor burden as identified by fluciclovine (metabolic) and/or PSMA (angiogenesis) PET.

1.2 Schema

We will undertake a single arm feasibility study with n=20 patients with treatment naïve biopsy proven ILC who have either: a) clinical or imaging suspicion of metastatic disease; b) biopsy proven metastatic disease but in whom there is suspicion of an even greater tumor burden that could change therapy approach. All patients will be recruited from the Winship Glenn Family Breast Cancer Program at any Emory facility and they will have already undergone best standard of care conventional imaging (CT or MR and possibly bone scan depending on clinical presentation). **Standard of care conventional imaging typically does not include FDG PET, since FDG PET is not recommended for lobular cancer due to low sensitivity.** We will then complete both fluciclovine PET and Ga PSMA PET scans on the same patient on separate days separated by at least 10 half-lives to allow for radioactive decay. There is no need for randomization since each patient will undergo both imaging techniques, and the results will be interpreted blindly. Thus, radiopharmacy scheduling will dictate the order of the radiotracers to ensure the patient will undergo both imaging modalities in the shortest time frame. Sometimes this will mean PSMA is completed first, followed by fluciclovine, and other times the opposite. After interpretation of both studies, careful correlation to already obtained conventional imaging will then be done to determine if a previously unsuspected anatomic correlate can be identified which would impact therapy. One or two metastases (depending on scan findings) will then undergo biopsy in the safest manner possible as standard of care (if not already done) to determine the verified detection rate of metastatic disease. Uncommonly, definitive imaging such as MR for skeletal or liver lesions may be accepted in lieu of biopsy, if biopsy is not feasible. We will then compare verified detection rate of each PET study to conventional imaging and to each other. In addition, we will collect peripheral blood for ctDNA in all patients and determine if ctDNA correlates with presence or absence of metastases and tumor burden as identified by conventional imaging, fluciclovine and/or PSMA PET. Study Specific Experimental Procedures will only consist of both PET studies and ctDNA analysis. All other procedures will be conducted per standard of care. See Figure 1.

Figure 1: Trial Schema, Flow diagram



1.3 Schedule of Activities

Procedures	**Enrollment/ Screening (-60 days)**	**Study Visit 1** PET 1 **(within 30 days of enrollment)**	**Study Visit 2** PET2 **(within 15 days of Visit 1)**	**Follow up** [e] (Up to 5 years)
Informed consent	X			
Demographics	X			
Medical history	X			X
Administer study intervention		X	X	
Pregnancy test [b]		X	X	
AE Assessment		X	X	
Optional biopsy [c]		X	X	X
Follow medical history				X
Peripheral blood sample (ctDNA)[d]		X		
Complete Case Report Forms (CRFs)	X	X	X	X

[a] Study intervention is either the PSMA or fluciclovine PET

[b] Pregnancy test within 24 hours prior to each PET as applicable

[c] Uncommonly, definitive imaging such as MR for skeletal or liver lesions may be accepted in lieu of biopsy, if biopsy is not feasible.

[d] Blood collection may also occur at other visit

[e] There will be no further study specific visits beyond visit 2 (except for followup phone call from study nurse at 5-10 business days inquiring as to AE which does not require a “visit”). All further followup will be via medical record of subsequent history, biopsy/histology, imaging, etc.

2. Introduction

2.1 Study Rationale

Lobular breast cancer is a lethal disease. It is difficult to detect and accurately stage, especially distant metastases. This difficulty is in part secondary to the discohesive growth pattern due to loss of E-cadherin which results in diffuse spread of cancer cells in atypical and sometimes unexpected patterns which may resemble inflammation. ILC may not demonstrate significant FDG uptake and also may be present in difficult to image locations such as peritoneum and central nervous system [2]. Thus, with ILC there may be significant understaging leading to inappropriate therapeutic decisions. Finally, since lobular breast cancer is not mass forming as is IDC, response to therapy may be difficult to evaluate. For these same reasons recurrent disease is difficult to detect with ILC, thus potentially limiting salvage therapy options. Therefore, the scientific justification for improved detection of metastatic disease is compelling.

Fluciclovine

Tumor glucose metabolism, specifically the shift away from oxidative phosphorylation to aerobic glycolysis (Warburg Effect), has formed the basis of advanced molecular imaging of breast cancer. Other metabolic activity upregulated in breast cancer includes use of amino acids. The amino acid transporters, specifically LAT1, ASCT2, $ATB^{0,+}$ SNAT1 and XCT, are overexpressed in breast cancer and reported to be associated with tumor growth, metastasis and hormone receptor status [13-16]. Shennan and coauthors demonstrated that certain human breast cancer cell lines, MDA-MB-231 and MCF-7, express system L (large neutral amino acid) transporters [17, 18]. In fact, Shennan has also demonstrated that growth of cultured human breast cancer cells may be decreased via inhibition of system L transporters [14]. Fluciclovine is a synthetic amino acid analog PET radiotracer transported via system L (LAT1) and ASCT (ASCT2) and has shown promise in the imaging of prostate and other cancers [19]. Fluciclovine uptake has been shown in vitro in breast carcinoma cell lines as well as in animal models of breast cancer [16]. This has led to the study of amino acid transport as a basis for superior detection of breast cancer by our group. As noted in the preliminary data section of this proposal, we unexpectedly discovered that ILC was better detected with amino acid transport-based PET with fluciclovine compared to glycolytic based FDG PET. Preliminary studies understandably focused on the detection of primary and locoregional disease. We propose to expand this concept to also include detection of distant disease which could alter the stage of the patient and thus therapeutic approach. Improved initial staging and treatment selection could impact the survival of nearly 40,000 women with ILC per year, and potentially enhance the evaluation of therapy response in women living with metastatic ILC.

PSMA

PSMA receptors are upregulated in many cancers besides prostate cancer since tumor associated neovasculature has upregulated PSMA expression [8]. It is likely that PSMA cleavage of vitamin B9 (folic acid) stimulates oncogenic signaling through glutamate receptors with downstream activation of the PI3K-Akt-mTOR signaling pathway [20]. Utilizing PSMA as a biologic marker of tumor neovasculature thus has a solid scientific underpinning and may yield significant biologic information in reference to lobular cancer. Many non-prostate ^{68}Ga-PSMA avid malignancies have been reported in the literature including breast cancer, yet there is little information specifically on ILC [10]. Furthermore, if we can demonstrate that ILC is sufficiently PSMA avid, there is a possibility that a theranostic approach with Lu-177 PSMA radiotherapy may be investigated as is currently occurring with metastatic prostate cancer [21]. In fact, there has been one case report of metastatic IDC triple negative breast cancer treated with Lu-177 PSMA [11]. While ^{68}Ga-PSMA is FDA approved for prostate cancer, it is not FDA approved for breast cancer. Ga-PSMA is currently produced at the Emory Center for Systems Imaging for an ongoing prostate cancer trial, and Dr. Schuster holds an IND allowing research applications.

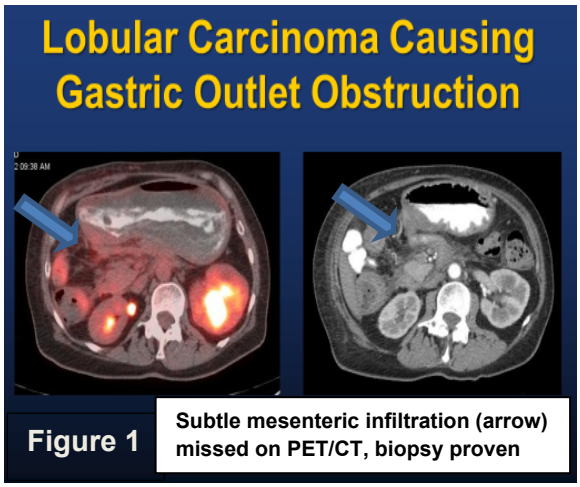
ctDNA

The role of ctDNA specifically in regard to ILC has been little studied, especially the ability of ctDNA in predicting distant disease. The premise in this exploratory aim is specifically studying the association of ctDNA targeted to ILC with imaging that is more sensitive to ILC compared with FDG PET. If there is an association, this would be a significant area for future research since ctDNA expression could then be utilized to select patients who should undergo this advanced imaging or serve to improve specificity in lieu of confirmatory biopsy if the association with verified detection rate of metastasis was particularly

robust. Finally, understanding if this correlation is associated with upregulated amino acid metabolism and/or tumor neoangiogenesis as characterized by molecular imaging could yield valuable insights into the biologic behavior of ILC.

