

REVIEW

Advances in molecular imaging for breast cancer detection and characterization

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Abstract

Advances in our ability to assay molecular processes, including gene expression, protein expression, and molecular and cellular biochemistry, have fueled advances in our understanding of breast cancer biology and have led to the identification of new treatments for patients with breast cancer. The ability to measure biologic processes without perturbing them *in vivo* allows the opportunity to better characterize tumor biology and to assess how biologic and cytotoxic therapies alter critical pathways of tumor response and resistance. By accurately characterizing tumor properties and biologic processes, molecular imaging plays an increasing role in breast cancer science, clinical care in diagnosis and staging, assessment of therapeutic targets, and evaluation of responses to therapies. This review describes the current role and potential of molecular imaging modalities for detection and characterization of breast cancer and focuses primarily on radionuclide-based methods.

Introduction

Progress in the ability to assay molecular processes, including gene expression, protein expression, and molecular and cellular biochemistry, has fueled advances in our understanding of breast cancer biology and has led to the identification of new treatments for patients with breast cancer. The ability to measure biologic processes without perturbing them *in vivo* by using advanced imaging methods provides the opportunity to better characterize tumor biology and to assess how biologic and cytotoxic therapies alter critical pathways of tumor response and resistance. Traditionally, imaging has relied

on structural and anatomic features to detect breast cancer and determine its extent (that is, anatomic imaging). By contrast, molecular imaging modalities allow for imaging of regional biochemistry and molecular biology. Molecular imaging further provides information complementary to that obtained by traditional, tissue-based assay methods. By accurately characterizing tumor properties and biologic processes, molecular imaging plays a pivotal role in breast cancer science and clinical care in diagnosis and staging, assessment of therapeutic targets, and evaluation of responses to therapies [1]. This review describes the current role and potential of molecular imaging modalities for detection and characterization of breast cancer and focuses specifically on radionuclide imaging techniques.

Overview of molecular imaging methods applied to breast cancer

Most imaging modalities used in clinical practice are largely anatomic in nature, using tissue features such as size, shape, and density to identify breast cancer. Anatomic imaging modalities commonly used for detecting both primary breast cancer and metastatic breast cancer (MBC) include mammography, x-ray computed tomography (CT), ultrasound, and magnetic resonance imaging (MRI). Alternatively, molecular imaging measures regional *in vivo* biochemical, cellular, and molecular properties of tumors and normal tissues. By targeting underlying molecular processes, molecular imaging modalities can image biologic processes specific to cancer and this may aid in cancer detection and characterization and complement traditional anatomic imaging methods. Table 1 summarizes current molecular imaging modalities that have been used in clinical practice and in human research settings applied to breast cancer. In this review, we focus primarily on radionuclide-based molecular imaging methods but briefly mention applications of other molecular imaging modalities.

Radionuclide imaging relies on the use of imaging probes tagged with radioactive nuclei [2]. Position-sensitive radiation detectors identify emitted photons and generate images of regional radiopharmaceutical concentration. Radionuclide imaging can be performed

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Table 1. Molecular imaging for breast cancer

Modality	Indication	Advantages	Disadvantages
Radionuclide imaging			
Positron emission tomography	Detection Response evaluation Tumor characterization	Wide range of molecular imaging probes Tracer imaging without perturbing biologic system	Limited spatial resolution (improved with use of non-contrast computed tomography) Some radiation exposure
Positron emission mammography	Detection Tumor characterization	More sensitive for smaller tumors Higher spatial resolution	Increased radiation dose Visualization of posterior lesions Variable uptake of ¹⁸ F-fluorodeoxyglucose (FDG) in small and less metabolically active tumors
Breast-specific gamma imaging	Detection	More sensitive for smaller tumors Heavy compression of breast tissue not required	Associated with radiation exposure Best combined with anatomic imaging (mammography) for optimal screening Longer imaging time Some radiation exposure
Magnetic resonance			
Magnetic resonance imaging (MRI), especially dynamic contrast-enhanced MRI and targeted contrast agents	Tumor characterization	Quantification of tumor perfusion and tumor capillary permeability	Confined space Contrast design limited by need for magnetic atom
Magnetic resonance spectroscopy	Tumor characterization	Can measure wide range of molecules No contrast necessary	Limited spatial resolution Challenging to obtain high-quality spectra in routine imaging
Ultrasound, especially with contrast enhancement	Detection Tumor characterization	Highly portable, inexpensive Molecular microbubble agents possible	Operator dependence Contrast agents confined to vascular space thus far
Optical imaging	Tumor characterization	Inexpensive, highly portable, and does not necessarily require a contrast agent	Limited depth penetration, challenging spatial localization, and operator dependence

by using single-photon-emitting isotopes and is termed single-photon emission CT (SPECT), in which images are collected and reconstructed as tomographic images. The most commonly used single-photon radiopharmaceutical used for breast imaging is ^{99m}Tc-sestamibi (MIBI). More recently, high-resolution, small-field-of-view γ cameras specific to breast imaging, sometimes called breast-specific γ imaging (BSGI) or molecular breast imaging, have been developed [3,4].

