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[^{18}F]-3′Deoxy-3′-Fluorothymidine Positron Emission Tomography and Breast Cancer Response to Docetaxel

Kaiyumars B. Contractor[1], Laura M. Kenny[1], Justin Stebbing[1], Lula Rosso[2], Rizvana Ahmad[1], Jimmy Jacob[1], Amarnath Challapalli[1], Federico Turkheimer[2], Adil Al-Nahhas[3], Rohini Sharma[1], R. Charles Coombes[1], and Eric O. Aboagye[1]

Abstract

Purpose: To establish biomarkers indicating clinical response to taxanes, we determined whether early changes in [^{18}F]-3′deoxy-3′-fluorothymidine positron emission tomography (FLT-PET) can predict benefit from docetaxel therapy in breast cancer.

Experimental Design: This was a prospective unblinded study in 20 patients with American Joint Committee on Cancer (AJCC) stage II–IV breast cancer unresponsive to first-line chemotherapy or progressing on previous therapy. Individuals underwent a baseline dynamic FLT-PET scan followed by a scan 2 weeks after initiating the first or second cycle of docetaxel. PET variables were compared with anatomic response midtherapy (after 3 cycles).

Results: Average and maximum tumor standardized uptake values at 60 minutes ($SUV_{60,av}$ and $SUV_{60,max}$) normalized to body surface area ranged between 1.7 and 17.0 and 5.6 and 26.9 $\times$ 10^{-5} m^2/mL, respectively. Docetaxel treatment resulted in a significant decrease in FLT uptake ($P = 0.0003$ for $SUV_{60,av}$ and $P = 0.0002$ for $SUV_{60,max}$). Reduction in tumor $SUV_{60,av}$ was associated with target lesion size changes midtherapy (Pearson R for $SUV_{60,av} = 0.64$; $P = 0.004$) and predicted midtherapy target lesion response (0.85 sensitivity and 0.80 specificity). Decreases in $SUV_{60,av}$ in responders were due, at least in part, to reduced net intracellular trapping of FLT (rate constant, K_i). Docetaxel significantly reduced K_i by 51.1% ($\pm$28.4%, $P = 0.0009$).

Conclusion: Changes in tumor proliferation assessed by FLT-PET early after initiating docetaxel chemotherapy can predict lesion response midtherapy with good sensitivity warranting prospective trials to assess the ability to stop therapy in the event of non–FLT-PET response. *Clin Cancer Res; 17(24); 7664–72. ©2011 AACR.*

Introduction

Taxanes are some of the most commonly used drugs in the treatment of breast cancer today. A molecular imaging biomarker which could provide early accurate prediction of response or resistance *in vivo* would be of great benefit to oncologists. The cytotoxic activity of taxanes is promoted by stabilizing cellular microtubule assembly (1, 2). This leads to inhibition of mitotic cell division (the G_2–M phase of the cell cycle; ref. 2) and cell death by apoptosis (3). Docetaxel is approved for the treatment of patients with locally advanced or metastatic breast and non–small cell lung cancer (4) who have undergone anthracycline-based chemotherapy and failed because of tumor progression or relapse. The drug has shown effectiveness against tumors resistant to anthracyclines or paclitaxel (5, 6) and good response rates (55%–68%) in the neoadjuvant setting (7, 8), at the expense of well-known myelosuppressive and other toxicities.

In patients undergoing chemotherapy, systemic toxicity remains the major limitation to adequate dosing. The ability to predict early response after 1 to 2 cycles of docetaxel would avoid unnecessary toxicities in patients unlikely to respond with potential impact on clinical outcomes and quality of life, particularly in the palliative setting. We explored the performance of [^{18}F]-3′deoxy-3′-fluorothymidine positron emission tomography (FLT-PET) as an early biomarker of tumor response in this setting. FLT uptake into tumor cells is regulated by equilibrative nucleoside transporter (ENT-1) and thymidine kinase1 (TK1; refs. 9–11); the latter controlled during cell division

Authors' Affiliations: Departments of [1]Surgery and Cancer, [2]Neuroscience, and [3]Nuclear Medicine, Imperial College London and Imperial College Healthcare NHS Trust, Hammersmith and Charing Cross Hospitals, London, United Kingdom

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Corresponding Author: Eric O. Aboagye, Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom. Phone: 4420-8383-3759; Fax: 4420-8383-1783; E-mail: eric.aboagye@imperial.ac.uk

