Molecular imaging for monitoring treatment response in breast cancer patients

Frederike Bensch¹, Michel van Kruchten¹, Laetitia E. Lamberts¹, Carolien P. Schröder¹, Geke A.P. Hospers¹, Adrienne H. Brouwers², Marcel A.T.M. van Vugt¹, Elisabeth G.E. de Vries¹

¹Department of Medical Oncology, ²Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands.

ABSTRACT

Currently, tumor response following drug treatment is based on measurement of anatomical size changes. This is often done according to Response Evaluation Criteria in Solid Tumors (RECIST) and is generally performed every 2-3 cycles. Bone metastases, being the most common site of distant metastases in breast cancer, are not measurable by RECIST. The standard response measurement provides no insight in changes of molecular characteristics. In the era of targeted medicine, knowledge of specific molecular tumor characteristics becomes more important. A potential way to assess this is by means of molecular imaging. Molecular imaging can visualize general tumor processes, such as glucose metabolism with $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) and DNA synthesis with $^{18}$F-fluorodeoxythymidine ($^{18}$F-FLT). In addition, an increasing number of more specific targets, such as hormone receptors, growth factor receptors, and growth factors can be visualized. In the future molecular imaging may thus be of value for personalized treatment-selection by providing insight in the expression of these drug targets. Additionally, when molecular changes can be detected early during therapy, this may serve as early predictor of response. However, in order to define clinical utility of this approach results from (ongoing) clinical trials is required.

In this review we summarize the potential role of molecular imaging of general tumor processes as well as hormone receptors, growth factor receptors, and tumor micro-environment for predicting and monitoring treatment response in breast cancer patients.
1. INTRODUCTION

Treatment decision-making in locally advanced and metastatic breast cancer is currently based on the extent and sites of disease, and the expression of hormone receptors as well as the human epidermal growth factor receptor 2 (HER2). To assess the effect of the initiated therapy, response monitoring is performed. The European Society of Medical Oncology (ESMO) and National Comprehensive Cancer Network (NCCN) guidelines therefore advise serial conventional radiography, with chest X-ray, computed tomography (CT) or magnetic resonance imaging (MRI).\(^1\,^2\)

The most commonly used criteria for defining response are provided in the Response Evaluation Criteria for Solid Tumors (RECIST v1.1).\(^3\) These criteria use anatomical measurements and are based on a warehouse filled with data from chemotherapy trials. Objective tumor response with tumor shrinkage of \(\geq 30\%\) and progression with tumor growth of \(\geq 20\%\) or new lesions, are widely applied endpoints in clinical trials. The only exception of non-anatomical imaging in RECIST criteria is new metastases detected on \(^{18}\)F-fluorodeoxyglucose positron emission tomography \((^{18}\)F-FDG PET).

With the rising number of targeted drugs for breast cancer treatment, there is an increasing need for reliable predictive biomarkers to select the most suitable therapy for the individual patient. For targeted therapies, the precise value of RECIST criteria is not yet known. Especially in case of targeted agents, visualization of distinct molecular targets or drug behavior may also be of interest.

Anatomical imaging determines response by evaluating measurable lesions. This approach excludes many breast cancer patients from response evaluation according to RECIST, as bone metastases are the most common site of distant metastases. They occur in \(\sim 70\%\) of the patients and are in 17-34% of the patients the only site of presentation at the initial diagnosis of metastatic disease.\(^4\) Currently bone scintigraphy is the standard staging method to detect bone metastases. However, for response evaluation bone scintigraphy is not valuable, since it takes 6 months or longer to reliably detect a response.\(^5\) Therefore patients with bone-only disease are often excluded from clinical trials. This is undesirable as bone metastases apart from being frequently occurring, can cause symptoms.\(^6\) It would therefore be valuable to have a tool that allows also response evaluation in bone metastases.

Finally, response monitoring by conventional methods is recommended after 2-3 months for endocrine therapy and 2-3 cycles of chemotherapy.\(^1\) This period is needed since size changes often occur at a modest pace. It would clearly be a big advantage when before or early during treatment anti-tumor efficacy could be predicted. Similarly, patients with locally-advanced breast cancer receiving neo-adjuvant chemotherapy may benefit from early response monitoring, since ineffective treatment could be timely replaced.
In breast cancer, estrogen receptor (ER) and HER2 expression, in general measured immunohistochemically, are proven biomarkers. Presence of these receptors is predictive for respectively response to endocrine and HER2-targeted therapy. Recent guidelines advise re-evaluation of receptor status in metastatic patients, since discordances between primary tumor and metastases can occur in up to 40% of the patients. Therefore ESMO and NCCN guidelines advise that histology and receptor expression should be repeated at relapse. A biopsy can however not always be obtained, and tumor characteristics can be heterogeneously expressed within and across metastases. It might therefore well be valuable to obtain whole-body information about the expression of relevant drug targets in all tumor lesions within an individual patient.

A potential novel way of response monitoring is by molecular imaging with single photon emission computed tomography (SPECT) or PET imaging with radio-labeled tracers. This allows serial measurements of general tumor processes such as glucose metabolism with 18F-FDG PET or DNA synthesis with 18F-fluorodeoxy-L-thymidine (18F-FLT PET). Increasingly, also relevant drug targets can be visualized such as hormone receptors, growth factor receptors, and growth factors. Molecular imaging of drug-specific targets can in the future potentially support selection of patients for certain therapies and measure early treatment-specific changes in tumors. The recently developed multimodality scanners, such as PET/CT and PET/MRI, combine anatomical and molecular information (Fig. 1). In this review, we summarize the available literature on PET imaging of general tumor processes, hormone receptors, growth factor receptors, and tumor micro-environment, for predicting and monitoring treatment response in breast cancer.