2.2 Background

Invasive lobular carcinoma (ILC) comprises 10-15% of breast cancer. While mammography, ultrasound, breast MRI, and whole body FDG PET form the backbone of breast imaging, there are limitations in the imaging of lobular cancer histology, especially for distant disease (figure 1) [22, 23]**.** ILC is diagnosed at a later and more advanced stage compared with IDC and is typically hormone receptor positive [1]. Up to 50% of patients with the CDH1 genetic mutation will develop ILC [1]. The development of metastatic disease is likely facilitated by the loss of E-cadherin in most ILC cells [24]. Similarly, while (^{18}F) fluorodeoxyglucose positron emission tomography (FDG-PET) has also assumed an important role in whole body staging, detection of recurrent disease and monitoring therapy response, limitations for lobular cancer are well described in the literature [2, 25]. These limitations are secondary to indolent growth and diffuse growth patterns, making lobular cancer metastasis difficult to distinguish from background activity. In a study by Ulaner in 146 patients with ILC, FDG PET had only a modest effect on upstaging and did not reveal additional nodal disease. Compared with IDC patients who were upstaged in 22% of cases to stage 4 in a separate cohort, only 6% of ILC patients had unsuspected distant metastasis that were FDG PET positive [26].

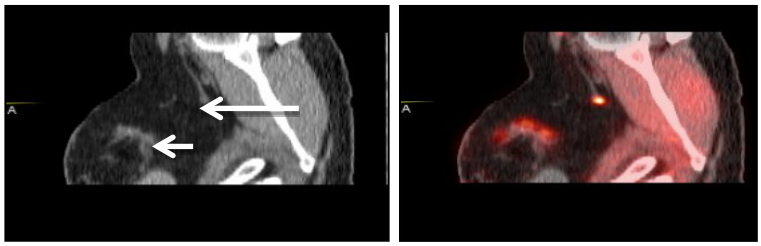


Fluciclovine

Due to the limitations of FDG PET for the staging of breast cancer in earlier studies, we have investigated molecular imaging targeting other aspects of the metabolome [27, 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 [13]. Since amino acid metabolism is also upregulated in breast cancer, molecular imaging utilizing natural or synthetic amino acid radiotracers is an attractive approach [28, 29]. Emory University is a center of innovation for amino acid transport PET imaging, having developed *anti*-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-3-[^{18}F]FACBC or fluciclovine), a synthetic amino acid analog PET radiotracer, which was subsequently FDA approved for restaging of prostate cancer [30]. Liang reported fluciclovine uptake in breast carcinoma cell lines which correlated with malignant potential as well as in orthotopic MDA-MB-231 breast carcinoma xenografts [16]. With funds from a Winship Glenn Family Breast Center Pilot Grant in 2014-2015, we studied uptake characteristics of fluciclovine in breast carcinoma, confining the study to evaluation of the primary breast lesions and locoregional disease.

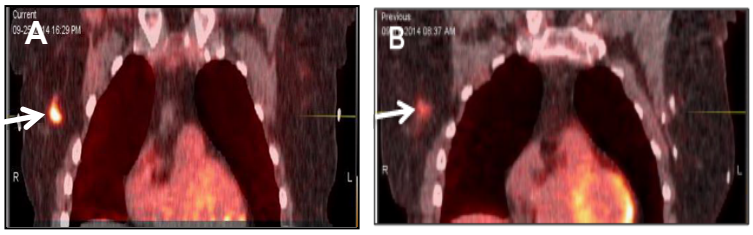
A total of 12 patients with 13 primary lesions were studied at Emory [5]. At diagnosis, 9/12 patients had primary breast cancer while 3/12 had local recurrence. Of the malignant lesions, there were 7/13 invasive ductal carcinoma (IDC), 5/13 invasive lobular carcinoma (ILC) and 1/13 invasive metaplastic carcinoma with sarcomatous differentiation (IMC). Histologic sampling occurred in 9 locoregional lymph nodes (4 benign and 5 malignant). Of the malignant nodes, 3 were ILC and 2 were IDC. Although fluciclovine uptake in IDC and ILC were both higher than in benign breast lesions, IDC had higher uptake than ILC in the primary tumor. **However, this was reversed with nodal disease in which metastatic ILC nodes had higher uptake than IDC nodes** (ILC mean SUVmax (±SD) of 6.2±0.8; IDC mean SUVmax of 3.3±2.6). **Interestingly, uptake by fluciclovine in ILC locoregional metastatic disease was also greater than uptake in the primary lesion.** Figure 2 is an example of a patient with ILC who had fluciclovine uptake in a known primary breast lesion and a biopsy proven metastatic axillary node reported negative on outside MR and ultrasound.

Figure 2. CT [A] and coregistered fluciclovine PET-CT [B] in a 49 yr old woman with left ILC. SUVmax = 3.5 in the primary mass (short arrow) and 5.6 in biopsy proven metastasis to a 6 x 11mm left axillary lymph node (long arrow).



Four patients also underwent clinical FDG PET-CT. Fluciclovine and FDG activity were compared in 8 biopsy proven lesions (5 malignant and 2 benign primary, 1 node). The malignant lesions included 3 IDC, 2 ILC and 1 IMC. For ILC, mean SUVmax was 4.6 (±3.5) with fluciclovine compared to 2.6 (±1.0) for FDG. **This finding was surprising and intriguing since uptake was greater in ILC with fluciclovine PET than with FDG.** Figure 3 is an example of greater uptake in an ILC intramammary nodal metastasis with fluciclovine than with FDG PET-CT.

Figure 3. Coregistered fluciclovine [A] and FDG [B] PET-CT in a 54 yr old woman with a 2.1cm x 1.1cm right ILC. Fluciclovine [A] had higher uptake (SUVmax = 7.1) compared to FDG [B] (SUVmax = 3.3) in metastasis to an intramammary node (arrows).



In our pilot study, **we unexpectedly discovered greater uptake in ILC with fluciclovine than with FDG PET, and greater uptake with fluciclovine in metastasis versus the primary lesion, and this observation was not limited to our center.** Simultaneously, fluciclovine PET was also being studied in breast cancer by the Ulaner Group at Memorial Sloan Kettering. Results were published in the same issue of the Journal of Nuclear Medicine as our paper along with an accompanying editorial [6, 31]. In that series 27 women with locally advanced IDC (n = 19) or ILC (n = 8) underwent fluciclovine PET/CT. All primary tumors were radiotracer avid. Fluciclovine PET detected 20/21 patients with axillary nodal metastases and also detected pathologically proven extraaxillary nodal disease in 3 patients, including 2 previously unsuspected internal mammary nodes. In the 4 patients with ILC who underwent FDG and fluciclovine PET, primary ILCs demonstrated fluciclovine avidity (median SUVmax, 6.1; range, 4.5–10.9) greater than FDG avidity (median SUVmax, 3.7; range, 1.8–6.0). Thus, in total from both cohorts, 13 patients with ILC were studied. **There was greater uptake in ILC lesions on fluciclovine versus FDG PET. And while IDC had greater fluciclovine uptake than ILC in the primary breast lesion; in nodal metastases, this relationship reversed and ILC in nodal disease had greater fluciclovine uptake than IDC. Furthermore, ILC nodal metastasis had greater fluciclovine uptake than in the primary ILC breast lesion. ILC also seems to have better correlation with complete metabolic response to neoadjuvant chemotherapy as reported in a separate study [4]. Critically, since these were pilot studies, only images of the thorax were obtained. Detection of distant disease was not studied. Our proposed trial will specifically address the detection of distant disease and locoregional staging.**