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Translational Relevance

This study investigated the use of [^{18}F]-3′deoxy-3′-fluorothymidine (FLT) PET as an imaging biomarker to measure early response to docetaxel chemotherapy in breast cancer. We found that early changes in tumor FLT uptake predicted lesion size reduction midtherapy. This technology will be particularly useful in those patients where early response to therapy may aid definitive treatment. Further verification of this work in a larger population, could permit the ability to stop therapy in the event of non–FLT-PET response sparing patients of cumulative toxicity.

transcriptionally in the G_1 to S-phase of the cell cycle (12). FLT uptake has been shown to strongly correlate with cell proliferation in untreated patients with breast cancer and further that response by PET imaging is detectable as early as 1 week following fluorouracil-epirubicin-cyclophosphamide (FEC) chemotherapy (13, 14). It is, however, unknown whether the antiproliferative activity of taxanes, an effect that is largely G_2–M in nature, could be detected in patients by FLT-PET. We studied the effects of docetaxel on FLT-PET parameters in this setting.

Methods

Patients

This was a prospective, unblinded, single institution study in patients with American Joint Committee on Cancer (AJCC) stage II–IV breast cancer, including primary breast cancer being unresponsive to first-line chemotherapy (via ultrasound scanning done by the same radiologist to minimize operator-dependent errors), or metastatic disease progressing on previous therapy (with either new lesions appearing or >20% increase in maximum diameter of previous target lesions). Patients underwent a baseline dynamic FLT-PET scan for 66.5 minutes followed by a similar posttreatment scan conducted approximately 14 days after the first or second cycle of docetaxel therapy (dose of docetaxel: 100 mg/m^2). Primary patients were selected if they were not responding or progressing on chemotherapy. Metastatic patients were those who were documented to have progressive disease on last computed tomographic (CT) scan from prior therapy. The main inclusion criteria were histologically confirmed invasive breast cancer with at least one measurable lesion outside the bone or liver, accurately measured in one dimension (longest diameter recorded) as 20 mm or greater using conventional techniques. This was done to reduce the effect of partial volume effect (15, 16). No chemotherapy or radiotherapy was permitted for at least 3 weeks before undergoing first FLT-PET scan. Patients having only bone or liver metastasis were excluded as physiologic uptake of FLT is high in bone and liver and would have obscured any tumor(s) in these organs. The study received ethical approval from the Hammersmith Hospital Research Ethics Committee and a license to inject FLT into subjects from the Administration of Radioactive Substances Advisory Committee. The study was conducted in accordance with the Declaration of Helsinki. All patients gave written informed consent.

Study design and statistical considerations

Previous test–retest reproducibility studies showed that changes in FLT standardized uptake value (SUV) of more than 20% or net irreversible uptake (K_i) of more than 31% could be classified as radiotracer responsive with 95% confidence interval (SD: 10%–15%; ref. 14). Published data indicate a 40% response rate (worse case) to docetaxel therapy in patients with metastatic cancer (6, 8). This would dilute the expected "group" effect from 0.31 to 0.124 and inflate the variability for measuring change to 18%. Power calculation (paired statistics) suggested that a number ($n = 20$) of patients would give to the design a minimum power of 0.80 to detect a group difference in FLT at a 0.05 error rate. Comparisons of tumor results were done on a lesion-by-lesion basis assuming non-Gaussian distribution (Wilcoxon matched pairs test or Mann–Whitney test). The association between FLT retention variables and midtherapy lesion response was explored using Pearson correlation coefficient (R, for continuous data) or χ^2 and Bland–Altman plots (for categorical data). Statistical analyses were conducted using GraphPad Prism version 4.00 for Windows (GraphPad Software; www.graphpad.com).

PET imaging protocol and radiology

FLT was manufactured as previously described (13) with 99.6% radiochemical purity and a mean specific radioactivity of 147,584 MBq/μmol. PET scanning was conducted on the GE-Discovery PET-CT scanner (GE-Healthcare) with a 15.7 cm field of view. Patient preparation for PET has been previously described (13). FLT [mean: 210 (±8) MBq] was injected as a slow bolus in about 5 mL of saline followed by emission scanning for 66.5 minutes. The dose of radioactivity used was in accordance with suggested limits described by Vesselle and colleagues (17). Data were binned into 28 frames and reconstructed. Volumes of interest were drawn on at least 4 slices of tumor (using the combined PET and CT volume) to obtain the activity concentration within the volumes of interest. SUV was calculated by normalizing tumor radiotracer concentration (kBq/mL) to injected radioactivity (in kBq) and body surface area as previously described (13, 14). Average SUV ($SUV_{60,av}$) or the maximum pixel value ($SUV_{60,max}$) were determined. Kinetic modeling of PET data was conducted to calculate K_i (13, 14).