**Figure 1** PET imaging (left) can acquire molecular information, while on CT scan (middle) anatomical information can be obtained. Fusion of both techniques (right) allows simultaneous visualization of molecular and anatomical information. In this example of a patient with metastatic breast cancer, areas with increased 18F-FDG uptake can be observed in a right axillary lymph node, sternum, and chest wall metastasis.
2. **18F-FDG PET**

The possibility to visualize glucose metabolism and thereby metabolic activity of malignancies with 18F-FDG PET has led to a wide range of studies evaluating it for primary tumor detection, diagnosis, (re)staging and monitoring therapy response. 18F-FDG PET is currently not recommended for primary breast cancer staging. The resolution of the PET-camera does not permit detection of small primary lesions as well as nodal sites, and tracer uptake characteristics vary within different (breast) cancers. \(^{10-12}\) To limit overuse of 18F-FDG PET in primary staging and follow-up it is included as 2 of the 5 don’ts of the American Society of Clinical Oncology in the American Board of Internal Medicine Foundation’s Choosing Wisely® campaign.\(^ {13}\) NCCN and ESMO guidelines advise to consider 18F-FDG PET as additional work up in locally advanced, inflammatory, recurrent or metastatic disease, especially when standard imaging remains equivocal or suspicious, and if lesions are inaccessible for biopsy.\(^ {1,2}\)

Several trials studied 18F-FDG PET to monitor treatment response (Table 1). In 1999, the European Organization for Research and Treatment of Cancer (EORTC) PET study group developed criteria to measure lesions at baseline and to objectify treatment response using 18F-FDG PET, based on hypothetical considerations as well as literature review with limited published and unpublished data available.\(^ {14}\) The change in standardized uptake value (SUV) within the tumor lesion compared to a previous scan is used for the assessment. Complete metabolic response is defined as complete resolution of the lesion’s 18F-FDG signal against its surrounding tissue, partial metabolic response as a reduction of \(\geq 15\%\) SUV after one cycle or \(\geq 25\%\) after 2 or more cycles. It is emphasized that also standardized imaging protocols are required to be able to evaluate response. Another attempt to introduce a standard method for PET interpretation is formulated in the PERCIST criteria.\(^ {15}\) After an extensive literature review, response criteria were proposed and conclusions were obtained using a Delphi-like approach. The PERCIST criteria advise to correct the SUV for lean body mass (SUL). Here partial metabolic response is defined as a SUL peak reduction of \(\geq 30\%\). Furthermore, signal of the target lesion must be less than mean liver activity and indistinguishable from surrounding blood-pool levels to be evaluated as complete metabolic response.

Several studies evaluated response by measuring metabolic activity with 18F-FDG PET. In a meta-analysis, 19 mainly prospective \((n = 17)\) studies with in total 786 breast cancer patients who received neo-adjuvant treatment were included.\(^ {16}\) In 15 studies 18F-FDG PET scan was performed before and at different moments during chemotherapy. The pooled analysis showed that 18F-FDG PET with a sensitivity of 84%, a negative predictive value of 91% and a diagnostic odds ratio (DOR) of 11.9 has a beneficial value to forecast pathological response after neo-adjuvant chemotherapy. However, because of relatively low specificity (66%) and positive predictive value (50%) 18F-FDG PET has to be interpreted carefully in the clinic. In a subgroup analysis 18F-FDG
PET after 1-2 cycles of neo-adjuvant treatment had a better DOR (21.8), sensitivity (88%) and specificity (70%) than PET scanning after 3 cycles or later (DOR 5.1, sensitivity 81%, specificity 61%). In this data set, complete response defined by a SUV decrease of ≥ 55-65% would predict response to neo-adjuvant therapy in primary breast cancer more accurately.

In metastatic breast cancer 18 F-FDG PET has been evaluated for its ability to predict (early) response and survival. In several prospective studies with in total 61 locally advanced or metastatic breast cancer patients receiving chemotherapy, 18 F-FDG uptake decreased in almost all responding lesions already after the first cycle.17-21 Analysis of 11 patients showed a SUV

<table>
<thead>
<tr>
<th>Ref #</th>
<th>Treatment</th>
<th>No. pts</th>
<th>Clinical endpoint</th>
<th>PET endpoints</th>
<th>PPV/NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Tamoxifen</td>
<td>40</td>
<td>CR+PR+SD</td>
<td>≥ 10% increase in 18F-FDG-uptake 7-10 days after therapy initiation (^a)</td>
<td>91%/94%</td>
</tr>
<tr>
<td>28</td>
<td>AI (n = 40) FUL (n = 11)</td>
<td>51</td>
<td>CR+PR+SD</td>
<td>≥ 12% increase in 18F-FDG-uptake 1 day after 30 mg oestradiol (^a)</td>
<td>100%/94%</td>
</tr>
<tr>
<td>20</td>
<td>Estradiol 6 mg or 30 mg</td>
<td>46</td>
<td>CR or PR or SD according to RECIST after 24 weeks</td>
<td>≥ 12% increase in 18F-FDG-uptake 1 day after therapy initiation</td>
<td>80%/87%</td>
</tr>
<tr>
<td>30</td>
<td>Anti-hormonal (various)</td>
<td>22</td>
<td>PFS</td>
<td>PFS 28 months in metabolic responders and stable disease vs. PFS 6 months in metabolic non-responders (EORTC criteria)</td>
<td>NA</td>
</tr>
<tr>
<td>18</td>
<td>TAG vs. AT</td>
<td>9</td>
<td>CR or PR according to WHO criteria, after 6 cycles</td>
<td>&gt; 10% decrease in 18F-FDG-uptake after the 1st cycle of chemotherapy (^a)</td>
<td>100%/100%</td>
</tr>
<tr>
<td>20</td>
<td>AC vs. AT</td>
<td>11</td>
<td>CR or PR or SD according to WHO criteria, after 6 cycles</td>
<td>≥ 20% decrease in 18F-FDG uptake after the 1st cycle of chemotherapy (^a)</td>
<td>86%/71%</td>
</tr>
<tr>
<td>21</td>
<td>A (n = 4) and T (n = 16)</td>
<td>20</td>
<td>CR or PR according to RECIST and/or clinical assessment, after 6 cycles</td>
<td>EORTC criteria (≥ 15% decrease in 18F-FDG uptake) after the 1st cycle of chemotherapy</td>
<td>75%/75%</td>
</tr>
</tbody>
</table>

