Molecular Imaging and Precision Medicine in Breast Cancer

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Synopsis

Precision medicine, namely basing treatment approaches on patient traits and specific molecular features of disease processes, has an increasingly important role in the management of breast cancer patients as targeted therapies continue to improve. PET imaging offers noninvasive information that is complementary to traditional tissue biomarkers, including information about tumor burden, tumor metabolism, receptor status and proliferation. Several PET agents that image breast cancer receptors can visually demonstrate the extent and heterogeneity of receptor positive disease and can help predict which tumors are likely to respond to targeted treatments. Furthermore, PET agents can aid in drug development by demonstrating successful drug binding to target and early response of specific therapies. Molecular imaging may also provide a key indicator of outcome at later time points in breast cancer treatment. This review will present an overview of applications of PET imaging in the targeted treatment of breast cancer.

Key Words

Breast cancer, precision medicine, biomarkers, fluorodeoxyglucose (FDG), 16α - $[^{18}\text{F}]$ fluoro- 17β -estradiol (FES)

Key Points:

- Molecular imaging can serve as a biomarker in breast cancer and provide a complementary, non-invasive global evaluation of the disease process and information to guide treatment.

- Imaging of estrogen, progesterone, and human epidermal growth factor (HER2) receptors may help predict which patients are the most likely to endocrine or HER2 targeted therapy.
- PET imaging can be used in breast cancer drug development, drug dose optimization, and assessment of early response to therapy.
- Molecular imaging may also provide a key indicator of outcome at later time points in breast cancer treatment.

Introduction

Breast cancer is the most common malignancy in women, with an estimated 234,000 new cases diagnosed in 2015 and approximately 5-9% of patients with metastatic disease at presentation (1). Biomarkers have become increasingly important in breast cancer treatment for prognosis and for guiding treatment decisions (2). Precision medicine involves the use of biomarkers to create individualized and targeted treatments (2). In addition to traditional tissue-based biomarkers, molecular imaging can also serve as a biomarker and provides a complementary, non-invasive global evaluation of the disease process and can provide information to guide treatment, such as prognosis, drug pharmacodynamics and response to treatment.

Precision medicine in breast cancer has traditionally been based on results of tissue sampling and assay of the primary breast tumor and metastatic sites - for example, assaying the estrogen receptor (ER) to select patients who may benefit from endocrine therapy (3). However there are limitations of biopsy, such as sampling error and invasiveness. Biopsy of the primary neoplasm is not always representative of the entire tumor because of intratumor heterogeneity (4). Tissue sampling of metastatic lesions is performed when possible but certain metastatic sites, such as bone and brain, are not always amenable to biopsy and receptor status is often inferred from the primary tumor. However receptor status can vary from the primary tumor and metastatic lesions in 25-40% of patients (5). Even when biopsy of metastatic sites is possible, tissue sampling of metastatic lesions is not always representative of the entirety of the disease because of tumor heterogeneity (4). Monitoring response to targeted treatment to evaluate for changes in the tumor biology would require resampling of primary and metastatic lesions

and is invasive and often painful. Furthermore there are risks associated with biopsy, such as bleeding and infection.

Imaging of biomarkers through positron emission tomography/computed tomography (PET/CT) offers a complementary, non-invasive method to obtain biological information regarding breast cancer, including tumor burden, tumor metabolic activity, receptor status and proliferation index. This review focuses on the use of molecular imaging as a biomarker in breast cancer precision medicine, with a focus on: 1) imaging as a prognostic biomarker, 2) imaging as a predictive biomarker, 3) imaging to evaluate drug pharmacodynamics, 4) imaging to determine early response to therapy, and 5) imaging to predict biological response (**Table 1**). Through example of the use of molecular imaging to accomplish each of these tasks, we will highlight several different PET radiopharmaceuticals used either clinically or in clinical trials and describe recent clinical studies that demonstrate the impact and promising future role of imaging in precision medicine.