PSMA

Prostate specific membrane antigen (PSMA) is a Type 2 transmembrane glycoprotein of the M28 peptidase family and is overexpressed on the cell surface of prostate cancer [32]. The name prostate-specific is a misnomer, since other cancers including breast cancer have upregulated PSMA receptors due to tumor angiogenesis. Most data regarding PSMA PET with breast cancer pertains to IDC. Wernicke studied 92 samples in 106 patients with breast cancer [7]. Immunohistochemistry (IHC) for tumor associated vascular endothelial cell PSMA receptors revealed staining in 74% of primary and 100% of brain metastasis. There was absence of PSMA staining in 98% of normal breast tissue. Wernicke noted there was no difference between PSMA staining for IDC and ILC, yet only 10 ILC samples were included. In another study of 72 patients with breast cancer, moderate to strong tumor associated neovasculature PSMA staining was present in 67% of distant metastasis versus 36% in primary tumor cells [9]. Yet, only 12 ILC patients were included and it was not clear if these patients had metastases. In a study of 315

cases by Tolkach of which 64 tumors were ILC, 64.6% of patients with IDC had PSMA staining, versus 42.2% of those with ILC [11]. Finally, in one of the few imaging studies, Sathekge reported in 81 lesions in 19 breast carcinoma patients that PSMA PET visualized 84% of lesions; yet again, only 2 ILC patients were included in this cohort. In a major review in the Journal of Nuclear Medicine, Salas-Fragomeni argues "this potential application of PSMA-targeted imaging in breast cancer should perhaps be most urgently explored, as the need for reliable agents for systemic staging in lobular breast cancer has been long-standing" [8]. Our study will address this urgent question, that of the utility of PSMA PET explicitly for ILC metastatic disease, as well as that of fluciclovine PET for ILC.

ctDNA

The absolute number of circulating tumor cells has been associated with metastatic breast cancer [33, 34]. A more refined technique, that of circulating tumor DNA (ctDNA), holds promise as a "liquid biopsy," since tumor DNA circulating in the blood is more specific for carcinoma. High levels of ctDNA are reported to correlate with tumor size, lymph node involvement, histopathological grade, and clinical staging [35-40]. Though there have been anecdotal reports of ctDNA and ILC or inclusion of a small number of ILC related ctDNA in a larger study, ILC has not been systematically studied [41]. Probing the association of ctDNA to tumor burden in ILC with two novel imaging biomarkers (amino acid transport/fluciclovine and angiogenesis/PSMA) with more sensitivity than FDG PET for ILC will provide biologic insights in the context of this exploratory aim.

2.3 Potential Risks and Benefits

Benefits:

The primary benefit of participation in this study is more accurate identification of metastatic disease which may impact appropriate staging and therapy decisions. If occult metastasis are present but are not identified on conventional imaging this would lead to understaging and possible futile curative therapy. On the other hand, if conventional imaging identifies metastasis which are in fact false positive for neoplasia, and which could have been more definitively characterized by molecular imaging, this may lead to overstaging and inappropriate withholding of curative therapy.

Study Specific Procedure Risks:

1) Radiation Risks: The principal risk associated with a radiation dose is the possibility of developing a radiation-induced cancer later in life. However, the additional risk of radiation-induced cancer from these diagnostic procedures is low compared to the risks from the disease itself as well as systemic therapy or radiation therapy.

Human dosimetry data for fluciclovine from FDA package insert: The (radiation absorbed) effective dose resulting from the administration of the recommended activity of 370 MBq (10 mCi) of fluciclovine is 8 mSv (22 microSv/MBq). For an administered activity of 370 MBq (10 mCi), the highest-magnitude radiation doses are delivered to the pancreas, cardiac wall, and uterine wall: 38 mGy, 19 mGy, and 17 mGy, respectively.

Human dosimetry data for Ga-PSMA from Joint EANM and SNMMI Procedure Guideline for Prostate Cancer Imaging: Version 1.0 [42]. Based on the available studies, the coefficient for effective dose from ^{68}Ga-PSMA averages is 2.0 × 10−2 mSv/MBq, resulting in an average effective radiation dose of 3 mSv for an administered activity of 150 MBq. For a typical dose of 5 mCi (185 MBq) that we will be administering, the effective dose equivalent would be 3.7 mSv. The organs with the highest dose are urinary bladder wall (0.13-0.173 mSv/MBq) and kidney (0.122-0.262 mSv/MBq).

2) Allergic or other reactions to radiotracer: Although the risk is extremely small, it is possible to develop an allergic reaction to the fluciclovine or Ga-PSMA. This can result in hives, rash, itching and difficulty breathing which may require emergency medical treatment. There have been no previous instances of allergic reaction. In prior studies with both radiotracers, the risk of adverse events which can be attributed to the radiotracer is extremely low, and of minimal medical impact such as burning at IV site or dysgeusia.

3) Risk related to IV for PET scan: A small amount of radioactive material will be injected by either a hand-held needle or a machine. Such injections are generally quite safe, but any injection involves some risks. The injection could harm a nerve, artery or vein, or cause infection. The radioactive material could also leak from veins, causing swelling and discomfort.

4) False positive on PET: It is possible that a lesion identified on the experimental PET procedures may be false positive, thus potentially leading to an inappropriate change in stage. Yet, no lesion will be acted upon which could change therapeutic intent unless confirmed via histology or advanced imaging such as MR for certain bone lesions. See below for risks associated with biopsy or MR.

Non-Study Specific Procedure Risks:

1) Biopsy associated risk: If a suspect lesion is identified on either or both PET scans, definitive histologic proof in the safest manner possible will be sought if clinically indicated. Risks of biopsy, though small in expert hands, include bleeding, infection and even rarely death. Note that before biopsy is considered, careful scrutiny of the already obtained CT or MR will be undertaken to determine if an unsuspected anatomic correlate can be identified. This will then prompt a discussion between the patient and primary clinician on the benefits versus risks to definitively determine if a metastasis is present which could affect therapy. Biopsy will be undertaken in the least invasive manner possible as a standard of care procedure, reimbursed by third party payers.

2) MR: If a lesion is not deemed safe or possible to biopsy, MR may then be performed (e.g. for bone or liver). MR, even with the administration of Gadolinium is considered a safe and effective noninvasive imaging procedure. In addition to the small possible reactions to contrast material if used, claustrophobia while in the magnet is a potential risk, as well as reaction from metal within the body. Yet at Emory, special screening is performed before MR to determine if the patient has tattoos or any metal items in the body such as implants, pacemakers, clips or shrapnel, to make sure the MRI scan is done safely.

3. Objectives and Endpoints

3.1 Study Objectives:

Our major goal in this proposed investigation is to use advanced molecular imaging to better detect metastases in patients with invasive lobular cancer.

Specific Aim 1: Improve detection of metastasis with fluciclovine and Ga-PSMA PET versus best standard of care conventional imaging, as confirmed with histology. We will image 20 patients with ILC who have either: a) clinical or imaging suspicion of metastatic disease; or b) proven metastatic disease but in whom there is suspicion of an even greater tumor burden that could change therapy approach. Abnormal foci which correlate to anatomic lesions previously unsuspected on conventional imaging will undergo optional biopsy as clinically indicated in the safest manner possible (if not already done) to determine the verified detection rate of metastatic disease.

Specific Aim 2: **Determine concordance and discordance of ILC detection with Ga-PSMA versus fluciclovine PET, as confirmed with histology.** We will compare verified detection rates for metastasis between fluciclovine and PSMA PET modalities. This may necessitate a second biopsy of a distant lesion discordant between the 2 PET techniques. We will also analyze images for biodistribution in the primary lesion(s) for both radiotracers.

Exploratory Aim 3: Establish the role of molecular detection by ctDNA mutations including PIK3CA, ESR1, HER2, and AKT1 in characterizing the degree of tumor burden as identified by metabolic amino acid transport and/or tumor neovasculature receptor imaging [12]. We will collect peripheral blood for ctDNA in all patients and determine if ctDNA correlates with presence or absence of metastases and tumor burden as identified by fluciclovine (metabolic) and/or PSMA (angiogenesis) PET.