|               | EORTC criteria (> 25% decrease in 18F-FDG uptake) after the 3rd cycle of chemotherapy | 63%/75% |

\(^a\) Retrospectively defined optimal threshold. CR, complete response; PR, partial response; SD, stable disease; PPV, positive predictive value; NPV, negative predictive value; AI, aromatase inhibitor; FUL, fulvestrant; G, gemcitabine; A, anthracycline; T, taxane; C, cyclophosphamide; NA, not available.
decrease of 38% ± 21% in responding and 6% ± 19% in non-responding lesions after the first course. After the second and third course 31 patients in two studies experienced a SUV decrease in responders of 46% ± 16% in one and 52-56% in the other study and in non-responders 21% ± 9% and 16-26%, respectively. The ability to predict survival was evaluated prospective and retrospective in 4 studies in a total of 306 patients. The second 18F-FDG PET scan was done as mid-therapy scan or at the end of treatment. About half of the patients had bone dominant or bony disease and all patients received different systemic therapies. Whereas 18F-FDG PET was predictive for survival in all four data sets, just in two studies this ability remained present in a multivariate analysis.

The main focus for the role of 18F-FDG PET during hormonal treatment has been on the metabolic flare phenomenon as measured by an initial increase in tumor 18F-FDG uptake. This phenomenon can already be observed 24 h after therapy initiation. In two studies in a total of 40 metastatic breast cancer patients, 18F-FDG PET was performed prior to and 7-10 days after start of tamoxifen. Response was based on a combination of RECIST and clinical assessment. While in the responding patients (complete or partial response, or stable disease ≥ 6 months) a higher tumor 18F-FDG uptake was noted 7-10 days after the initiation of tamoxifen, in the non-responding patients tumor 18F-FDG uptake decreased (28 ± 23% vs. 10 ± 16%, P = 0.0002). An arbitrary 10% increase in tumor 18F-FDG uptake would have resulted in a 91% positive predictive value and 94% negative predictive value for response to tamoxifen.

In another study in 51 postmenopausal metastatic breast cancer patients, 18F-FDG PET was performed prior to and 1 day after 30 mg estradiol orally. Thereafter the patients received an aromatase inhibitor (n = 40) or fulvestrant (n = 11). 18F-FDG tumor uptake (SUVmax) increased in patients that subsequently responded to endocrine therapy compared to a slight decrease in non-responding patients (21 ± 24% vs. -4 ± 11%; P < 0.0001). Receiver-operating-characteristic (ROC) analysis revealed an optimal threshold of 12% increase in 18F-FDG uptake to differentiate between responders and non-responders. Finally, this threshold was prospectively evaluated in 66 metastatic patients randomized to estradiol 6 mg or 30 mg daily. Forty-six patients underwent serial 18F-FDG PET. The prospectively defined 12% increase in tumor 18F-FDG uptake, 1 day after initiation of the assigned dose of estradiol, positively predicted response in 80% (12 of 15 such patients responded), and negatively predicted response in 87% (27 of 31 such patients did not respond, P < 0.001).

A few small studies evaluated 18F-FDG PET after a longer period of endocrine therapy. In 22 ER-positive metastatic breast cancer patients on various endocrine drugs, 18F-FDG PET was performed within 7 days prior to therapy initiation and at a mean of 10 ± 4 weeks later. Mean progression free survival (PFS) was 27.5 months in the group with metabolic response or stable metabolic disease, compared to 5.8 months in patients with progressive metabolic disease (P < 0.0001) according to EORTC criteria. In a neo-adjuvant study in 11 patients with ER-positive
breast tumors, $^{18}$F-FDG PET was performed prior to and 4 weeks after start of letrozole, followed
by surgery at 12 weeks.\textsuperscript{31} Metabolic response, defined as > 40% decrease in tumor $^{18}$F-FDG
uptake (SUVmax), did not correlate with morphologic and pathologic response. Metabolic
responders did however have a clear decrease in Ki-67 labeling index (91% ± 11% relative
decrease) compared to non-responders.