Another important class of radionuclide imaging procedures uses positron-emitting isotopes and is termed positron emission tomography (PET). Compared with SPECT, PET offers the potential for better spatial resolution, a more accurate image quantification, and a wide range of possible imaging probes. PET has proven to be a very useful tool in the staging of advanced breast cancer, in assessing response to therapy, and is widely used in clinical care in the form of PET-CT. Although a wide range of radiopharmaceutical tracers have been developed for use with PET, most breast cancer imaging to date has been done with ¹⁸F-fluorodeoxyglucose (FDG) [5]. FDG is a glucose analog that is transported via glucose transporters into the cells, where it is phosphorylated by hexokinase in proportion to the rate of glucose phosphorylation. Further catabolism of FDG is

not possible, because it lacks a hydroxyl group at the C-2 position. FDG becomes 'metabolically trapped' in tumor cells at a rate proportional to glucose utilization. FDG PET therefore provides an effective way to measure glucose metabolism. Most PET imaging is performed by using devices designed for torso imaging; however, dedicated devices designed specifically for positron emission of the breast, termed positron emission mammography (PEM), represent a promising breast imaging modality [6].

Of other modalities used for molecular imaging, MRI is the most widely used in current breast cancer practice [7]. MRI relies upon the interaction of atomic nuclei with radiofrequency signals in the presence of strong magnetic fields and can generate high-resolution, three-dimensional images with excellent soft tissue contrast. In current clinical practice, MRI is most often used for anatomic imaging; however, with a more detailed analysis of contrast enhancement kinetics or the use of contrast agents that are more molecularly targeted or both, MRI can be used to measure physiologic and molecular properties [8-11]. Magnetic resonance methods can also be used to measure the regional concentration of specific biochemical species (for example, products of metabolism or membrane lipids, often termed magnetic resonance

spectroscopy (MRS) or magnetic resonance spectroscopic imaging) [12]. For example, increased levels of choline in breast cancer versus normal breast tissue can be measured by MRS, and changes in choline levels with treatment can provide an early indication of therapeutic efficacy [13].

Other molecular imaging modalities that have been tested in breast cancer include optical imaging and contrast-enhanced ultrasound. Optical imaging relies on visible light to generate images that reflect breast tissue properties [14]. Optical imaging can also employ molecularly targeted optical contrast agents for a more specific delineation of molecular features. Pilot studies suggest that optical imaging methods (for example, diffuse optical spectroscopy) can provide an early readout of treatment efficacy [15], and larger, multicenter trials of optical imaging are under way. Though currently used as a largely anatomic imaging method in breast cancer, ultrasound can provide molecular information through the use of molecule-labeled microbubble contrast agents [16]. Molecularly targeted contrast ultrasound is largely at the preclinical stage of investigation; however, some early trials of target ultrasound contrast agents in patients are under way.

Radionuclide molecular imaging for primary breast cancer detection and diagnosis

Anatomic imaging is widely used in breast cancer screening and detection, and mammography is still the gold standard. While anatomic techniques continue to evolve with improvements in spatial resolution and image quality (examples include computer-aided detection and digital mammography as well as other anatomic imaging methods such as MRI and ultrasound), molecular imaging may provide a more specific targeting of breast cancer tissue and greater contrast between tumor and normal tissue. We briefly review studies that use radionuclide methods for primary breast cancer detection and diagnosis.

Single-photon radionuclide breast imaging

The most commonly used single-photon radiopharmaceutical used for breast imaging is MIBI. MIBI is a cationic compound whose uptake and retention in the breast tumor are dependent on regional blood flow, plasma, and mitochondrial membrane potential [17,18]. MIBI retention in tumors may also be affected by the efflux transporter, P-glycoprotein [19]. Early breast imaging using MIBI used standard nuclear medicine γ cameras and was termed scintimammography. A meta-analysis by Liberman and colleagues [20] on the diagnostic accuracy of scintimammography found a sensitivity of 85%, a specificity of 87%, a positive predictive value of 88%, a negative predictive value of 81%, and an

accuracy of 86%. The primary limitations of this approach were poor detection of breast lesions of less than 1 cm, lower sensitivity in non-palpable lesions, and some false-positive uptake in benign breast lesions, inflammation, hematoma, and fat necrosis. Scintimammography generated early interest in clinical breast cancer diagnosis, but the problems noted above limited its clinical use [21].