Prognostic Factors and Breast Cancer

Prognostic factors help to distinguish which tumors are most likely to progress to tumor spread and death. Some examples of prognostic factors in breast cancer are ER, PR, and HER2 receptor status, proliferation index, and Oncotype DX 21-gene panel (6–8). Most of these prognostic indicators have traditionally been evaluated through tissue assay, typically via immunohistochemistry. An important prognostic factor for breast cancer is the size of the tumor and its extent of spread – i.e., staging. As such, imaging serves to help assess breast cancer prognosis by determining the stage of breast cancer.

The National Comprehensive Cancer Network (NCCN) guidelines currently lists fluorodeoxyglucose (FDG)-PET/CT as a consideration in staging clinical stage III or higher breast cancer (9). FDG-PET/CT has been shown as a tool for prognostic stratification through staging (10,11). Cochet et al prospectively evaluated 142 patients with at least grade T2 tumor, comparing conventional imaging against FDG-PET/CT and found FDG-PET/CT provided stronger prognostic stratification of progression free survival compared to conventional imaging ($p < 0.0001$) and FDG-PET/CT was an independent predictor of recurrence or progression ($p < 0.001$) (11). Groheux et al showed in a prospective study of 189 patients with clinical stage IIB disease, 47 patients with M1 disease discovered on FDG-PET/CT had statistically significant shorter three-year disease specific survival, 57% vs 88% ($p < .001$) (10). In this same group of patients, M1-disease and triple-negative phenotypes were found to be two statistically significant independent prognostic variables (10).

Additionally, several studies have also demonstrated that the level of FDG uptake in the primary breast tumor, typically measured as the standardized uptake value (SUV), and in metastases serves as a prognostic factor itself and is indicative of histological grade, triple negative status, and high proliferation index (Ki-67 expression) (11–16). Although FDG-PET/CT is used for diagnosis and staging, the intensity of FDG uptake on PET/CT in the primary tumor and in metastatic lesions also serves as an important prognostic factor (11–14). As an analog of glucose, FDG uptake is a measures glycolytic rate. Studies using FDG-PET/CT have shown that elevated glycolysis is associated with a number of aggressive cancer features, such as proliferation and enhanced survival (17). High FDG uptake in the primary breast tumor has been associated with poor prognostic

factors including high histological grade, triple negative status, or p53 mutation (17,18). In the above-mentioned study, Cochet et al found that higher FDG uptake was associated with aggressive features, with a 3:1 ratio of baseline FDG uptake between triple negative and luminal A tumors (11). Koolen et al found in 203 primary stage II or III breast cancer patients evaluated with FDG-PET/CT, a higher SUV value was associated with aggressive features, such as distant metastases at time of staging, triple negative tumors, and grade 3 tumors (12).

Tumor as estimated by tissue sampling and the by Ki-67 immunochemistry index also provides prognostic information. Several studies have demonstrated a correlation between high proliferation index (Ki-67 expression) and higher FDG uptake (12,15). Furthermore, invasive breast tumors with higher grades demonstrated higher FDG uptake compared to lower grade tumors (14,15). Triple negative breast cancer patients typically have higher FDG avidity (14,16).

Precision Medicine and Predictive Factors

Predictive factors indicate whether or not a specific treatment is likely to be beneficial to the patient, for example, whether a patient may benefit from endocrine therapy.

Predictive biomarkers such as ER, PR, and HER2 based upon tissue assays are widely used to direct breast cancer systemic therapy (4). However the results may not be reflective of the entire burden of disease due to intratumoral and metastatic heterogeneity. Imaging biomarkers offer an approach that is complementary to tissue sampling to help guide treatment decisions.

Approximately 70% of breast cancers express estrogen receptors and treatment with endocrine therapy has been one of the key factors improving breast cancer mortality. Endocrine therapy is considered the preferred first line treatment and is the most effective treatment for metastatic ER+ breast cancer, but only 50 to 75% of ER+ breast cancer patients will respond to first line endocrine therapy (19). Beyond first line therapy, response to endocrine therapy decreases to 25% due to resistance through various mechanisms (19).