Few clinical and preclinical studies addressed response evaluation with $^{18}$F-FDG PET after
administration of HER2-targeting drugs in breast cancer. In patients with advanced malignancies,
SUV decreased > 25% after 1 month of lapatinib treatment in 4 out of 8 patients (breast cancer
\( n = 1 \)), one of whom had partial response, while the other 3 had stable (\( n = 2 \)) or progressive
disease.\textsuperscript{32}

A number of preclinical studies evaluated effects of targeted drugs on $^{18}$F-FDG PET. However, studies evaluating $^{18}$F-FDG PET in mouse models need to be interpreted cautiously. Biodistribution in mice is dependent on dietary status, ambient temperature and muscle activity, and tumor uptake often seems low because of high background uptake of normal tissue.\textsuperscript{33}

Mice with HER2-over-expressing or with low HER2-expressing human xenografts were
treated with trastuzumab or phosphate-buffered saline (PBS) on day 1, 2, 7 and 14.\textsuperscript{34} $^{18}$F-FDG
uptake was lower in trastuzumab treated HER2-over-expressing mice than in the PBS treated
control group after 16, but not after 2 or 9 days. Tracer uptake was not influenced in trastuzumab
and PBS treated mice with low HER2-expression. In another study, mice with tumors transplanted
from MMTV/HER2 transgenic mice or with BT474 human xenografts were treated and imaged
twice weekly for 3 weeks with trastuzumab or PBS.\textsuperscript{35} In contrast to the former study, independent
of tumor response $^{18}$F-FDG uptake did not change. Heat shock protein 90 (HSP90) inhibitors, such
as 17-AAG and NVP-AUY922 have mostly been tested combined with $^{18}$F-FLT PET in preclinical
studies (see section 3). In a BT474 human xenograft mouse model treated with 17-AAG once,
there was no change in $^{18}$F-FDG uptake during the first 22 days thereafter.\textsuperscript{36}

In conclusion, FGD PET can be of value in case of advanced stage and problematic staging
of breast cancer. For response monitoring several studies suggest a potential role in (metastatic)
breast cancer. But given several unsolved issues, it is not yet part of standard tumor response
measurement guidelines.

3. $^{18}$F-FLT PET

Uptake of $^{18}$F-FLT is determined by the activity of the enzyme thymidine kinase, which is
involved in DNA synthesis and reflects therefore indirectly the proliferative state of cells. This
was confirmed in a meta-analysis where $^{18}$F-FLT uptake correlated with Ki-67 staining with
sufficient data available for at least brain, lung and breast cancer.\textsuperscript{37} Over the last years small
studies evaluated the ability of this tracer to monitor response in breast cancer. Fourteen patients
with metastatic breast cancer, treated by chemotherapy \((n = 9)\) or hormonal therapy \((n = 5)\) underwent \(^{18}\)F-FLT PET scans at baseline, 2 weeks after the first and 2 weeks after the last course of therapy or maximal 1 year after the initial scan.\(^{18}\) Here, \(^{18}\)F-FLT uptake correlated with overall response, based on change in tumor marker \((\text{CA27.29}; r = 0.79)\) and tumor size measured on CT \((r = 0.74)\). In another prospective study \(^{18}\)F-FLT PET and response was analyzed in 12 breast cancer patients.\(^{39}\) Scans were performed at baseline and one week after the first administration of 5-fluorouracil, epirubicin and cyclophosphamide (FEC). Tumors were assessed according to the RECIST criteria and proliferation was scored as response when \(^{18}\)F-FLT SUV decreased at least 18%. With these prospectively defined criteria, \(^{18}\)F-FLT PET showed response in 12 out of 17 lesions. In these lesions, mean SUV change 1 week after the first course of chemotherapy was higher than in non-responding lesions (-41.3% vs. +3.1%). The 6 clinically responding patients were identified correctly by \(^{18}\)F-FLT PET. Response assessment in another 18 patients after the first or second cycles of docetaxel has been performed.\(^{40}\) Response criteria were prospectively defined either according to RECIST in case of conventional imaging or a SUV change of ≥ 20%. Change of the \(^{18}\)F-FLT signal after 1-2 cycles correlated with the size of the lesion after the third cycle \((r = 0.64)\). Eleven out of 13 responders and 4 out of 5 non-responders were correctly identified with \(^{18}\)F-FLT PET. Sensitivity of \(^{18}\)F-FLT PET was 85% with a specificity of 80%. Six patients with locally advanced or metastatic breast cancer treated with capecitabine were scanned 2-10 days before and 1 hour after the first drug administration.\(^{41}\) Interestingly, tracer uptake increased 3.4%-84.5% in 9 out of 10 lesions. Other parameters like blood flow and \(^{18}\)F-FLT delivery variables were largely unchanged. Increased influx of nucleosides due to redistribution of nucleoside transporters and increased activity of thymidine kinase 1 induced by thymidylate synthase inhibition may explain this flare phenomenon.\(^{41,42}\)

Preclinical studies addressed response evaluation on targeted agents with \(^{18}\)F-FLT PET, mainly in comparison to \(^{18}\)F-FDG PET. \(^{18}\)F-FLT, but not \(^{18}\)F-FDG, 3 days after pulse treatment with NVP-AUY922 in BT474 multilayer spheroids showed, in accordance with growth inhibition, a dose-dependent decrease in tracer uptake.\(^{43}\) A second study confirmed this positive correlation between changes in \(^{18}\)F-FLT uptake and growth inhibition in BT474, MCF-7, U87MG and HCT116 cell spheroids.\(^{44}\) \(^{18}\)F-FDG uptake only correlated highly in BT474 spheroids and poorly in MCF-7 cells. Trastuzumab treatment in mice with BT474 human xenografts reduced \(^{18}\)F-FLT tumor uptake, whereas uptake was not changed in mice with tumors transplanted from MMTV/HER2 transgenic mice after treatment.\(^{35}\)