3.2 Study Endpoints:

Primary endpoint:

Determine verified detection rate for metastasis of invasive lobular cancer using fluciclovine PET and PSMA PET for imaging (Specific Aims 1 and 2)

Secondary endpoints:

Correlation of ctDNA results with presence or absence of metastasis (Specific Aim 3)
Correlation of ctDNA results with results of imaging (Specific Aim 3)

4. Study Design

4.1 Overall Design

We will undertake a study with 20 patients with treatment naïve biopsy proven ILC who have either: a) clinical or imaging suspicion of metastatic disease; b) biopsy proven metastatic disease but in whom there is suspicion of an even greater tumor burden that could change therapy approach. All patients will be recruited from the Winship Glenn Family Breast Cancer Program at any Emory facility. All patients will undergo Ga PSMA PET followed at minimum of 10 hours (to allow for radiotracer decay) by fluciclovine PET. (This may also happen in the opposite order, in which case 20 hours after fluciclovine will be needed to allow for decay.) There is no need for randomization since each patient will undergo both imaging techniques, and the results will be interpreted blindly. Thus, radiopharmacy scheduling will dictate the order of the radiotracers to ensure the patient will undergo both imaging modalities in the shortest time frame. Two nuclear medicine physician co-investigators, Drs. Schuster and Muzahir, will independently interpret the 20 cases, each reading half (the other reading the other half) of the fluciclovine and PSMA images (to avoid bias in always reading the same type of imaging). The readers will be blinded to all imaging including the other PET. All suspicious lesions with nonphysiologic activity above background for locoregional spread and distant disease will be identified and rated for suspicion of malignancy on a 6 point Likert scale (where 1 = definitely benign and 6 = definitely malignant). Both the primary lesion and all suspected metastases will be measured on an advanced workstation and metabolic uptake parameters (SUV, MTV, TLA) recorded. After both studies have been interpreted blindly, the third nuclear medicine physician co-investigator, Dr. Ulaner, will blindly interpret **deidentified** PET studies on every patient (alternating for a total of 10 each fluciclovine and Ga-PSMA PET). Afterward, an unblinded reconciliation interpretation will take place with all 3 readers. To ensure balance, 10 of the studies will be reviewed for consensus first showing Ga-PSMA, then fluciclovine; 10 of the studies will be reviewed in the opposite manner. In this manner, a preliminary sense of kappa can be developed between the third and the other 2 readers, yet will guarantee the highest degree of patient care as there are currently no validated criteria for interpreting fluciclovine or PSMA PET for breast cancer. This approach will help us adapt existing prostate specific criteria to that of breast cancer. Concordant and discordant lesions between each PET study and conventional imaging will be reported and potential biopsy sites, if any, recommended. The most distant and accessible lesion may undergo biopsy in the safest manner possible (if not already done) as clinically indicated after discussion between the primary oncologist and patient.

Preference for biopsy will be given to discordant lesions. Uncommonly, in some lesions not deemed safe to biopsy, characteristic appearance on correlative imaging will be accepted as truth (e.g., MRI for certain skeletal or liver lesions). In select cases, a second biopsy of a discordant distant lesion may be required. For example, if there are 2 potentially impactful lesions which are fluciclovine positive/PSMA negative and fluciclovine negative/PSMA positive, respectively. Note that before biopsy is considered, careful scrutiny of the already obtained CT or MR will be undertaken to determine if an unsuspected anatomic correlate can be identified. This will then prompt a discussion between the patient and the referring clinician co-investigator on the benefits versus risks to definitively determine if a metastasis is present which could affect therapy. Biopsy may be undertaken in the least invasive manner as standard of care as clinically indicated, reimbursed by third party payers. Uptake parameters will be correlated with all proven primary, locoregional, and distant lesions. Verified detection rates for metastasis will be calculated for each modality.

In addition, we will collect 20 ml peripheral blood for ctDNA analysis in all patients at visit 1 (or possibly 2) to be initially processed and stored by Dr. Torres' lab for off-site specialty analysis. Plasma levels of ctDNA will be measured using a targeted 28 gene panel to detect point mutations including mutations in PIK3CA, ESR1, HER2, and AKT1, genes associated with hormone therapy resistance and ILC [38, 39]. We will examine ctDNA results for association with presence or absence of locoregional metastasis on imaging as confirmed with histology, presence or absence of distant metastasis on imaging as confirmed with histology, correlation of presence or absence and absolute ctDNA with PET uptake.

4.2 Scientific Rationale for Study Design

Our preliminary data support a key scientific premise that metabolic imaging with amino acid PET has shown promise in the detection of ILC. There is also emerging data that tumor neovasculature imaging with PSMA PET may improve detection of ILC. **We hypothesize that metabolic imaging with amino acid transporter PET will improve staging of ILC, particularly for distant metastases, compared to best standard of care conventional imaging (usually CT or MR and possible bone scan), and which typically does not include FDG PET due to low sensitivity. We also hypothesize that receptor directed PSMA imaging of tumor associated neovasculature in ILC will reveal unique information to complement metabolic interrogation with fluciclovine PET. Improved staging will facilitate more appropriate management decisions.**

To test these hypotheses with the highest scientific rigor, we propose a feasibility trial with fluciclovine and Ga-PSMA PET strategies centered on detection of metastasis in patients with advanced ILC using histology as the gold standard. As an exploratory aim, we will also correlate the occurrence of circulating tumor DNA (ctDNA) mutations including PIK3CA, ESR1, HER2, and AKT1 with presence of metastasis and tumor burden. We will explore if a multiparametric strategy with ctDNA and imaging will help define which patients should undergo molecular imaging and also identify those who may forego biopsy if distant lesions are detected on imaging. We expect this pilot trial will generate sufficient preliminary data to determine feasibility for a definitive NIH sponsored trial developing more accurate staging techniques to help modify current practice for imaging of ILC.

4.3 Justification for Dose

The doses administered for each radiotracer: 10 mCi fluciclovine (^{18}F) and 5 mCi for Ga PSMA are based upon well established guidelines referenced below.

4.4 End of Study Definition

The study will be closed to accrual once all 20 patients have been recruited and scanned with PET. End of analysis will occur once sufficient followup data has been collected for each patient to determine study endpoints. Study will be formally closed once all manuscripts have been published. Though it is likely most relevant standard of care followup will be acquired within 1 year, patients will be consented for 5 year followup through the medical record in the case of future required analysis. There will be no other study specific visits after the second PET scan (visit 2), though the patient will be called by the study nurse at 5-10 business days to ensure no delayed AEs occurred.

5. Study Population

Approximately 1400 breast cancer patients are seen yearly at Winship, with approximately 20% of lobular histology (280). Of these, approximately 100-150 are advanced. Thus at a conservative recruitment rate of 10% (10-15 patients/year), successful accrual is highly feasible. Lobular cancer tends to present at a higher stage. Based on historical averages, we expect that 31% of patients will present with Stage 3 and 37% of patients with Stage 4 disease. We expect approximately 10% dropout either before completion of both PET scans or after PET scanning and before biopsy.

5.1 Inclusion Criteria

___(Y) 1. Treatment naïve biopsy proven ILC Patients with ILC

___(Y) 2. Either: a) clinical or imaging suspicion of metastatic disease; or b) proven metastatic disease but in whom there is suspicion of an even greater tumor burden that could change therapy approach.

___(Y) 3. Ability and willingness to undergo biopsy if needed per standard of care for possible metastasis which could change therapy approach.

___(Y) 4. Age over 18

Non-English-speaking patients may be considered for enrollment in this trial. Subjects with Limited English Proficiency LEP may be enrolled and study team members will use Emory IRB approved short forms to conduct the consent process.

5.2 Exclusion Criteria

___(N) 1. Pregnancy. Qualitative or quantitative serum or urine pregnancy test will be done in women of childbearing potential within 24 hours before PET[1].

[1] A female of childbearing potential (FCBP) is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 12 consecutive months (if age > 55 years); if the female subject is < 55 years and she has been naturally postmenopausal for > 1 year her reproductive status has to be verified by additional lab tests (< 20 estradiol OR estradiol < 40 with FSH > 40 in women not on estrogen replacement therapy).

6. Registration Procedures

Informed consent is required prior to participation in the study. Patients will sign the written informed consent, if possible, at the time of enrollment. In instances where this is not possible, verbal informed consent will be first obtained in order to instruct the patient to fast in preparation for the fluciclovine PET scan (not required for PSMA). However final written informed consent will be obtained before the PET scan. Participants will also be assigned an identification number for screening purposes; data collected during the screening process will also be recorded using that number. Patients will then be registered in OnCore through usual Winship and department of Radiology and Imaging Sciences guidelines. No randomization will be performed. Blinding will only take place for initial study interpretation before consensus interpretation by Nuclear Medicine imagers.