More recently, high-resolution, small-field-of-view γ cameras specific to breast imaging, sometimes called BSGI or molecular breast imaging, have been developed [3,4]. With the use of breast-specific γ cameras, lesions of less than 1 cm and non-palpable and *in situ* carcinoma can be visualized [4]. Because the uptake of MIBI is independent of breast density, BSGI may serve as a valuable imaging technique for women with mammographically dense breasts. In one study, 1,007 patients with heterogeneously or extremely dense breasts by mammography were screened with mammography and BSGI. The addition of BSGI to mammography significantly increased detection of node-negative breast cancer in dense breasts by 7.5 per 1,000 women screened over mammography and was able to detect cancers as small as 0.4 cm [22]. Limitations to BSGI include the long imaging time (four 10-minute images), the radiation dose associated with injection of the MIBI, and the uncertain ability of BSGI to detect breast microcalcifications [23]. Through optimization of detector technology and innovative noise reduction algorithms, it is anticipated that the dose of radiation required for BSGI could be comparable to that associated with a screening mammogram. BSGI may have a role in the evaluation of patients for whom breast MRI is contraindicated. Finally, although MIBI is the principal radiopharmaceutical used with BSGI to date, a number of other radiopharmaceuticals that target other facets of cancer biology are in development [23]. BSGI has a limited role in clinical practice; however, it is being actively investigated in clinical trials for breast cancer detection and characterization.

Positron emission tomography breast imaging

Compared with SPECT, PET offers the potential for better spatial resolution, a more accurate image quantification, and a wide range of possible imaging probes. The combination of PET with CT yields co-registered molecular and anatomic images and the opportunity to image molecular biology and anatomy simultaneously [24-27]. Although several radiopharmaceutical tracers for use with PET exist, only two are approved in the US for clinical use in cancer: FDG and ^{18}F -fluoride, the latter of which is used primarily for bone imaging. Almost all clinical cancer imaging performed currently is done using FDG. Because accelerated glycolysis is a key feature of many cancers (including breast cancer), FDG generally has high tumor uptake compared with background in

most normal tissues (including the normal breast), making it an attractive agent for cancer detection [5]. Studies have suggested that the degree of FDG uptake in breast cancer is variable and correlated with several phenotypic features such as histologic type (higher uptake in ductal versus lobular cancers), tumor histologic grade (higher uptake in higher grade), steroid receptor expression (higher uptake in steroid receptor-negative tumors), and indices of cellular proliferation (higher uptake in the more proliferative tumors) [28,29].

Early studies using whole-body PET imaging devices showed that FDG PET has high sensitivity and specificity for the detection of larger, palpable breast cancers [30]. Overall, the sensitivity of FDG PET using devices designed for whole-body imaging to detect primary breast cancer was 64% to 96%, specificity was 73% to 100%, positive predictive value was 81% to 100%, and negative predictive value was 52% to 89% [30]. However, sensitivity for smaller and non-palpable lesions was more limited, as was the detection of low-grade or non-invasive cancers [31], which are of significant importance for a breast cancer detection modality. There has therefore been fairly widespread agreement that whole-body FDG PET does not have a clinical role in detecting primary breast cancer, nor is it an alternative to histologic sampling to establish or exclude a primary breast cancer, because of the well-documented inability of FDG PET to consistently demonstrate small and low-grade lesions [31].

Positron emission mammography breast imaging

To overcome the limitations of whole-body FDG PET, dedicated devices for positron emission imaging designed to image the breast only, often termed PEM, have been developed. The advantages of PEM over whole-body FDG PET include higher spatial resolution, reduced attenuation, and possibly lower radiopharmaceutical doses [6]. By mounting the positron detectors on a mammography unit, anatomical and molecular images are co-registered in a fashion analogous to PET-CT (Figure 1). The correlation of mammographic and PEM images allows for PEM-guided biopsy [32].