Moreover, it is important to take into account that \(^{18}\)F-FLT uptake in rodents is influenced by a higher thymidine plasma level as compared to humans. Competition of endogenous thymidine and \(^{18}\)F-FLT can be neutralized by administration of thymidine phosphorylase right before tracer injection, leading to increased tracer accumulation in the tumor.\(^{45}\) If neutralization was no part of the imaging protocol, interpretation of \(^{18}\)F-FLT uptake in rodents must be done carefully. However, in the latter study it is not reported that thymidine neutralization was done.
Table 2 (Ongoing) clinical trials with $^{18}$F-FDG PET and $^{18}$F-FLT PET for determination of predictive value before and/or during breast cancer therapy

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Therapy</th>
<th>Scan planning</th>
<th>Planned no. of pts</th>
<th>Aim of the study</th>
<th>Clin. Trial ID (NCT#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{18}$F-FDG</td>
<td>Trastuzumab-DM1</td>
<td>After cycle 1 and 3 palliative trastuzumab-DM1</td>
<td>60</td>
<td>Negative predictive value of the early $^{18}$F-FDG PET for response on trastuzumab-DM1 therapy</td>
<td>01565200</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>NAC</td>
<td>Before start, 15 days after 1 cycle</td>
<td>80</td>
<td>Value of $^{18}$F-FDG PET after 1 course of NAC in prediction of pathological response</td>
<td>01038258</td>
</tr>
<tr>
<td>$^{18}$F-FDG/ $^{18}$F-FLT</td>
<td>NAC</td>
<td>Before start, after cycle 1 and just before cycle 2</td>
<td>30</td>
<td>Value of changes in the SUV as a predictor of complete pathologic response</td>
<td>01222416</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>NAC</td>
<td>Before start of therapy and after cycle 1 and 6</td>
<td>50</td>
<td>Efficacy of multi-parametric MRI, $^{18}$F-FDG PET, and PET-MR fusion imaging in the prediction and monitoring response to NAC</td>
<td>01190566</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>PEM</td>
<td>Bilateral and axillary PEM and MRI at baseline, after 1-2 weeks and after 3-4 weeks of NAC</td>
<td>50</td>
<td>Response to NAC</td>
<td>01012440</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>HER2-targeted or hormonal therapy</td>
<td>Before and 2 weeks after neo-adjuvant or palliative therapy</td>
<td>40</td>
<td>Correlation between the % change in $^{18}$F-FDG PET SUV and % change in cell proliferation (assessed in tumor biopsy)</td>
<td>00362973</td>
</tr>
<tr>
<td>$^{18}$F-FDG/ $^{18}$F-FLT</td>
<td>NAC</td>
<td>Prior to and after completion of NAC before definitive surgery</td>
<td>20</td>
<td>Sensitivity and specificity of $^{18}$F-FLT PET compared to $^{18}$F-FDG PET. Correlation between PET and % Ki-67.</td>
<td>01018251</td>
</tr>
<tr>
<td>$^{18}$F-FLT</td>
<td>NAC</td>
<td>Before start, after 1 cycle, and at the end of NAC</td>
<td>45</td>
<td>Correlation between change in tumor $^{18}$F-FLT uptake and % Ki-67</td>
<td>01015131</td>
</tr>
<tr>
<td>$^{18}$F-FLT/ $^{18}$F-FDG/ MRI</td>
<td>NAC</td>
<td>At initial staging, 3 times during NAC and prior to surgery</td>
<td>60</td>
<td>Sensitivity and specificity of the three imaging modalities for prediction of response to NAC</td>
<td>00236275</td>
</tr>
<tr>
<td>$^{18}$F-FLT</td>
<td>NAC</td>
<td>Before start, mid-treatment, and prior to surgery</td>
<td>36</td>
<td>Predictive value of $^{18}$F-FLT PET for response to NAC</td>
<td>00572728</td>
</tr>
<tr>
<td>$^{18}$F-FLT</td>
<td>NAC</td>
<td>Before start, before cycle 2 and prior to surgery</td>
<td>100</td>
<td>Predictive value of $^{18}$F-FLT PET for response to NAC according to Sataloff criteria 94</td>
<td>00534274</td>
</tr>
<tr>
<td>$^{18}$F-FDG</td>
<td>Hormonal therapy</td>
<td>Before start</td>
<td>100</td>
<td>Predictive value of $^{18}$F-FDG PET for response to hormonal therapy</td>
<td>00358098</td>
</tr>
</tbody>
</table>

NAC, neo-adjuvant chemotherapy; PEM, positron emission mammography; PET, positron emission tomography; SUV, standardized uptake value; $^{18}$F-FDG, $^{18}$F-fluorodeoxyglucose; $^{18}$F-FLT, $^{18}$F-fluorodeoxythymidine.
There is currently no role for $^{18}$F-FLT PET in standard clinical breast cancer care. More information is expected from 6 ongoing trials in over 250 patients. In these trials results are compared between $^{18}$F-FLT PET and $^{18}$F-FDG PET, MRI and CT as well as to histological parameters like Ki-67, grade and tumor type in the neo-adjuvant setting (Table 2).

4. HORMONE RECEPTOR IMAGING

Estrogen receptor

The most relevant hormone receptor in breast cancer is the ER. It is expressed in ~ 75% of the patients. For the patients with an ER-positive tumor, endocrine therapy can be an important treatment option.