Tumor target lesion size was measured by ultrasound (for primary lesions) or CT (for metastatic disease) prior to treatment and after 3 cycles of docetaxel, and response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (18). Response by FLT-PET was

Table 1. Patient demographics ($n = 20$) showing prior treatment before FLT-PET was initiated and tumor characteristics

				Tumor				
Patient no.	Age, y	Previous treatment	Primary or metastatic disease	Site	Grade	Type	ER/PR	Size, mm
1	67	3 × FEC	Primary	Breast	II	IDC	+/+	30
2	65	3 × FEC	Primary	Breast	III	IDC	+/+	64
3[a]	45	3 × FEC	Primary	Breast	I	IDC	+/+	22
4[a]	56	Arimidex	Metastatic	Breast	III	IDC	+/+	35
5[a]	44	3 × FEC	Primary	Breast	III	IDC	−/−	30
6	41	None	Metastatic	Neck node	III	IDC	−/−	32
7	59	3 × FEC	Primary	Breast	III	IDC	−/−	28
8	44	5 × FEC	Primary	Breast	II	IDC	+/+	21
9[a]	58	2 × FEC	Primary	Breast	II	IDC	−/+	32
10	62	Faslodex	Metastatic	Breast	II	IDC	+/+	18
11	50	Faslodex	Metastatic	Chest wall	II	IDC	+/+	100
12	49	3 × FEC	Primary	breast	II	IDC	+/+	20
13	49	Tamoxifen	Metastatic	chest	II	IDC	+/−	85
14	44	None	Metastatic	breast	III	IDC	−/+	35
15[a]	63	Arimidex	Metastatic	pleura	III	IDC	+/+	28
16	59	None	Metastatic	breast	II	IDC	−/−	30
17[a]	46	None	Metastatic	lung	III	IDC	+/+	20
18	46	3 × FEC	Primary	breast	II	IDC	+/+	37
19	58	3 × FEC	Primary	breast	II	IDC	+/+	31
20	69	Faslodex	Primary	breast	II	IDC	+/+	70

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; +, positive; −, negative; IDC, infiltrative ductal carcinoma.
[a]Cases who underwent posttreatment FLT-PET after 2 cycles of chemotherapy.

defined as more than 20% change for SUV (>31% change for K_i; ref. 14).

Results

Clinical results and visualization of lesions by PET

Twenty patients (11 primary and 9 metastatic; average age, 54 years; average tumor size, 38.4 mm) successfully completed both pre- and posttreatment FLT-PET scans (Table 1). All lesions were seen on FLT-PET (Fig. 1). In most patients, posttreatment FLT-PET was conducted approximately 14 days after initiating cycle 1; in 6 patients (patient no. 3, 4, 5, 9, 15, and 17), the posttreatment PET was conducted 2 weeks after initiating cycle 2 of docetaxel either due to patients being extremely unwell from chemotherapy or failure of FLT production. Two individuals (no. 11 and 12) developed grade III toxicity to docetaxel after the first cycle (needing hospitalization for neutropenic sepsis) and treatment was then changed to weekly paclitaxel based on the clinicians decision. Patient no. 11 had 1 cycle of docetaxel and 3 cycles of paclitaxel and patient no. 12 had 1 cycle docetaxel and 2 cycles of paclitaxel prior to RECIST assessment. PET uptake data for these 2 cases have been presented, but these data were not used for further comparison with RECIST response.