7. Study Intervention

Radiotracer production: Blue Earth Diagnostics, Ltd manufacturers of fluciclovine has pledged support with gratis radiotracer unit doses supplied from PETNET Solutions.

Telix Pharmaceuticals (US) Inc, manufacturer of Ga-PSMA, has pledged support with gratis radiotracer kits which will be compounded by the Emory CSI radiopharmacy.

Fluciclovine

PETNET Solutions, located on the Emory campus, produces commercial doses of fluciclovine which is FDA-approved for the detection of recurrent prostate cancer. Unit doses of this material will be provided for the study. The IRB has decided that a formal IND exemption is not required.

The following is provided as justification for IND exemption: This is an early phase study in which 20 female patients will undergo fluciclovine and PSMA PET on separate days. We have already studied 12 female patients with fluciclovine in the past for breast cancer under an Emory IRB approved protocol without safety concerns. At the time, the fluciclovine radiotracer was manufactured at the Emory CSI radiopharmacy under Dr. Schuster's existing IND 072437. Yet, we will now be administering FDA approved commercial doses supplied by the commercial radiopharmacy PETNET to which our IND would not be applicable. Thus, this is off-label research of an FDA approved product for recurrent prostate cancer. The patient will undergo pregnancy testing within 24 hours before PET as appropriate per Winship and Radiology regulations. Note also that 27 women had also been studied for this indication at Sloan Kettering in the past without complication. Three female normal volunteers and a number of other female patients with other cancers have been studied with fluciclovine under our IND without complication and this data had been used to obtain FDA approval in terms of safety. Thus, we believe an IND exemption is in order since: 1) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug; 2) The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the investigation is not intended to support a significant change in the advertising for the product; 3) The investigation is conducted in compliance with the requirements concerning the promotion and sale of drugs; 4) The investigation is conducted in compliance with the requirements for IRB review and informed consent; 5) The investigation does not intend to invoke 21 CFR 50.24, a waiver of informed consent in an emergency room setting.

PSMA

^{68}Ga-PSMA will be produced under Dr. Schuster's IND #143137 via a lyophilized kit produced by Telix Pharmaceuticals. Briefly, ^{68}Ga will be eluted from our Eckerd and Ziegler 50 mCi Pharmaceutical Grade ^{68}Ge/^{68}Ga Generator (or equivalent) located at our in-house radiopharmacy and combined in a vial with the premixed lyophilized product including peptide. Our existing PSMA IND #143137 has been amended with the study protocol.

7.1 Study Specific Experimental Procedures

A. Imaging (PET acquisition)

Note that this order may be reversed in certain patients depending on patient and radiopharmacy schedule.

PSMA
Patients will undergo PSMA PET-CT after 5 mci (3-7 mCi) IV dose of ^{68}Ga-PSMA. Then after an uptake period of approximately 60 minutes, the patient will be encouraged to void. A PET-CT will then be completed from pelvis to skull base at approximately 5 minutes per bed position. Fasting is not required for this study.

Fluciclovine
On a separate day to allow for radiotracer decay, and within 15 days of PET Visit 1, a fluciclovine PET-CT at approximately 4 min post IV injection of 10 mCi fluciclovine will be completed from pelvis to skull base at approximately 3 minutes per table position. Fasting except for water (and medications) will be required for at least 4 hours before the PET scan.

B. Circulating Tumor DNA

Step #1 - Blood Draw will typically occur on day of first PET scan.

1. 20ml of peripheral blood (or two 10 ml Cell-Free DNA BCT Streck tubes) is collected by any standard phlebotomy technique from a peripheral access point or from a central line by trained personnel.

2. Tubes are inverted about 10 times immediately after collection.

3. Samples are then prepared for transportation to the laboratory for processing (within 24 hours).

Step #2 - Plasma Processing (in Laboratory)

1. Perform this step once for each patient: transfer 1 mL whole blood with a pipette to a pre-labeled 2 mL cryogenic vial, round bottom, self-standing. This 1 ml will be stored frozen per below as a backup sample. Then the remainder gets processed as following.

2. Streck tubes are centrifuged at room temperature for 10 min at 1600 (±150) g.

3. After centrifugation, remove tubes from centrifuge and transfer supernatant of the Streck tubes to one fresh 10 ml polypropylene centrifuge tube without disturbing the cellular layer using a disposable serological pipette or disposable bulb pipette.

4. Centrifuge the plasma in the 10 ml centrifuge tube at room temperature for 10 min at 3000 (±150) g.

5. After centrifugation, remove tubes from centrifuge and transfer supernatant to a fresh 10 ml centrifuge tube without disturbing the cellular layer using a disposable serological pipette or disposable bulb pipette. After transferring the plasma to a new 10 ml centrifuge tube as described, gently mix plasma and record total plasma volume (~8-10 ml plasma per 20 ml blood).

6. Transfer 1 ml plasma aliquots with a pipette to 2 ml pre-labeled cryogenic vials.

7. Place plasma tubes into storage box and freeze plasma in freezer upright in storage box at -80°C or colder. Six-hour time storage at -20°C is possible.

Step #3 - Specimen Storage

1. Sample are maintained continuously at -80°C or colder.

2. When outside the freezer, such as when transferring to a different freezer in another location or preparing for shipment, boxes containing tubes should be covered with dry ice.

3. Freezer or dry ice specimen storage container temperature must be checked and monitored. Document any deviation from protocol.

4. The freezer or dry ice storage box containing the specimens will either be locked or in a secure area accessible only to authorized study staff.

5. A backup storage plan will be in place in the event of freezer failure.

8. Study assessments and Procedures.

8.1 Schedule of Procedures

See above schedule of activities in Section 1.3 for **study specific experimental procedures**.

Standard of Care Procedures
1) Conventional imaging is broadly defined as mammography, ultrasound, bone scan, FDG PET, CT and/or MR: Any or all of these imaging studies may have been performed as standard of care at enrollment. These are not under control of study design. Conventional imaging and reports within 6 months of enrollment (ideally 2 months) will be reviewed to correlate to PET imaging. No additional interpretation will take place. Imaging reports for these studies will be utilized for comparative analysis.

2) Biopsy: After the PET studies, patients with abnormal findings on the PET scans will have the most distant and accessible lesion identified so that the patient may undergo biopsy in the safest manner possible (if not already done) as clinically indicated. Preference for biopsy will be given to discordant lesions. In some lesions not deemed safe to biopsy, characteristic appearance on correlative imaging will be accepted as truth (e.g., MRI for certain skeletal or liver lesions). In select cases, a second biopsy of a discordant distant lesion may be required. Note that before biopsy is considered, careful scrutiny of the already obtained CT or MR will be undertaken to determine if an unsuspected anatomic correlate can be identified. This will then prompt a discussion between the patient and the referring clinician co-investigator on the benefits versus risks to definitively determine if a metastasis is present which could affect therapy. Biopsy will be undertaken in the least invasive manner as standard of care, reimbursed by third party payers.

3) Therapy: All subsequent therapy for breast cancer will be standard of care and beyond the scope of this investigation.

9. Measurement of Effect

Image Analysis: Two nuclear medicine physician co-investigators, Drs. Schuster and Muzahir, will independently interpret the 20 cases, each reading half (the other reading the other half) of the fluciclovine and PSMA images (to avoid bias in always reading the same type of imaging). The readers will be blinded to all imaging including the other PET. All suspicious lesions with nonphysiologic activity above background for locoregional spread and distant disease will be identified and rated for suspicion of malignancy on a 6 point Likert scale (where 1 = definitely benign and 6 = definitely malignant). Both the primary lesion and all suspected metastases will be measured on an advanced workstation and metabolic uptake parameters (SUV, MTV, TLA) recorded. After both studies have been interpreted blindly, the third nuclear medicine physician co-investigator, Dr. Ulaner, will blindly interpret deidentified PET studies on every patient (alternating for a total of 10 each fluciclovine and PSMA PET). Afterward, an unblinded reconciliation interpretation will take place with all 3 readers. To ensure balance, 10 of the studies will be reviewed for consensus first showing Ga-PSMA, then fluciclovine; 10 of the studies will be reviewed in the opposite manner. In this manner, a preliminary sense of kappa can be developed between the third and the other 2 readers, yet will guarantee the highest degree of patient care as there are currently no validated criteria for interpreting fluciclovine or PSMA PET for breast cancer. This approach will help us adapt existing prostate specific criteria to that of breast cancer. Concordant and discordant lesions between each PET study and conventional imaging will be reported and potential biopsy sites, if any, recommended.