A novel way to evaluate ER expression is by $^{18}$F-fluoroestradiol ($^{18}$F-FES) PET. $^{18}$F-FES PET measures tumor ER-expression with a 69-100% sensitivity and 80-100% specificity when compared to in vitro assays. These results support future trials to examine $^{18}$F-FES PET to re-evaluate ER-expression non-invasively in patients that cannot be biopsied, and thus may support treatment decision-making.

$^{18}$F-FES PET has been evaluated as biomarker to predict response in four relatively small studies. Here, the positive predictive value of increased $^{18}$F-FES uptake at baseline was limited and ranged 34-79%, while the negative predictive value of low $^{18}$F-FES uptake was relatively good (81-100%). Although these results show the potential of $^{18}$F-FES PET to guide therapy decisions, still many aspects are unresolved. Most importantly, aforementioned studies have used different, often retrospectively defined, thresholds to dichotomize $^{18}$F-FES PET results.

Serial $^{18}$F-FES PET imaging was studied to measure effect of endocrine therapy in two small studies. A retrospective study in 30 metastatic breast cancer patients evaluated $^{18}$F-FES uptake prior to and 1-18 weeks after endocrine therapy initiation. Drugs that competitively bind the ER (tamoxifen and fulvestrant) blocked tumor $^{18}$F-FES uptake, although partially with an average decrease of 54%. Aromatase inhibitors, which affect circulating estrogen levels, hardly affected tumor $^{18}$F-FES uptake (< 15% decrease). Correlation between changes in $^{18}$F-FES uptake and clinical outcome was not evaluated. In a prospective study in 40 metastatic breast cancer patients, $^{18}$F-FES PET was performed prior to and 7-10 days after the initiation of tamoxifen. Response was defined as objective tumor response or stable disease (< 50% decrease and < 25% increase in lesion diameter) ≥ 6 months. Responders had a larger decrease in $^{18}$F-FES uptake than non-responders (-55% vs. -19%, $P = 0.0003$). However, the threshold to optimally differentiate between responders and non-responders, as well as the corresponding positive and negative predictive value, is still to be elucidated.
Androgen receptor

The androgen receptor (AR) is a key target in prostate cancer patients and various anti-androgens are available in the clinic. The AR is expressed in ~70% of all breast cancer patients, and 12-40% of the so-called triple-negative patients. Therefore, the AR is currently also being explored as a potential therapeutic target in breast cancer. The AR can be imaged by 18F-fluorodihydrotestosterone (FDHT) PET. Currently no information is available on FDHT PET in breast cancer patients. In metastatic prostate cancer patients, 18F-FDHT uptake occurs in the majority of metastases. This uptake can be blocked by the AR antagonists flutamide and MDV3100, illustrating the specificity of 18F-FDHT for the AR. With the emerging interest for anti-androgen therapy in breast cancer patients, this PET tracer may well show its value in the near future.

Progesterone receptor

The progesterone receptor (PR), although not a direct target of endocrine therapy in breast cancer itself, is a predictive marker for response to anti-estrogen therapy. Several attempts have been made to develop a PR-specific PET tracer, although with limited success. The best tracer available to date is 21-18F-fluoro-16α,17α-[(R)-1'-α-furylmethylidene)dioxo]-19-norpregn-4-ene-3,20-dione (18F-FFNP). In 22 patients 18F-FFNP PET showed visually increased uptake in 15 of 16 PR-positive primary breast tumors, while 18F-FFNP uptake was moderate-low in 5 of 6 PR-negative primary breast tumors. However, no correlation between quantitative 18F-FFNP uptake and PR status determined by immunohistochemistry was observed nor did 18F-FFNP uptake differ in PR-positive compared to PR-negative tumor lesions (SUVmax 2.5 ± 0.9 vs. 2.0 ± 1.3). The prognostic and predictive value of 18F-FFNP PET, and the use of 18F-FFNP PET to monitor treatment response, has not been evaluated in the clinic. In a preclinical study, however, in mice with ER/PR-positive murine mammary adenocarcinomas the ER-antagonist fulvestrant decreased 18F-FES-uptake in both fulvestrant-sensitive and fulvestrant-resistant tumors, while tumor 18F-FFNP-uptake only decreased in the fulvestrant-sensitive tumors. These early results suggest that serial 18F-FFNP PET may be a good read-out and predictor of endocrine therapy efficacy.

5. GROWTH FACTOR RECEPTOR IMAGING

Amplification of the HER2 gene results in over-expression of the HER2 protein, which occurs in 20-25% of primary breast cancers. HER2 is a member of the cell surface receptor HER family with tyrosine kinase activity, involved in transmission of signals controlling cell growth and proliferation. HER2-over-expression, when left untreated, is associated with aggressive growth and poor prognosis. The anti-HER2 monoclonal antibody trastuzumab, which targets the
extracellular domain of HER2, is part of treatment in the adjuvant as well as in the metastatic setting of HER2-positive breast cancer.\textsuperscript{67,68}

We used radio-labeled trastuzumab for molecular imaging of the HER2 status in breast cancer patients with SPECT and PET. First the SPECT tracer \textsuperscript{111}In-trastuzumab was developed for clinical use. Subsequently in 15 HER2-expressing metastatic breast cancer patients, specific uptake of \textsuperscript{111}In-trastuzumab was shown in HER2-positive tumor lesions. In addition, new HER2-positive lesions were identified in 13 of 15 patients.\textsuperscript{69} The next step in HER2 imaging was labeling of trastuzumab with the radioisotope \textsuperscript{89}Zr for PET imaging of HER2 (Fig. 2).\textsuperscript{70,71} PET imaging reaches higher spatial resolution than SPECT and tumor uptake of the tracer is easier to quantify. \textsuperscript{89}Zr has a half-life of 78.4 hours, which is compatible with the relative long biological half-life of the trastuzumab antibody. In the first clinical trial with \textsuperscript{89}Zr-trastuzumab 14 metastatic breast cancer