The PET radiopharmaceutical 16α -[^{18}F]fluoro-17 β -estradiol (FES), an estradiol analog, is the most researched radioligand for imaging the estrogen receptor. Measurement of SUV on FES-PET/CT has been shown to correlate with ER expression when compared to immunohistochemistry (20,21). Several studies have addressed FES as a predictive biomarker. Prior FES studies have shown that FES-PET/CT, using both qualitative and quantitative measures can identify which patients are most likely to benefit from endocrine therapy (20,22–24). For example, Dehdashti et al found in 11 patients, a baseline FES SUV in responders was ≥ 2.2 in patients who responded to Tamoxifen therapy at 2 months and ≤ 1.7 in the nonresponders (22). Mortimer et al found in 40 ER+ breast cancer patients images with FES-PET/CT and FDG-PET/CT before and 7-10 days after Tamoxifen therapy, baseline SUV of FES uptake in responders was 4.3 ± 2.4 compared to non-responders with SUV of 1.8 ± 1.3 ; $p = 0.0007$ (25). Linden et al found in 47 pretreated patients with ER+ tumors, 0 of 15 patients with baseline SUV < 1.5 responded to endocrine therapy versus 11 out of 32 patients with SUV > 1.5 ($p < 0.01$) (**Figure 1**) (24). In assessing studies published across heterogeneous populations, the FES SUV threshold of 1.5 retained predictive value, but with imperfect sensitivity or

specificity for predicting response endocrine to therapy (26). Further studies are necessary to determine the sensitivity and specificity of baseline FES-PET/CT SUV value to predict response to endocrine therapy and identify optimal thresholds. This question is actively being addressed in a phase II trial enrolling patients with ER+ metastatic breast cancer receiving first line endocrine therapy (NCT02398773) (27).

Progesterone receptor, an estrogen-regulated gene, also serves as a predictive biomarker and is routinely evaluated in immunohistochemical assays. The presence of expression of PR with expression of ER increases the likelihood to respond to endocrine therapy (7). 21-(¹⁸F)-fluoro-16 α ,17 α -[(R)-(1'- α -furylmethylidene)dioxy]-19-norpregn-4-ene-3,20-dione (FFNP) is the radioligand with high affinity and selectivity for the PR and shows the most promise for PR imaging (28,29). Preclinical breast models have demonstrated decreased uptake of FFNP predicts tumors that will respond to fulvestrant and estrogen-deprivation therapy. (30). Although less research has been performed on FFNP-PET/CT as a predictive biomarker, a current clinical trial (NCT02455453) is addressing this topic (31). In this trial, FFNP-PET/CT scans are being performed in ER+ postmenopausal breast cancer patients before and after the administration of estradiol for one day (estrogen challenge) to determine look at changes in in FFNP maximum SUV measurement.

Human epidermal growth factor receptor type 2 (HER2) is overexpressed in approximately 15-25% of invasive breast cancer and is associated with aggressive disease (32-35). HER2 is routinely assayed through immunohistochemistry or fluorescence in situ hybridization to determine which patients may benefit from HER2 directed therapy. However an estimated 50% of patients with overexpression of HER2

breast cancer do not respond to HER2 directed therapy (36). The Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (NEO-ALTTO) study enrolled 455 women with HER-2 positive breast cancer, of which 77 patients underwent serial FDG-PET/CTs and found that early FDG-PET/CT was able to identify patients more likely to have complete response to neoadjuvant trastuzumab and/or lapatinib with paclitaxel (37).

Directly imaging HER2 disease has been studied to visualize the tumor burden of HER2+ disease (38). Several imaging agents have been created to bind to the HER2 receptor and examples of the positron emitting radionuclides are ^{64}Cu -trastuzumab, ^{64}Cu -DOTA- $\text{Z}_{\text{HER2}:477}$, ^{68}Ga -trastuzumab F(ab' b₂ fragments, ^{68}Ga -ABY-002, and ^{89}Zr -trastuzumab (39,40). The ZEPHIR study was the first large prospective trial evaluating ^{89}Zr -trastuzumab-PET/CT (HER2-PET/CT) as a predictive biomarker for advanced HER2+ breast cancer patients for response to Trastuzumab emtansine (T-DM1), an antibody-drug conjugate to target HER2 receptor in patients who progress after prior line of trastuzumab-based therapy (**Figure 2**) (41). They found in 56 patients with advanced HER2 positive breast cancer, pretreatment imaging with HER2-PET/CT and imaging with FDG-PET/CT after 1 month demonstrated a high negative predictive value when compared to RECIST1.1 (88%) (41). There are several prospective clinical trials underway to evaluate the clinical utility of HER2 molecular imaging, including 1 study at Institut Jules Bordet (NCT01420146) using ^{89}Zr -Trastuzumab in HER2+ metastatic breast cancer correlated to FDG-PET/CT and two studies at the University Medical Center Groningen (NCT01832051 and NCT01957332) (42–44).