PEM allows for detection of breast lesions as small as 2 mm and small foci of ductal carcinoma *in situ*. The results of a multicenter study examining the efficacy of PEM reported 91% sensitivity and 93% specificity [33]. The reported limitations of PEM include a radiation dose that is higher than that of a mammogram [34], potential difficulty imaging breast lesions that are in a posterior location, the variable uptake of FDG in small and less metabolically active tumors, and false-positive findings from prior biopsy [33,35]. In a large trial of patients who had newly diagnosed early breast cancer and who were undergoing conventional imaging, PEM, and MRI,

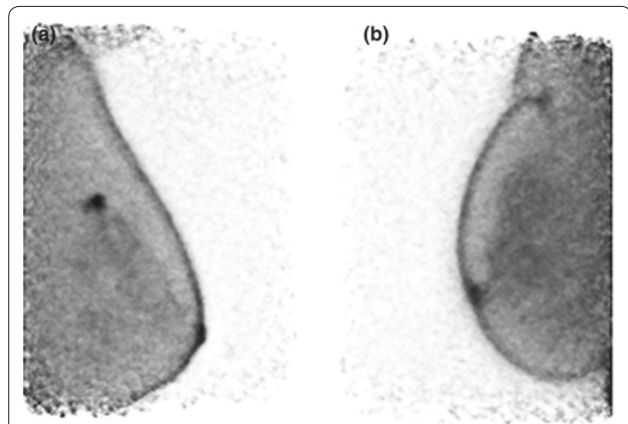


Figure 1. Demonstration of invasive breast carcinomas with ¹⁸F-fluorodeoxyglucose (FDG) positron emission mammography (PEM). Images were made with dedicated breast PEM (Naviscan system; Naviscan, San Diego, CA, USA). PEM images show a 23-mm infiltrating ductal carcinoma in the right breast on medio-lateral oblique (MLO) view (a) and a left breast 12-mm infiltrating ductal carcinoma also on MLO view (b). Images are courtesy of James Rogers, of the Swedish Cancer Institute, and Lawrence MacDonald, of the University of Washington.

conventional imaging plus PEM depicted additional disease in 14% of patients, which is not significantly different from the detection achieved with conventional imaging and MRI (15%, $P = 0.26$). PEM showed improved specificity compared with MRI and may be less likely to prompt unnecessary biopsies and may be an alternative for individuals who cannot tolerate MRI [36]. Currently, PEM uses an approved radionuclide tracer, namely FDG, but is not considered a routine diagnostic modality; however, clinical trials to better define its clinical role are ongoing.

Breast cancer staging and response evaluation

Most FDG PET performed in current clinical practice is performed as whole-body PET-CT, largely for staging and response evaluation. Early studies demonstrating abnormal FDG uptake on PET in metastatic axillary lymph nodes of patients with breast cancer [37,38] prompted a prospective multicenter trial to evaluate the ability to stage the axilla with FDG PET before surgery [39]. The results of this study, which included patients with earlier-stage disease of the type more typically found with modern screening methods, were disappointing. Overall, FDG PET was 61% sensitive and 80% specific for axillary metastases. This trial and subsequent studies showed that sensitivity of FDG PET for axillary metastases in early-stage breast cancer was not sufficient to preclude tissue sampling, usually performed with sentinel lymph node mapping and biopsy, which is highly sensitive for even microscopic foci of tumor. There is currently no clinical role for routine FDG PET axillary

staging in women with newly diagnosed, early-stage breast cancer. Owing to the low likelihood of distant metastases and the not insignificant rate of false-positive findings in low-risk patient populations, whole-body FDG PET or PET-CT is not recommended for systemic staging in breast cancer patients with early-stage disease [40].

Although FDG PET or PET-CT is not recommended in the staging evaluation of patients with early-stage breast cancer, numerous studies have supported the role of FDG PET and PET-CT in regional and systemic staging for patients with locally advanced breast cancer (LABC) [41-43]. In the setting of LABC, the risk of axillary and internal mammary node metastases is higher, as is the risk for occult sites of malignant disease which may affect therapeutic recommendations. FDG PET can also be useful in assessing cancer spread to the internal mammary node chain. In patients with LABC, FDG uptake in the internal mammary nodes is frequently demonstrated (25%) and such uptake is predictive of both the likelihood and pattern of treatment failure [44]. A recent, randomized clinical trial evaluating the role of regional nodal radiation (including internal mammary nodes) in patients treated with breast-conserving surgical therapy showed improvement in disease-free survival with comprehensive nodal radiation [45]. Such findings emphasize the importance of identification of regional nodal disease in breast cancer.

FDG PET-CT is an accepted and reimbursed staging tool for patients with recurrent, suspected, or documented stage IV disease and in these settings has been shown to be both sensitive and specific for accurately determining the extent of disease [21,46-49]. It is important to remember that the CT obtained for attenuation correction in a clinical PET-CT is not necessarily the same as a dedicated, diagnostic, contrast CT that is breath-held.

Serial FDG PET has been widely studied as a method for assessing tumor response to neoadjuvant chemotherapy by using comparison with histopathology assessment of response from the pathology specimen as the gold standard. Studies evaluating change in FDG uptake early in the course of neoadjuvant therapy demonstrate that early declines in FDG uptake are predictive of pathologic response to therapy [50-52]. Molecular imaging by FDG PET may serve as an early predictor of chemotherapy response and, perhaps more importantly, accurately identify those tumors with lack of response, which is clinically relevant as the number of options for systemic therapies increases.