Correlation of histology with images: Uptake parameters as above will be correlated with all proven primary, locoregional, and distant lesions.

ctDNA analysis: Plasma levels of ctDNA will be measured using targeted assays to detect point mutations including mutations in PIK3CA, ESR1, HER2, and AKT1, genes associated with hormone therapy resistance and ILC [12, 38, 39, 43]. Because ILC are predominantly hormone receptor positive, hormone resistance is particularly relevant because it predicts for higher rate of distant metastasis. Dr. Mylin Torres, will provide expertise in ctDNA analyses. **Deidentified** specimens will be shipped to the Circulating Tumor Cell Center at Massachusetts General Hospital for fee for service ctDNA processing.

Correlation of ctDNA results with results of imaging: We will examine ctDNA results for association with presence or absence of locoregional metastasis on imaging as confirmed with histology, presence or absence of distant metastasis on imaging as confirmed with histology, correlation of presence or absence and absolute ctDNA with PET uptake as determined by the following indices: SUVmax, SUVmean, SUVpeak, Total Lesion Activity for the index primary, locoregional and distant metastatic lesion if any, and correlation to summed indices as a reflection of total metabolic tumor burden. ctDNA will also be correlated with CI.

10. Statistical considerations

Specific Aims 1 and 2

Analysis plan: The objectives of Aims 1 and 2 are to compare verified detection rates between fluciclovine and PSMA PET versus conventional imaging, as confirmed with histology (Aim 1), and determine concordance and discordance of ILC detection with PSMA versus fluciclovine PET, as confirmed with histology (Aim 2). Paired detection rates will be assessed using McNemar's test. Detection rates will be reported for each method, and 95% confidence intervals will be estimated using the Clopper-Pearson approach. Rates of concordant/discordant observations will be reported. The Likert rating-scale data also allows for determination of sensitivity, specificity, PPV, NPV and Receiver Operating Characteristic (ROC) Area Under the Curve (AUC) values to compare.

Sample size and power: We will image 20 patients with ILC for this study. This is a small, exploratory study, with a goal of obtaining preliminary data to estimate effect size. Thus, there is no power analysis for the study aims. If we find a 20% difference in detection rate with a 40% discordant rate, we would require 67 patients in a larger study to achieve 80% power with a Type I error rate of 0.05 using McNemar's test. Discordant rates of 50% and 60% with a difference in detection rate of 20% would require 83 and 99 patients, respectively, to achieve 80% power. If the difference in detection rate is 15%, the required sample sizes would range from 118 to 175 for discordant rates of 40% to 60% to achieve 80% power. If the difference in detection rate is 25%, the required sample sizes would range from 42 to 63 for discordant rates of 40% to 60% to achieve 80% power.

Exploratory Aim 3

Analysis plan: In Aim 3, ctDNA parameters will be summarized descriptively using mean, median, min/max, IQR, and standard deviation. ctDNA will be correlated with PET uptake parameters using Pearson's or Spearman's correlation coefficient (where appropriate), as well as chi-squared tests, Fisher's exact tests, or ANOVA if any variables are further categorized. In addition, generalized linear models will be fit to evaluate the relationship between ctDNA parameters and PET uptake parameters, controlling for relevant characteristics.

Sample size and power: We will image 20 patients with ILC for this study. This is a small, exploratory study, with a goal of obtaining preliminary data to estimate effect size. Thus, there is no power analysis for the study aims. If we find a correlation coefficient of 0.3 between a specific ctDNA parameter and PET update parameter, we would need 84 patients to detect that correlation coefficient vs. a null value of 0 with 80% power and a Type I error of 0.05. We would need 46 patients to detect a correlation coefficient of 0.4 vs. a null value of 0 to achieve 80% power.

11. Adverse Events: List and Reporting Requirements

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (please follow directions for routine reporting provided in the full Data Safety and Monitoring Plan (DSMP)). Additionally, certain adverse events must be reported in an expedited manner for timely monitoring of patient safety and care. The following sections provide information about expedited reporting.

> **Special note for radiotracers given during this trial**: **The risk of AEs or SAEs from PET radiotracers is exceedingly low. 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 for fluciclovine and 10 hours for Ga-PSMA will not be considered a related to study procedure AE since the radiotracer has effectively decayed by 20 hours and 10 hours respectively.** Reporting will follow relevant FDA and manufacturer guidelines as outlined below.

A serious adverse event is any medical occurrence which is fatal, is immediately life threatening, requires hospitalization (or prolongs an existing hospitalization), results in persistent significant disability or incapacity, is a congenital abnormality or a birth defect, or is considered medically significant by a physician.

The definition of serious adverse event (experience) also includes *important medical event.* Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Attribution: The determination of whether an adverse event is related to a study imaging agent. The categories are:
Definite: The adverse event is clearly related to the study imaging agent.
Probable: The adverse event is likely related to the study imaging agent.
Possible: The adverse event may be related to the study imaging agent.
Unlikely: The adverse event is doubtfully related to the study imaging agent.
Unrelated: The adverse event is clearly not related to the study imaging agent.

Unexpected Adverse Event**:** An adverse event, the nature or severity of which is not consistent with the applicable product information.
Investigational Agent: A protocol drug administered under an Investigational New Drug Application (IND).
Commercial Agent: An agent not provided under an IND but obtained from a commercial source.
Determination of Reporting Requirements
Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the grade (severity), the relationship to the study imaging agent (attribution), and the prior experience (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

Steps to determine if an adverse event is to be reported in an expedited manner:
Step 1: *Identify the type of event using the NCI Common Toxicity Criteria for Adverse Event Reporting Version 5.0 (CTCAE v5.0).* The CTCAE v5.0 provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE v5.0 can be downloaded from the CTEP home page (**http://ctep.cancer.gov**). Additionally, if assistance is needed, the NCI has an Index to the CTCAE v5.0 that provides help for classifying and locating terms. All appropriate treatment locations should have access to a copy of the CTCAE v5.0.
Step 2: *Grade the event using the NCI CTCAE v5.0.*
Step 3: *Determine whether the adverse event is related to the protocol imaging agent (investigational or commercial)*.
Step 4: *Determine the prior experience of the adverse event*. *Expected* events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered *unexpected*, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in the investigator brochure for an investigational agent or the drug package insert for a commercial agent.
Step 5: Review Expedited reporting requirements below to determine if there are any protocol-specific requirements for expedited reporting of specific adverse events that require special monitoring.
Step 6: Determine if the protocol treatment given prior to the adverse event included investigational agent(s), a commercial agent(s), or a combination of investigational and commercial agents.

Reporting Methods:
The Principal Investigator is responsible for evaluating all reports of adverse events which occur in patients enrolled on this study and reporting those events which are serious to Telix Pharmaceuticals or

to Blue Earth as outlined below and the Emory University IRB according to the guidelines below. SAEs must be reported as required by the WCI DSMP.

Expedited reporting requirements: All grade 4-5 adverse events (unexpected and expected) and grade 2-3 adverse events that are unexpected must be reported as above within 24 hours of occurrence.

Reporting to Institutional Review Board (IRB): All SAEs must be reported by the Principal Investigator to the Emory University IRB as required by their regulations and the conditions of approval for the protocol. Reporting to FDA: The Sponsor- Investigator must notify the FDA in a written IND safety report of any adverse event which is both serious and unexpected. Each written notification must be made as soon as possible but no later than 15 calendar days of first becoming aware of the event. Reports should be submitted using the FDA Form 3500A (available on the FDA website at www.fda.gov/medwatch. Reporting may be done online or via mail or fax.