Using molecular imaging to evaluate drug binding to target

Molecular imaging can be used as a tool to evaluate binding of drug to target in the development of new drugs. For example, FES-PET/CT has been shown as an effective tool to assess endocrine therapy on ER binding (45). A retrospective study of 30 patients with metastatic breast cancer (predominately bone dominant) measured FES uptake before and after endocrine-targeted treatments, including aromatase inhibitors, tamoxifen, and fulvestrant (45). They found that estrogen blocking therapies (tamoxifen, fulvestrant) decreased FES binding over serial PET/CTs compared to aromatase inhibitors, demonstrating that FES-PET/CT might be a valuable clinical tool to visualize activity of endocrine therapy (45). The authors also noted differences in the ER blockade between the ER blocking agents tamoxifen and fulvestrant and hypothesized that the incomplete ER blockade in patients receiving fulvestrant was due to inadequate dosing of this medication (loading dose of 500 mg followed by 250mg at 2 weeks x 2) (45). This hypothesis was further supported by clinical studies showing increased efficacy of fulvestrant at higher doses (46,47). A prospective study studied the efficacy of a higher dose of fulvestrant (500 mg) to determine if this dose was sufficient for complete ER blockade using FES-PET/CT (48). 38% of patients treated with fulvestrant (6 out of 16) were found to residual FES uptake, which was associated with early clinical disease progression (48). These studies demonstrate that FES-PET/CT can be used for determining optimal ER blockade for drug development and optimization of therapeutic dose(49).

Another example of using FES-PET/CT in the evaluation of new drug development is evaluating ARN-810, a newer endocrine directed therapy to treat advanced ER+ breast cancer by blocking and degrading ER (50,51). A recent multicenter, Phase I clinical trial

evaluated targeting of ER by ARN-810 in 30 patients with advanced or metastatic ER+ breast cancer. Baseline and subsequent FES PET scans after initiation of ARN-810 demonstrated come to near complete (>90%) suppression of FES uptake to background levels, indicating successful drug target binding (50).

HER2 imaging agents have been used in the evaluation of drug binding of HER2 directed therapies (8). For example, ^{89}Zr -trastuzumab and ^{89}Zr -bevacizumab PET scans were used for quantitative assessment in the evaluation of NVP-AUY922, a HER2 targeted therapy that inhibits HSP90, a molecular chaperone with client proteins that play a role in metastatic breast cancer through HER2, hypoxia-inducible factor-1 α , and ER (52). ^{89}Zr -trastuzumab PET was found to positively correlate to tumor size after 3 weeks of HSP90 inhibitor treatment, indicating that PET probes such as ^{89}Zr -trastuzumab may be used for evaluation of HER2 targeted therapies. (52).

Assessment of early response (pharmacodynamics)

A pharmacodynamic response indicates some action of the drug on its target and provides an indication of efficacy in targeted cancer therapy. A pharmacodynamic response can indicate some likelihood of subsequent response to therapy, and perhaps more importantly, a lack of a pharmacodynamic response often indicates little chance of therapeutic success. Pharmacodynamics may also be used to determine the optimal dosing for therapy (6). Clinically, it may be difficult to infer the early impact of many targeted drugs, particularly those with a cytostatic rather than cytotoxic effect.