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Molecular imaging can provide information on glucose metabolism by \textsuperscript{18}F-FDG PET (left) and HER2-status by \textsuperscript{89}Zr-trastuzumab PET (right). Arrow heads indicate a mediastinal lesion with increased \textsuperscript{18}F-FDG and \textsuperscript{89}Zr-trastuzumab uptake in this patient with HER2-positive metastatic breast cancer. Note the differences in physiological uptake as a result of distribution, metabolism and excretion of both tracers.}
\end{figure}
patients with HER2-positive tumors received 37 MBq $^{89}$Zr-trastuzumab. Optimal PET scanning results were found at 4–5 days after tracer injection, with sufficient tumor uptake, less background signal and sufficient count-statistics. Most lesions were detected with excellent tumor uptake and visualization. Moreover, unknown brain metastases were detected in two patients, showing that trastuzumab can penetrate the brain in case of brain metastases. In a patient with both a HER2-positive and a HER2-negative breast cancer who developed metastases, standard work up failed to determine HER2 status. The $^{89}$Zr-trastuzumab PET scan showed uptake of $^{89}$Zr-trastuzumab in the metastases, leading to initiation of anti-HER2 therapy. The predictive value of the $^{89}$Zr-trastuzumab PET scan still deserves further studies.

Another role of imaging may be to determine the negative predictive value of $^{89}$Zr-trastuzumab PET in HER2-positive metastatic breast cancer patients who receive the antibody drug conjugate trastuzumab-DM1 (T-DM1). This is part of an ongoing trial (NCT 01565200). T-DM1 was recently assessed in a phase III trial in patients with HER2-positive advanced breast cancer. T-DM1 improved progression free survival as well as overall survival compared to lapatinib and capecitabine, with less toxicity.

Trastuzumab was also labeled to $^{64}$Cu for PET imaging. The half-life of $^{64}$Cu is 12.7 hours. This leads to less radiation exposure compared to $^{89}$Zr imaging, but may also have a relatively low physical half-life compared to the long biological half-life of trastuzumab. $^{64}$Cu-DOTA-trastuzumab PET detected primary breast cancer, lymph node and lung metastases in 15 HER2-positive breast cancer patients on trastuzumab therapy. Currently two clinical trials are ongoing investigating $^{64}$Cu-DOTA-trastuzumab in HER2-positive metastatic breast cancer patients to determine the optimal imaging dose and biodistribution and to assess the correlation of tumor tracer uptake with HER2 expression by immunohistochemistry (NCT01093612; NCT00605397). There is as yet no head to head comparison available between $^{64}$Cu-trastuzumab and $^{89}$Zr-trastuzumab PET.

Besides a more intensive role of non-invasive PET imaging with $^{89}$Zr-trastuzumab for selecting the most suitable patients for anti-HER2 therapy, this technique might also be able to facilitate early response measurement of targeted therapy in breast cancer. HER2 is degraded upon HSP90 inhibition and is therefore a rational candidate for treatment monitoring during HSP90 inhibition. One study used a $^{68}$Ga labeled F(ab$^\prime$)$_2$ fragment of trastuzumab in HER2 expressing breast cancer xenografts before and during therapy with the HSP90 inhibitor 17-AAG. HER2 expression lowered 80% 24 h after treatment, and increased to 50% of the initial expression 2 to 7 days after treatment. The other study was performed with $^{89}$Zr-trastuzumab in HER2-overexpressing tumor bearing mice treated with the HSP90 inhibitor NVP-AUY922. $^{89}$Zr-trastuzumab tumor uptake was reduced 41% after three doses of NVP-AUY922. These results led to the initiation of a clinical study investigating the role of $^{89}$Zr-trastuzumab PET to monitor treatment effects of the HSP90 inhibitor NVP-AUY922 in metastatic breast cancer patients with HER2 positive tumors (NCT01081600).
6. **IMAGING OF TUMOR MICRO ENVIRONMENT**

Not only tumor cell membrane receptors and proteins, but also soluble tumor specific targets present in the tumor micro-environment can be visualized with molecular imaging. Vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGFβ) are such targets. VEGF is an important factor involved in tumor angiogenesis. VEGF is produced by tumor cells and over-expression is present in many human tumor types, making it a rational target for anti-angiogenic therapy. VEGF signaling can be blocked with neutralizing antibodies, inhibiting VEGF-receptor tyrosine kinases on endothelial cells and by inhibiting cellular tumor signaling pathways. Bevacizumab is a humanized monoclonal antibody that binds and inactivates VEGF-A, thereby inhibiting VEGF-mediated angiogenesis. Multiple randomized phase 3 trials with bevacizumab were conducted in metastatic breast cancer patients. They demonstrated modest improvements in PFS for bevacizumab combined with chemotherapy, without improvement in overall survival. The addition of bevacizumab in a phase 3 randomized trial to neo-adjuvant chemotherapy increased the rate of complete pathological response only in a subpopulation of triple negative patients. A complete pathological response rate of 34.5% following the addition of bevacizumab to standard chemotherapy in HER2-negative breast cancer patients vs. 28.2% without bevacizumab was shown in another study. Proper selection of patients who might benefit of bevacizumab would be very helpful. However, robust, predictive, biologic or clinical markers for bevacizumab are currently lacking.