Fluciclovine: The Investigator will report all Serious Adverse Events occurring in a subject on the day of or within XX days following the Agent administration to Bracco, the parent company for Blue Earth Diagnostics ("Bracco") by telephone (+44 1483 212151), FAX (+44 1483 212178) or e mail (drugsafetyus@blueearthdx.com).

Events will be reportedBracco **within 24 hours** of the investigator becoming aware of the events occurrence. Bracco will prepare an individual single case report (ISCR) in compliance with applicable regulations. A copy of the ISCR will be sent by Bracco to the Investigator. The Investigator is responsible for informing the ethics committee of serious events occurring during the study in compliance with local regulations. Bracco will report the event, if appropriate, to the regulatory authority in compliance with local regulations. The sponsor will also report SAEs to the FDA per regulations as noted in Section 10 above.

Ga PSMA: Emory has provided a copy of the detailed Clinical Trial protocol to Telix Pharmaceuticals, as amended from time-to-time, as well as a copy of the annual safety reporting (**Safety Reporting**) involving the use of the Kit. Emory shall provide an annual report to Telix, summarizing the Adverse Event (**AE**) profile in relation to the use of the Kit, specifically noting whether there was attribution of the AE (however serious) to the Kit.

In the event of a Serious Adverse Event (SAE) or death occurring any time within 7 days after administration of ^{68}Ga-PSMA-11 prepared using the Kit, and not related to disease progression, Emory shall report to Telix Designated Contact (Bernard Lambert; (571) 294 4646; bernard.lambert@telixpharma.com)

(a) Within seven (7) calendar days of the date of awareness of a life-threatening adverse event or death (**Date of Awareness**), regardless of apparent causality to the use of the Kit.
(b) Within fifteen (15) calendar days of the Date of Awareness, any other SAEs, regardless of apparent causality to the use of the Kit.
(c) Within seven (7) calendar days upon communication of any other safety-related report, issues or queries related to the Kit that are either raised by, or communicated to, regulatory authorities or ethics committees.
(d) Within seven (7) calendar days after submission, the Development Safety Update Report (DSUR) submitted to the FDA.

Any unexpected fatal (Grade 5) or unexpected, life-threatening (Grade 4) adverse event must be reported to the FDA as soon as possible but no later than 7 calendar days of first becoming aware of the event. Reports should be submitted using the FDA Form 3500A via fax at 1-800-332-0178

12. Data Reporting / Regulatory Requirements

Patients will be monitored by the technologists and/or 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). Basic monitoring of adverse events during the ^{68}Ga-PSMA-11 PET visit will be performed as pursuant to FDA agreement. This will consist of direct observation by our study staff during the visit with documentation of any adverse events or lack thereof in the electronic medical record.

The Data and Safety Monitoring Committee (DSMC) of the Winship Cancer Institute of Emory University will oversee the conduct of this study. This committee will review all pertinent aspects of study conduct including patient safety, compliance with protocol, data collection and efficacy.

The DSMC will review the charts of 10% of patients enrolled to the study and two of the first 5 patients entered to the study. Reviews will occur annually for studies that are moderate risk. High risk studies will be reviewed every 6 months. The committee reserves the right to conduct additional audits if necessary, at any time-point. The Principal Investigator is responsible for notifying the DSMC about the accrual of patients when the first 5 have been entered to the study.

The charter for the Winship DSMC is available upon request to the investigator or other study-related personnel.

As with our ongoing clinical trial, for the current proposal we will adhere strictly to Winship's Data Safety Monitoring Plan which is provided in detail at the following link:
https://winshipcancer.emory.edu/research/clinical-trials-office/data-and-safety-monitoring-committee.html

Data Safety Monitoring Board:
Note that our proposed trial is an early phase clinical trial that will be conducted completely within the Emory system, so a formal and independent Data Safety Monitoring Board (DSMB) [which is required for multi-site studies or Phase III trials] is not required for our proposal. As described above, the DSMC of Winship Cancer Institute of Emory University will oversee the conduct of our study.
Fidelity to Protocol and Data Integrity:
Clinical trial performance and fidelity to the protocol, will be monitored by the DSMC of Winship Cancer Institute of Emory University. Integrity of the data will be maintained by rigorous peer-review internally by all the clinical co-investigators in Radiation Oncology, Radiology/Nuclear Medicine, Surgical Oncology, and Medical Oncology.

13. Ethics and Protection Of Human Subjects

The study will be approved by the Emory IRB per standard institutional requirements and informed written consent obtained.

To maintain patient confidentiality, medical records will be accessed only by IRB approved study personnel (e.g. CRC). Partial HIPAA waiver will be obtained to allow screening of provider schedules for identification of potentially eligible research subjects. Upon medical record review and identification of a potentially eligible research subject, patient's full name, MRN, EMPI, and full dates (e.g. DOB, procedure dates, admission dates, etc.) will be stored on a screening log and used by study coordinators for the subsequent study activities. The screening log will be kept on the HIPAA compliant shared folder and will be accessible only to limited IRB approved study personnel (e.g CRC). Sensitive data will always be stored on the HIPAA compliant shared folder and will never be stored on local or portable drives that are not encrypted per Emory IS standards.

Each study participant will be assigned a unique study identification (ID) number at the time that informed consent is given. Personal identifying information, study data, including the unique study ID, will be entered in the Microsoft Database that will be kept on the HIPAA compliant shared folder accessible to approved study personnel only. Only de-identified data will be shared with those outside the study team to ensure adequate protection of sensitive data.

Study identifiers will be kept indefinitely.

To maintain participant confidentiality, no identifying information about any of the study participants will be published. Any data published (including demographic information about the study sample as a whole) will be in aggregate/summary form only.

Note also that Dr. Gary Ulaner of MSKCC and Dr. Aditya Bardia of the Circulating Tumor Cell Center at Massachusetts General Hospital, will only be exposed to deidentified data, samples, and images. As such they would not be considered "engaged" in human subjects research. As a result, they do not require inclusion on the IRB per our consultation with the Emory IRB.

Incidental Findings

a. All incidental findings noted by the imager will be discussed with the attending physician who is a co-investigator and documented in the electronic medical record.
b. The oncologist will educate the patient about the nature of the incidental finding, how to seek care from a clinician or specialist, obtaining health insurance to secure treatment, and/or referral to a clinical specialist, if one is required.
c. Language to this effect is present in the consent form.

PET findings will be used for research purposes within the context of the clinical trial as noted above. All incidental findings will be recorded in the clinical research form and emergent incidental findings will be communicated to the patient's physician who is also an investigator on this study.

Compensation for time and effort: Each patient will be compensated $50 as per diem for travel expenses per scan (total of $100 for both scans).