One example of an early pharmacodynamic effect can be seen in ER-targeted drugs with an agonist action. A “clinical flare reaction” is a clinical response in patients 2 weeks

after initiating endocrine therapy with drugs with transient agonist effect, such as tamoxifen, characterized by pain in osseous metastases and increase in size of soft tissue metastases and is predictive of response to endocrine treatment (25). However this response is not always seen or recognized and is furthermore difficult to differentiate from disease progression clinically when seen at later time points (23). Several studies have demonstrated that a metabolic flare reaction may be detected through FDG-PET/CT imaging, which is predictive of response to endocrine therapy (23,25,53). For example, Mortimer et al studied FDG-PET/CT before and after treatment with tamoxifen in postmenopausal ER+ breast cancer patients and found an increase in tumor FDG uptake after tamoxifen in 20 out of 21 responders versus no significant change in tumor FDG uptake in the 19 nonresponders ($p = 0.0002$) (25). A clinical flare reaction was seen in only 5 out of the 21 responders (25). Similarly, Dehadasti et al found that a metabolic flare reaction induced by an estradiol challenge could be detected as a significantly higher mean percent change in SUV for responders compared to non-responders on FDG-PET/CT, and patients with a metabolic flare who were subsequently treated with endocrine therapy had significantly longer overall survival compared to those without a metabolic flare (23). To further expand on these results, Kurland et al studied the metabolic flare reaction in patients treated with aromatase inhibitors, which decrease circulating estradiol levels, with correlation to proliferation index (Ki-67) assays (53). They found a decrease in FDG SUV values over a two week course of aromatase inhibitor therapy, which corresponded to the lower post-treatment Ki-67 (53). These studies demonstrate that the presence of a metabolic flare response indicates which patients are more likely to respond to endocrine treatment.

Tumor proliferation is most commonly performed by measuring Ki-67 labeling index through immunohistochemistry (54). 3'-deoxy-3'-¹⁸F-fluorothymidine (FLT), a thymidine analog, is the most widely used proliferation probe that is dependent on the activity of thymidine kinase-1 (TK-1) and correlates with Ki-67 expression (55–57). An early decline in cellular proliferation assayed by Ki-67 in a serial tissue biopsy has been shown to provide an early indication of successful therapy for both chemotherapy and endocrine therapy, as soon as 1-2 weeks after starting treatment (58,59). Similar findings have been seen using serial FLT-PET/CTs to measure early response to therapy in breast and other cancers (60,61). A recent multi-center trial performed in the US has confirmed their early single-center findings (**Figure 3**) (62).

FLT PET/CT can also be used to evaluate the pharmacodynamics of drugs impacting components of the thymidine metabolic pathway. For example, *thymidylate synthase (TS)* inhibitor drugs, such as capecitabine, inhibit the de novo thymidine synthesis pathway and have their greatest impact in tumors that rely on this pathway, versus the external (salvage) pathway traced by FLT (63). Interruption of the de novo pathway by *TS*-targeted drugs can cause a transient increase in thymidine flux through the external pathway, which can be detected by PET proliferation imaging. In a novel study, tumor FLT uptake was found to be increased in patients after treatment with capecitabine, indicating a transient increase in external pathway thymidine flux with *TS* inhibition measured by imaging, and a potential method to measure the pharmacodynamics of thymidylate synthase inhibitors (63).

Another target for imaging proliferation is the sigma-2 receptor, a biomarker of tumor cells that is overexpressed in proliferating tumors (64–66). The most promising

sigma-2 receptor radioligand is N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-(¹⁸F-fluoroethoxy)-5-methylbenzamide (¹⁸F-ISO-1), which has been found to correlate with laboratory measures of proliferation status in mouse mammary tumor model and with Ki-67 expression in 13 breast cancer patients (67,68). This imaging probe shows promise as a predictive biomarker and may be complementary to FLT-PET/CT in the evaluation of cell cycle-targeted therapies, such as selective cyclin dependent kinase inhibitors (69).

Many other PET radiotracers have been studied in breast cancer drug pharmacodynamics, including ¹¹[C]choline and L-methyl-¹¹C-methionine (¹¹C-MET) (54). ¹¹[C]choline has greater uptake in tumor cells due to increased intracellular choline kinase activity in tumor cells (54). ¹¹[C]choline PET uptake decreased over serial scans in patients treated with trastuzumab, indicating that this radiotracer can be used for monitoring response to therapy (70). L-methyl-¹¹C-methionine (¹¹C-MET) is an amino acid used in PET, and a small number of cases were found to have decreased ¹¹C-MET uptake after treatment with endocrine therapy or chemotherapy (71–73). Numerous others have a potential important role in the development and optimization of targeted therapies (7,54).