Molecular imaging modalities, primarily FDG PET, have also been used to evaluate response to therapy in metastatic (stage IV) breast cancer. Similar to observations made in the setting of neoadjuvant chemotherapy,

disease response is typically accompanied by substantial declines in FDG uptake by PET, typically 50% or more from pre-therapy baseline values [53,54].

The standard approach for response evaluation in MBC continues to rely on anatomic imaging and changes in tumor size by using standard criteria such as Response Evaluation Criteria in Solid Tumors (RECIST) and anatomic imaging, mostly CT [55,56]. The vast majority of clinical trials in MBC rely on RECIST to assess response and often have eligibility that is dependent on measurable disease by RECIST. While this approach works well for some disease sites like the lungs and liver, size-based anatomic imaging response for soft tissue disease and, particularly, bone metastases remains challenging and an opportunity for incorporation of molecular imaging modalities. Treatment stratification based on metabolic response by PET (PET Response Criteria in Solid Tumors, or PERCIST) has been proposed [57] and awaits validation but is an important advance in molecular imaging.

A particularly vexing clinical problem for breast cancer clinicians is the evaluation of response of bone metastases [58]. Bone is the most common site of breast cancer metastasis. Bone metastases may be detected by bone scintigraphy and MRI, which depict tumor sites largely on the basis of the tumor's effect on adjacent bone. However, in the setting of serial imaging to assess response, these techniques, particularly bone scintigraphy, can be problematic because of a lag in response and potential for 'flare' or transient increase in uptake in response to successful therapy [59]. Patients with bone-only or bone-dominant MBC are often excluded because of the lack of measurable disease.

Early studies have evaluated the role of serial FDG PET as an accurate means for assessing bone metastasis response as glucose metabolism measured in the bone metastasis itself might provide a more direct assessment of treatment response. The earliest studies showed that changes in FDG PET during therapy correlated with changes in serum tumor markers [60] and that percentage change in standard uptake value (SUV) predicted time to progression, a more robust clinical endpoint. Additionally, a higher initial SUV predicted a shorter time to skeleton-related events such as pathologic fracture, hypercalcemia, or need for radiation [61]. The combination of metabolic and anatomic features using PET-CT provides even greater insight. Tateishi and colleagues [62] showed that duration of response in bone-dominant MBC was associated with a decline in FDG PET SUV and an increase in sclerosis (a sign of bone 'healing' in response to therapy) as assessed by CT.

Breast cancer bone metastases present with a mixture of phenotypes ranging from osteoblastic to osteolytic lesions. Whereas FDG PET is a sensitive measure of

osteolytic bone destruction, bone scintigraphy using ^{99m}Tc -methylene diphosphonate (MDP) or ^{18}F -fluoride PET, which measures bone mineral deposition, is a preferred method for detection of osteoblastic lesions [63] and may offer some advantages for measuring response of these lesions, which can be difficult to visualize on FDG PET. Measurement of bone turnover kinetics by dynamic ^{18}F -fluoride PET has been shown to be feasible and offers the opportunity for quantitative assessment of bone metastasis response to therapy [64]. The prospective evaluation of the combination of ^{18}F -fluoride and FDG PET imaging may allow for validation of these imaging modalities as biomarkers for bone metastasis response that can be validated as endpoints for clinical trials (RECIST-like criteria for bone metastases) and shed light on the physiology of the breast cancer cells and their effects on adjacent bone turnover and thus may provide insights into novel therapies for bone metastases. Both FDG and ^{18}F -fluoride PET are approved tracers with increasing use in clinical response evaluation in the setting of MBC, especially in the setting of bone-dominant disease.