Bevacizumab has been radio-labeled for non-invasive tumor monitoring with 111In for SPECT imaging and with 89Zr for PET. Specific tumor accumulation occurred with both tracers. 89Zr-bevacizumab uptake could be quantified in VEGF expressing tumor bearing mice. This was translated to a study in primary breast cancer patients. Twenty-three patients with 26 tumors received 37 MBq 89Zr-bevacizumab at a protein dose of 5 mg followed by PET 4 days later, before surgery. Twenty-five of 26 tumors were detectable. VEGF expression was measured with ELISA after resection in 17 tumors and VEGF-A levels were higher in tumors than in normal breast tissue from the same patients.

89Zr-bevacizumab PET was used as a so called effect sensor to monitor treatment effects with the HSP90 inhibitor NVP-AUY922 in mice bearing ovarian cancer xenografts. Tumor uptake of 89Zr-bevacizumab decreased 44% after treatment with NVP-AUY922 measured with PET scans 144 h after tracer injection. The extent of the change in tracer uptake during treatment was related to the down-regulation of VEGF levels measured by quantitative ELISA. A study evaluating 89Zr-bevacizumab PET in ER-positive metastatic breast cancer patients treated with NVP-AUY922 is currently ongoing (NCT01081613).

The role of 89Zr-bevacizumab PET imaging as a biomarker of angiogenic changes during treatment with sunitinib or bevacizumab/interferon was determined in metastatic renal cell
cancer patients. Tracer uptake decreased 47% during bevacizumab, and only 15% during sunitinib.\(^9\)

7. DISCUSSION

This review shows that molecular imaging might support treatment decision making in the future. Especially PET imaging with tumor specific tracers like \(^{89}\)Zr-trastuzumab or \(^{18}\)F-FES has the potential to select the most suitable therapy for each individual patient. Furthermore, serial imaging of general tumor process with tracers such as \(^{18}\)F-FDG and \(^{18}\)F-FLT may provide early prediction of anti-tumor efficacy (Fig. 3). Up-front or early detection of non-responding patients can avoid unnecessary toxicities and reduce health care costs. However, studies performed until now are (too) small and mainly have retrospectively determined end points. Uptake characteristics for different breast cancer subtypes, as well as for different chemotherapy and/or targeted therapy regiments remain unclear. The optimal moment of scanning, the quantification method and validation of PET with conventional imaging and histology are issues, which further need to be dealt with. For PET to be implemented in the clinic robust and properly powered trials with clearly defined patient populations, standardized PET protocols and prospectively set endpoints need to be performed to prove its clinical utility.

Next to these future trials, more novel tracers are being developed for molecular imaging in breast cancer. Apart for patient selection, these tracers might also have an important role in response measurement. However, not for all molecular-targeted therapies it is clear which tracers can be used to measure response to treatment. It is therefore recommended to investigate which tumor characteristic correlates with therapeutic response and thus may be suitable as a starting point to develop tracers against.\(^8\)

New targeted therapies are designed with tyrosine kinase inhibitors, HSP90 inhibitors and phosphoinositide 3-kinase (PI3K) inhibitors. Moreover a new group of drugs are developed; the antibody-drug conjugates (ADCs). ADCs are monoclonal antibodies conjugated with a highly toxic component that is specifically delivered to the tumor since it is only released after intracellular tumor uptake. To determine the amount of toxin delivered to the tumor, PET might be used to calculate the targeting of the compound to the tumor by labeling the ‘naked’ antibody with \(^{89}\)Zr.

The knowledge obtained with nuclear molecular imaging of tumor lesions with SPECT and PET is currently translated to optical molecular imaging. With optical imaging, no radioactivity is administered to patients, creating a more important role of imaging in the diagnostic and intra-operative setting. Recently the near infrared fluorescent IRDye 800CW was labeled to the therapeutic monoclonal antibodies bevacizumab and trastuzumab targeting VEGF and HER2 respectively. In vivo both bevacizumab-800CW and trastuzumab-800CW showed specific tumor
Molecular imaging for monitoring treatment response in breast cancer patients

Figure 3: Next to standard imaging with CT scan, also additional imaging of general tumor processes with $^{18}$F-FDG or $^{18}$F-FLT PET, and molecular imaging of relevant drug targets (such as the ER or HER2 using $^{18}$F-FES or $^{89}$Zr-trastuzumab) before therapy initiation might aid to select the right patients for targeted therapies at an early time point.
detection in tumor-bearing mice using the real-time intra-operative clinical prototype camera system. Clinical testing with the fluorescent labeled antibodies has started based on a similar procedure as used for the radio-labeled antibodies (NCT01508572). Potentially the uptake of this tracer could be quantified serially with a handheld probe or endoscope measuring fluorescence in accessible tumor lesions. Furthermore the anti-EGFR nanobody 7D12 was labeled with IDRYe800CW and showed high tumor uptake with optical imaging in human tumor xenografts as early as 30 minutes after injection.

Acknowledgements
This research was supported by the Center for Translational Molecular Medicine – Mammary Carcinoma Molecular Imaging for Diagnosis and Therapeutics (CTMM - MAMMOTH) Project. We thank Esther van Straten for her assistance with designing Fig. 3.

Authors disclosures of potential conflicts of interest
Geke A.P. Hospers received a research grant from AstraZeneca, Elisabeth G.E. de Vries of Roche and Novartis.
REFERENCES