Biological response: How does imaging based response predict outcome?

In addition to measuring early response to treatment, molecular imaging may also provide a key indicator of outcome at later time points in breast cancer treatment. Molecular imaging may provide information complementary to size-based measures of response, particularly for disease sites that are difficult to evaluate by anatomic imaging.

One primary example of using PET imaging to predict outcome is in bone dominant breast cancer, a site of disease that has been difficult to evaluate by standard imaging **(Figure 4)** (74). In bone dominant metastatic breast cancer, FDG-PET/CT has been demonstrated to demonstrate overall time to progression and overall survival (75–78). In a retrospective study of 253 patients with metastatic breast cancer, there was a strong correlation of maximum SUV of bone metastases on baseline FDG-PET/CT and overall survival (75). Although not statistically significant, the presence of liver, nodal and pulmonary metastases were found to have a greater risk of death (75). A retrospective study of 28 patients undergoing treatment demonstrated that changes in serial FDG-PET/CT scans may predict time-to-progression and time to first skeletal-related event in bone-dominant metastatic breast cancer (76). A retrospective study of 102 women with metastatic breast cancer, a decrease in metabolic activity and increase in CT attenuation of osseous metastases after treatment was found as an independent predictor of response duration (77). A retrospective study of 35 patients with 146 identified osseous lesions, FDG uptake was found to correlate with tumor activity independent of the morphologic characteristics (78). Prospective studies are ongoing to confirm these retrospective studies.

Molecular imaging can also provide a robust indication of the impact of therapy in the neo-adjuvant setting, and measures of molecular imaging probes post-therapy have been shown to predict subsequent relapse and survival in a number of settings. Emmering and colleagues showed that the presence or absence of uptake post neoadjuvant chemotherapy predicted the likelihood of relapse (79), while Dunnwald showed that the change in quantitative FDG uptake measures mid-way through therapy

predicted relapse-free and overall survival (80). Another study by Dunnwald et al (81) also indicated that measures of blood flow from [^{15}O]-water PET predicted disease-free and overall survival, providing incremental value to established prognostic factors, echoing earlier work showing the ability of serial imaging with [$^{99\text{m}}\text{Tc}$]-sestamibi to predict similar measures (82). These examples indicate the ability of molecular imaging to provide biologically-based measures of response that are highly predictive of key downstream outcomes such as relapse and survival.

Conclusion

The role of PET in breast cancer continues to evolve with broader applications in diagnostics and therapeutics in metastatic breast cancer patients. As precision medicine leads to the development of further targeted therapies, PET imaging with new radiotracers provides a complimentary and noninvasive method for prognostic and predictive information about tumor burden, tumor metabolic activity, receptor status and proliferation index. Furthermore, with the development and evolution of targeted treatments, PET imaging offers a method to assess drug binding to the target and optimization of drug dosage and has the potential role for an increasingly important tool in clinical trials. As we have reviewed, molecular imaging has shown an important role in the assessment of breast cancer tumor biology, disease burden, and drug development and offers great promise in the future of breast cancer management.

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Table 1: Sample applications of molecular imaging in breast cancer

	Definition	Sample Applications
Prognostic Factors	Indicate which tumors are most likely to progress to tumor spread and death	-FDG-PET/CT for prognostic stratification through staging (10,11) -FDG-PET/CT SUV measurement in primary tumor for histological grade, triple negative status, proliferation index (11–16)
Predictive Factors	Whether or not treatment is likely to benefit the patient.	-FES-PET/CT for evaluation of ER expression to predict response to endocrine therapy (20,22–26) -FFNP-PET/CT for evaluation of PR expression to predict response to endocrine therapy (28–30) - ⁸⁹ Zr-trastuzumab-PET/CT for HER2 imaging to predict response to trastuzumab-based therapy (41)
Drug Binding to Target	Evaluate binding of drug to target in the development of new drugs.	-FES-PET/CT for evaluation of aromatase inhibitors, tamoxifen, and fulvestrant (45) -FES-PET/CT for optimizing dose of fulvestrant (48)