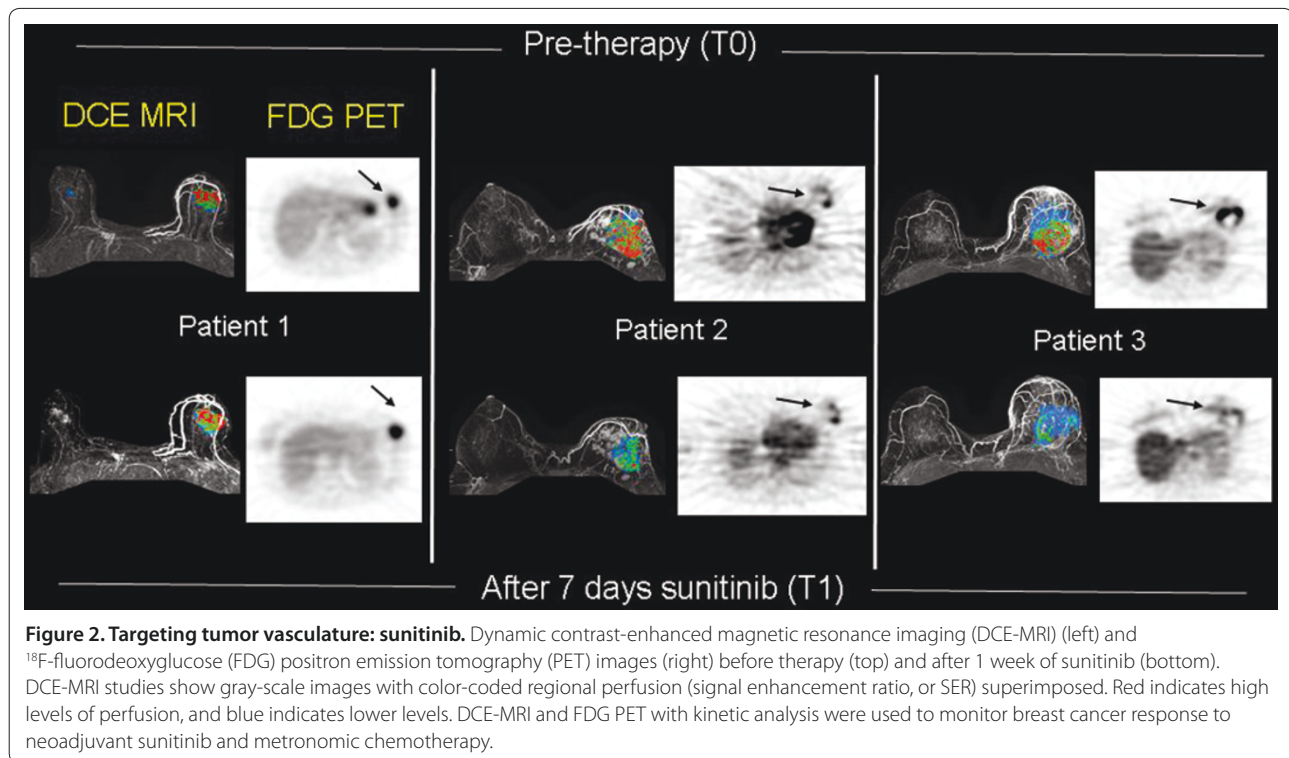
Molecular imaging for breast cancer characterization

Molecular imaging is ideally suited to measure *in vivo* tumor biology related to basic molecular and cellular processes such as metabolism, biosynthesis, cell proliferation, and cell death. This use of molecular imaging to elucidate mechanisms of tumor response and resistance and translation of observations from preclinical systems to patients is among the most exciting applications of molecular imaging. Given the difficulty in assaying some of these processes by tissue sampling, molecular imaging provides a unique and quantitative measure of important properties that may be assessed only *in vivo*. Molecular imaging thus can play an important role in directing breast cancer treatment by identifying regional target expression, documenting drug delivery, and measuring early pharmacodynamic responses to targeted therapy [65]. Selected examples of molecular imaging applied to understanding *in vivo* tumor biology, protein expression, and the tumor microenvironment are reviewed in this section. We emphasize that, although they have undergone preliminary testing in humans, almost all of the methods outlined in this section are investigational and have been tested only in small, single-center studies. The path to a more widespread availability and possible clinical use involves a number of steps that include commercialization of radiopharmaceutical production, rigorous prospective clinical trials, and regulatory approval. Below, we describe the current status of each example as well as any progress toward a more widespread availability.

Tumor perfusion is one of the earliest physiologic properties to be measured by molecular imaging, and advances in methodology have led to increasingly quantitative approaches. Measurement of freely diffusible imaging probes such as ^{15}O -water by PET is a robust quantitative measure of tumor blood flow. Studies by our group have shown that breast tumor blood flow and metabolism in LABC as evaluated by ^{15}O -water and FDG dynamic PET imaging are highly variable and that declines in blood flow and metabolism are predictive of response and survival in patients receiving neoadjuvant chemotherapy [66-68]. The studies suggested that changes in perfusion were highly predictive of response and subsequent relapse and were confirmed by other studies using dynamic contrast-enhanced MRI (DCE-MRI) to measure perfusion changes [8,69,70]. Combined metabolism/perfusion imaging also found that LABC tumors with pre-therapy flow-metabolism mismatch (high ratio of metabolic rate of FDG to blood flow) were most resistant to therapy, predicting a low likelihood of pathologic complete response and a high likelihood of early disease relapse [66,67]. In multivariate models, PET measures of predicted relapse and survival independently of other established prognostic measures, including pathologic complete response and post-therapy nodal status, and other investigators have confirmed the predictive value of metabolism/perfusion imaging in breast cancer and other tumors [71].