14. References

1. Dossus, L. and P.R. Benusiglio, *Lobular breast cancer: incidence and genetic and non-genetic risk factors.* Breast Cancer Res, 2015. **17**: p. 37.
2. He, H., et al., *Distant metastatic disease manifestations in infiltrating lobular carcinoma of the breast.* AJR Am J Roentgenol, 2014. **202**(5): p. 1140-8.
3. Ulaner, G.A. and D.M. Schuster, *Amino Acid Metabolism as a Target for Breast Cancer Imaging.* PET Clin, 2018. **13**(3): p. 437-444.
4. Ulaner, G.A., et al., *Prospective Clinical Trial of (18)F-Fluciclovine PET/CT for Determining the Response to Neoadjuvant Therapy in Invasive Ductal and Invasive Lobular Breast Cancers.* J Nucl Med, 2017. **58**(7): p. 1037-1042.
5. Tade, F.I., et al., *Anti-3-18F-FACBC (18F-Fluciclovine) PET/CT of Breast Cancer: An Exploratory Study.* J Nucl Med, 2016. **57**(9): p. 1357-63.
6. Ulaner, G.A., et al., *Initial Results of a Prospective Clinical Trial of 18F-Fluciclovine PET/CT in Newly Diagnosed Invasive Ductal and Invasive Lobular Breast Cancers.* J Nucl Med, 2016. **57**(9): p. 1350-6.
7. Wernicke, A.G., et al., *Prostate-specific membrane antigen expression in tumor-associated vasculature of breast cancers.* APMIS, 2014. **122**(6): p. 482-9.
8. Salas Fragomeni, R.A., et al., *Imaging of Nonprostate Cancers Using PSMA-Targeted Radiotracers: Rationale, Current State of the Field, and a Call to Arms.* J Nucl Med, 2018. **59**(6): p. 871-877.
9. Kasoha, M., et al., *Prostate-specific membrane antigen (PSMA) expression in breast cancer and its metastases.* Clin Exp Metastasis, 2017. **34**(8): p. 479-490.
10. Sathekge, M., et al., *(68)Ga-PSMA-HBED-CC PET imaging in breast carcinoma patients.* Eur J Nucl Med Mol Imaging, 2017. **44**(4): p. 689-694.
11. Tolkach, Y., et al., *Prostate-specific membrane antigen in breast cancer: a comprehensive evaluation of expression and a case report of radionuclide therapy.* Breast Cancer Res Treat, 2018. **169**(3): p. 447-455.
12. Desmedt, C., et al., *Genomic Characterization of Primary Invasive Lobular Breast Cancer.* J Clin Oncol, 2016. **34**(16): p. 1872-81.
13. Jager, P.L., et al., *Radiolabeled amino acids: basic aspects and clinical applications in oncology.* J Nucl Med, 2001. **42**(3): p. 432-45.
14. Shennan, D.B. and J. Thomson, *Inhibition of system L (LAT1/CD98hc) reduces the growth of cultured human breast cancer cells.* Oncol Rep, 2008. **20**(4): p. 885-9.
15. Wang, K., et al., *Activation of SNAT1/SLC38A1 in human breast cancer: correlation with p-Akt overexpression.* BMC Cancer, 2013. **13**: p. 343.
16. Liang, Z., et al., *Potential Biomarker of L-type Amino Acid Transporter 1 in Breast Cancer Progression.* Nucl Med Mol Imaging, 2011. **45**(2): p. 93-102.
17. Shennan, D.B., et al., *L-leucine transport in human breast cancer cells (MCF-7 and MDA-MB-231): kinetics, regulation by estrogen and molecular identity of the transporter.* Biochim Biophys Acta, 2004. **1664**(2): p. 206-16.
18. Shennan, D.B., et al., *Functional and molecular characteristics of system L in human breast cancer cells.* Biochim Biophys Acta, 2003. **1611**(1-2): p. 81-90.
19. Schuster, D.M., et al., *Anti-1-amino-3-18F-fluorocyclobutane-1-carboxylic acid: physiologic uptake patterns, incidental findings, and variants that may simulate disease.* J Nucl Med, 2014. **55**(12): p. 1986-92.
20. Kaittanis, C., et al., *Prostate-specific membrane antigen cleavage of vitamin B9 stimulates oncogenic signaling through metabotropic glutamate receptors.* J Exp Med, 2018. **215**(1): p. 159-175.
21. Yadav, M.P., et al., *Radioligand Therapy With (177)Lu-PSMA for Metastatic Castration-Resistant Prostate Cancer: A Systematic Review and Meta-Analysis.* AJR Am J Roentgenol, 2019. **213**(2): p. 275-285.

22. Glunde, K., et al., *Molecular and functional imaging of breast cancer.* NMR Biomed, 2009. **22**(1): p. 92-103.
23. Gore, J.C., et al., *Magnetic resonance in the era of molecular imaging of cancer.* Magn Reson Imaging, 2011. **29**(5): p. 587-600.
24. Ferlicot, S., et al., *Wide metastatic spreading in infiltrating lobular carcinoma of the breast.* Eur J Cancer, 2004. **40**(3): p. 336-41.
25. Groheux, D., et al., *Performance of FDG PET/CT in the clinical management of breast cancer.* Radiology, 2013. **266**(2): p. 388-405.
26. Hogan, M.P., et al., *Comparison of 18F-FDG PET/CT for Systemic Staging of Newly Diagnosed Invasive Lobular Carcinoma Versus Invasive Ductal Carcinoma.* J Nucl Med, 2015. **56**(11): p. 1674-80.
27. O'Connor, M., D. Rhodes, and C. Hruska, *Molecular breast imaging.* Expert Rev Anticancer Ther, 2009. **9**(8): p. 1073-80.
28. Schuster, D.M. and R.K. Halkar, *Molecular imaging in breast cancer.* Radiol Clin North Am, 2004. **42**(5): p. 885-908, vi-vii.
29. Plathow, C. and W.A. Weber, *Tumor cell metabolism imaging.* J Nucl Med, 2008. **49 Suppl 2**: p. 43S-63S.
30. Parent, E.E. and D.M. Schuster, *Update on (18)F-Fluciclovine PET for Prostate Cancer Imaging.* J Nucl Med, 2018. **59**(5): p. 733-739.
31. McConathy, J., *18F-Fluciclovine (FACBC) and Its Potential Use for Breast Cancer Imaging.* J Nucl Med, 2016. **57**(9): p. 1329-30.
32. Chang, S.S., *Overview of prostate-specific membrane antigen.* Rev Urol, 2004. **6 Suppl 10**: p. S13-8.
33. Paoletti, C., et al., *Circulating Tumor Cell Clusters in Metastatic Breast Cancer Patients: a SWOG S0500 Translational Medicine Study.* Clin Cancer Res, 2019.
34. De Giorgi, U., et al., *Circulating tumor cells and bone metastases as detected by FDG-PET/CT in patients with metastatic breast cancer.* Ann Oncol, 2010. **21**(1): p. 33-9.
35. Wang, R., et al., *Cell-free circulating tumor DNA analysis for breast cancer and its clinical utilization as a biomarker.* Oncotarget, 2017. **8**(43): p. 75742-75755.
36. Catarino, R., et al., *Quantification of free circulating tumor DNA as a diagnostic marker for breast cancer.* DNA Cell Biol, 2008. **27**(8): p. 415-21.
37. Canzoniero, J.V. and B.H. Park, *Use of cell free DNA in breast oncology.* Biochim Biophys Acta, 2016. **1865**(2): p. 266-74.
38. Kodahl, A.R., et al., *Correlation between circulating cell-free PIK3CA tumor DNA levels and treatment response in patients with PIK3CA-mutated metastatic breast cancer.* Mol Oncol, 2018. **12**(6): p. 925-935.
39. Lupini, L., et al., *High-sensitivity assay for monitoring ESR1 mutations in circulating cell-free DNA of breast cancer patients receiving endocrine therapy.* Sci Rep, 2018. **8**(1): p. 4371.
40. O'Leary, B., et al., *Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer.* Nat Commun, 2018. **9**(1): p. 896.
41. Zanetti-Dallenbach, R.A., et al., *Levels of circulating cell-free serum DNA in benign and malignant breast lesions.* Int J Biol Markers, 2007. **22**(2): p. 95-9.
42. Fendler, W.P., et al., *(68)Ga-PSMA PET/CT: Joint EANM and SNMMI procedure guideline for prostate cancer imaging: version 1.0.* Eur J Nucl Med Mol Imaging, 2017. **44**(6): p. 1014-1024.
43. Cheng, J., et al., *Clinical Validation of a Cell-Free DNA Gene Panel.* J Mol Diagn, 2019. **21**(4): p. 632-645.

APPENDIX A Abbreviations and definition of terms

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
AE	Adverse event
Akt	Protein kinase B (or PKB)
CRFs	Case Report Forms
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Event
ctDNA	Circulating tumor DNA
DSMB	Data Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
DSMP	Data Safety and Monitoring Plan
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
ESR1	Estrogen Receptor alpha
FCBP	Female of childbearing potential
FDG	fluorodeoxyglucose
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
hCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability Accountability Act
ICF	Informed consent form
IDC	invasive ductal carcinoma
IHC	Immunohistochemistry
IL	Interleukin
ILC	Invasive lobular carcinoma

Abbreviation or special term	Explanation
IMC	invasive metaplastic carcinoma
IND	Investigational New Drug
IRB	Institutional Review Board
ISCR	individual single case report
IV	Intravenous
MBq	Megabecquerel
mCi	Millicurie
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NIH	National Institutes of Health
PET	Positron Emission Tomography
PI3Ks	Phosphoinositide 3-kinases
PSMA	Prostate specific membrane antigen
RNA	Ribonucleic acid
SAE	Serious adverse event
SUV	Standardized uptake value
TLA	Total lesion activity
VEGF	Vascular endothelial growth factor