		<p>-FES-PET/CT in the evaluation of ARN-810 (50)</p> <p>-⁸⁹Zr-trastuzumab-PET/CT in the evaluation of HSP90 inhibitors (52)</p>
Assessment of Early Response	Indicates some action of the drug on its target and provides an indication of efficacy in targeted cancer therapy	<p>-FDG-PET/CT showing a “metabolic flare reaction” after treatment with tamoxifen or with estradiol challenge (23,25,53)</p> <p>-FLT-PET/CT for response to chemotherapy and endocrine therapy (58,59)</p>
Biological Response	Indicator of outcome at later time points in treatment	<p>-FDG-PET/CT to predict outcome is in bone dominant breast cancer (75–78)</p> <p>-FDG-PET/CT in the neo-adjuvant setting and post-therapy to predict subsequent relapse and survival (79–82)</p>

Figure 1: FES- and FDG- PET/CT in two patients demonstrating FES as a predictive marker for response to endocrine therapy. Row A demonstrates a patient with multiple FES- and

FDG-avid osseous metastases and post-treatment FDG scan demonstrates a favorable response at 6 months. Row B demonstrates non-FES avid osseous metastases which are FDG avid, but does not demonstrate a favorable response to endocrine therapy with progression of hypermetabolic osseous metastases noted on FDG post-treatment scan at 6 months. From Linden HM, Stekhova SA, Link JM, et al. Quantitative fluoroestradiol positron emission tomography imaging predicts response to endocrine treatment in breast cancer. *J Clin Oncol*. 2006;24:2793–2799, with permission.

Figure 2: FDG-PET/CT compared to ^{89}Zr -trastuzumab-PET/CT (HER2-PET/CT) MIP images in HER2+ breast cancer patients. (A) Example of similar distribution of metastases on FDG PET and HER2 PET. (B) The dominant portions of the tumor demonstrate uptake on HER2 PET noting that many of the lung lesions are not seen. (C) The majority of the tumor burden does not demonstrate uptake on HER2-PET/CT. (D) None of the tumor demonstrated uptake on the HER2-PET/CT. From Gebhart G, Lamberts LE, Wimana Z, et al. Molecular imaging as a tool to investigate heterogeneity of advanced HER2-positive breast cancer and to predict patient outcome under trastuzumab emtansine (T-DM1): the ZEPHIR trial. *Ann Oncol*. 2016;27:619–624, with permission.

Figure 3: FLT-PET/CT in a patient undergoing neoadjuvant treatment. The pretherapy scan in column FLT1 (left) demonstrates FLT uptake in primary mass in the left breast and left axillary lymph node. Column FLT2 (middle) demonstrates decreased tracer uptake after one cycle of neoadjuvant chemotherapy and column FLT3 (right) demonstrates complete metabolic response after completion of neoadjuvant chemotherapy. Pathologic

complete response was confirmed surgically. From Kostakoglu L, Duan F, Idowu MO, et al. A Phase II Study of 3'-Deoxy-3'-18F-Fluorothymidine PET in the Assessment of Early Response of Breast Cancer to Neoadjuvant Chemotherapy: Results from ACRIN 6688. *J Nucl Med*. 2015;56(11):1681-1689, with permission.

Figure 4: FDG-PET/CT used for assessing response in two patients with osseous dominant metastatic breast cancer. MIP whole body FDG-PET on top row and fused FDG-PET/CT on bottom row. Panel (A) demonstrating patient on tamoxifen without definite osseous metastases. Follow-up FDG-PET/CT performed 8 months later after switching to aromatase inhibitor (Letrozole) demonstrates new foci of FDG osseous uptake, reflecting progressive metabolic disease (arrows). Panel (B) demonstrating patient with ER+PR+/HER2- metastatic breast cancer with innumerable osseous metastases throughout axial and appendicular skeleton (arrows). Follow-up FDG-PET/CT performed after switching treatment to Capecitabine demonstrates significant partial metabolic response with near complete resolution of the majority of osseous lesions with a few scattered foci of FDG uptake reflecting residual disease (arrow).

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