^{15}O -water is an investigational tracer with a very short half-life (2 minutes) that requires an on-site cyclotron and rapid transit to the imaging site. Other modalities that provide measures of regional perfusion include DCE-MRI (often used to measure the effect of anti-angiogenic agents) [9,10], Doppler ultrasound [72,73], and optical imaging [15]. Agents for specific imaging of angiogenesis (for example, ^{18}F -galacto arginine-glycine-aspartate (RGD) peptide) have also undergone early testing in humans [74]. Combining measures of metabolism and perfusion (for example, FDG PET and DCE-MRI) may be useful to evaluate the effect of anti-vascular therapy combined with chemotherapy (Figure 2).

Breast tumor metabolism has also been widely studied with molecular imaging. Besides glucose metabolism measured by FDG PET, other PET pharmaceuticals can be used to measure other aspects of metabolism, including regional oxygen consumption using ^{15}O - O_2 inhalation and lipid metabolism using agents such as ^{11}C -choline [5,75]. MRS can also evaluate tumor metabolism by measuring concentrations of specific metabolites. Promising studies have used MRS to measure regional choline levels to characterize breast tumors [12]. For example, Moestue and colleagues [76] reported on distinct patterns of choline metabolism which are associated with different gene expression profiles in luminal and



basal-like breast cancers, and serial choline levels measured by MRS provide an early indicator of treatment response [13].

Aberrant cellular proliferation is a fundamental property of cancer, including breast cancer [77]. Historically, ^{14}C - and ^3H -thymidine have been important methods for measuring cellular proliferation through tissue sampling dating back more than 40 years [78]. More recently, Ki-67 (MIB-1) has provided a method of assessing breast tumor proliferation by immunohistochemistry and is commonly used in clinical practice [79]. A decline in Ki-67 assessed in serial breast tissue samples is an established prognostic marker, particularly in the setting of neoadjuvant endocrine therapy for breast cancer [80,81]. Early studies demonstrated the feasibility of PET imaging to measure cellular proliferation using ^{11}C -thymidine [82]; however, the short half-life of ^{11}C (approximately 20 minutes) requires an on-site cyclotron and limits a more widespread availability. More recent work using thymidine analogs labeled with ^{18}F (half-life of 109 minutes), specifically ^{18}F -fluorothymidine (FLT), has undergone considerable advances in recent years. FLT PET appears promising for measuring early effects of therapy on breast cancer growth [83,84] and has been validated against an *in vitro* assay of proliferation [85]. Currently, the American College of Radiology Imaging Network is completing a multicenter neoadjuvant imaging trial that will evaluate the relationship between FLT uptake parameters and pathologic complete response of

the primary tumor to neoadjuvant chemotherapy in patients with LABC [86]. FLT PET is also still an investigational tracer; however, a commercial supply network for FLT in the US and a National Cancer Institute-held (NCI-held) investigational new drug (IND) will lead to greater use in clinical trials and possible clinical use.

The ability to measure the expression of specific proteins that are gene products associated with breast cancer has led to important advances in breast cancer treatment. Examples include the archetypes of 'targeted therapy', the estrogen receptor (ER), a target for endocrine therapy [87], and human epidermal growth factor receptor 2 (HER2) [88]. Molecular imaging has also been applied to measuring specific protein expression [89,90]. Advantages of imaging include its non-invasiveness, the ability to measure receptor expression in the entire disease burden, and the potential for serial studies of *in vivo* drug effects on the target. Most of the work in this area of breast cancer research has been done for steroid receptors, particularly ER. The most successful ER imaging radiopharmaceutical is 16α - ^{18}F -fluoro- 17β -estradiol (FES) [91]. FES has binding characteristics similar to those of estradiol for both the ER and the transport protein SHBG (sex hormone-binding globulin) [92,93]. Regional estrogen binding is readily quantified by FES PET, and FES uptake has been validated as a measure of ER expression in breast tumors against ER expression assay of tissue samples by immunohistochemistry [94]. FES uptake is readily visualized and quantified in primary

breast cancer and MBC [95]. FES PET can identify heterogeneous ER expression [96] (Figure 3). The level of FES uptake has been shown to be predictive of response to endocrine therapy, and early increase in FDG uptake after administration of an ER agonist (metabolic flare) can also predict response to therapy [97,98]. Serial FES PET can also measure the pharmacokinetic effect of drugs on estradiol binding to the ER, yielding insights into determinants of drug efficacy, and has potential as an important tool for elucidating mechanisms of endocrine resistance [99]. FES is also an investigational tracer but is poised to be incorporated into multicenter cooperative group trials.

Molecular imaging also provides a unique opportunity to image the tumor microenvironment, which is challenging by more invasive means. Tumor hypoxia is an important factor mediating cancer aggressiveness and therapeutic resistance [100,101] and has gained renewed interest in the setting of increased use of anti-angiogenic therapies and with an improved understanding of aberrant patterns of breast tumor metabolism. Tumor hypoxia has been widely studied by imaging, mostly with PET and the agent ^{18}F -fluoromisonidazole (FMISO) [102,103]; however, other PET hypoxia probes have been developed and tested [104]. These are all investigational agents; however, there is a commercial supplier for FMISO in the US and an NCI-held IND facilitating its use. Other hypoxia imaging methods based upon MRI and optical approaches are in earlier stages of development but also appear promising [105].

An increasingly frequent application of molecular imaging to breast cancer treatment is as a pharmacodynamic measure of response to targeted therapy. Many biologically targeted anti-cancer agents can directly or indirectly affect the pathways of glucose metabolism, transport, and glycolysis, resulting in decreased FDG uptake in tumors with therapy [65]. Molecular imaging modalities, particularly FDG PET, are increasingly incorporated in phase I trials as changes in FDG uptake may provide early evidence of drug activity for many agents in development, such as insulin growth factor pathway (IGF1R) inhibitors, phosphatidylinositol 3-kinase (PI3K), mammalian target of rapamycin (mTOR) inhibitors, and others in which surrogate response biomarkers are not available or require tissue sampling that is not always feasible [106]. With the wide array of tracers capable of imaging of protein expression, tumor proliferation, tumor vascularity, and cell death, molecular imaging is perfectly poised as a surrogate response biomarker.

Conclusions

Breast cancer is a common disease in women and a leading cause of death. Molecular imaging plays an important role in the detection, diagnosis, staging, and

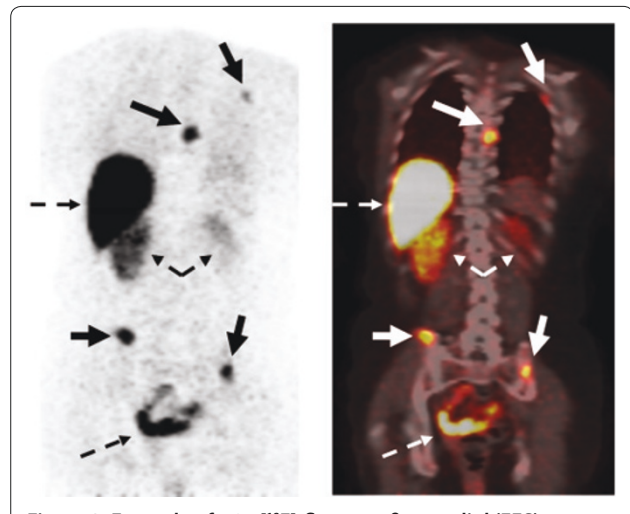


Figure 3. Example of 16α - ^{18}F -fluoro- 17β -estradiol (FES) positron emission tomography (PET) imaging with ^{18}F -fluoroestradiol. (Left) PET emission image. (Right) PET-computed tomography fusion image. In the emission image, increased tracer uptake appears dark, whereas in the fusion image, increased uptake appears white/yellow. Images show multiple sites of estrogen receptor-expressing bone metastases along with normal uptake and excretion of FES into the liver, kidneys, and bowel (dashed arrows).

response evaluation of breast cancer. As breast cancer diagnosis and therapies become increasingly molecular and individualized, molecular imaging will play a progressively more important role in breast cancer clinical care. Molecular imaging techniques offer exciting potential to translate tissue-based, genomic discoveries to the clinic and to further the development of new therapeutic agents for breast cancer.

Abbreviations

BSGI, breast-specific gamma imaging; CT, computed tomography; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; ER, estrogen receptor; FDG, ^{18}F -fluorodeoxyglucose; FES, 16α - ^{18}F -fluoro- 17β -estradiol; FLT, ^{18}F -fluorothymidine; FMISO, ^{18}F -fluoromisonidazole; IND, investigational new drug; LABC, locally advanced breast cancer; MBC, metastatic breast cancer; MIBI, $^{99\text{m}}\text{Tc}$ -sestamibi; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NCI, National Cancer Institute; PEM, positron emission mammography; PET, positron emission tomography; RECIST, Response Evaluation Criteria in Solid Tumors; SPECT, single-photon emission computed tomography; SUV, standard uptake value.

Competing interests

The authors declare that they have no competing interests.

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