PET imaging outcome

The median (±SD) pretreatment $SUV_{60,av}$ of tumors was 6.47×10^{-5} ($\pm 4.1 \times 10^{-5}$) m^2/mL and that for $SUV_{60,max}$ was 12.6×10^{-5} ($\pm 6.11 \times 10^{-5}$) m^2/mL (Supplementary Table S1). In contrast, the median $SUV_{60,av}$ in normal breast tissue and normal lung were 1.12×10^{-5} ($\pm 0.4 \times 10^{-5}$) m^2/mL and 0.99×10^{-5} ($\pm 0.33 \times 10^{-5}$) m^2/mL, respectively. The visual baseline uptake of FLT was low in 3 tumors (patient no. 4, 8, and 9). Of interest to use FLT-PET for assessing early response, single-agent docetaxel chemotherapy resulted in a significant decrease in FLT uptake (Fig. 1 and Table 2; $P = 0.0003$ for $SUV_{60,av}$ and $P = 0.0002$ for $SUV_{60,max}$; Wilcoxon matched pairs test, for all tumors). The median percentage decrease of $SUV_{60,av}$ for all tumors was 30.7% (median absolute decrease of 2.32×10^{-5} m^2/mL) and for $SUV_{60,max}$, a decrease of 17.5% was observed (median absolute decrease of 3.37×10^{-5} m^2/mL). The median decrease in $SUV_{60,av}$ in PET responders measured 40.2% versus 10.5% in nonresponders (Mann–Whitney test: $P = 0.01$). In one metastatic breast tumor of the lung (patient no. 17), the tumor had 30.2% and 41.8% reductions of $SUV_{60,av}$ and $SUV_{60,max}$, respectively, after 2 cycles of docetaxel with complete radiological response on CT after 3 cycles of docetaxel. For comparison, tumor SUV normalized to body weight rather than body surface area are shown in Supplementary Table S2.

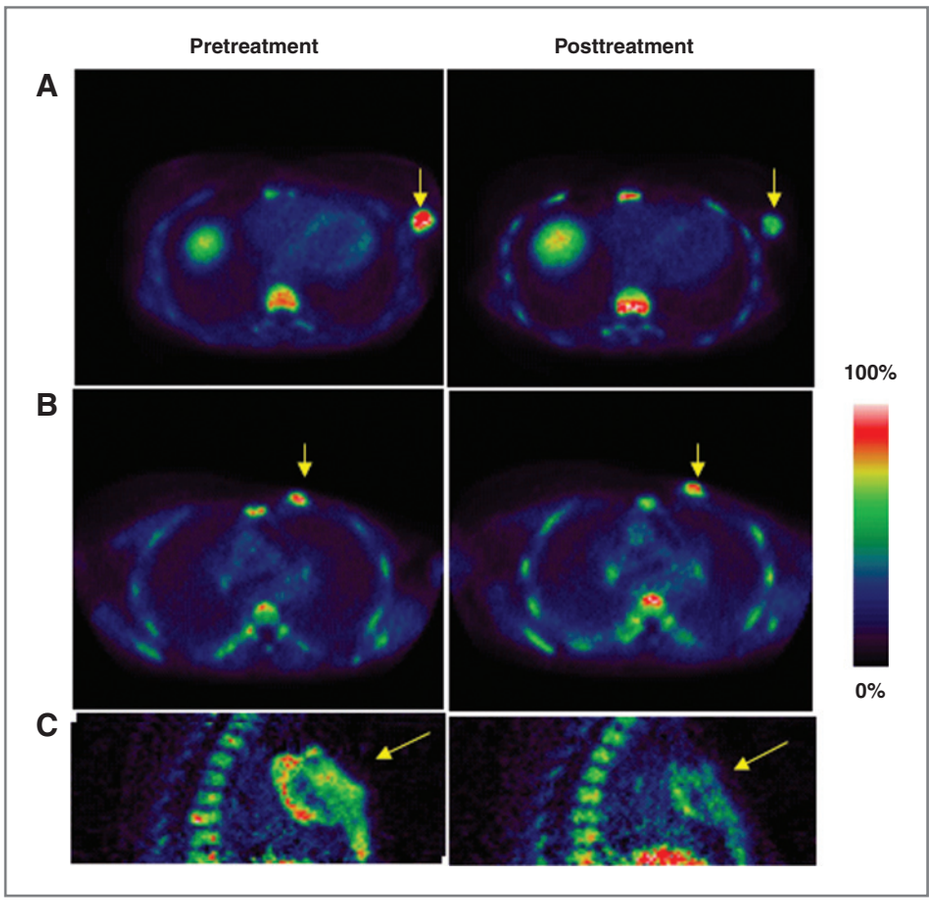

Figure 1. FLT-PET image planes of patients with breast tumors before (pretreatment) and 2 weeks after (posttreatment) the first cycle of docetaxel. A, transverse sections of a primary tumor responding after 1 cycle of docetaxel. B, transverse sections of a chest wall recurrence showing no response after 1 cycle of docetaxel. C, sagittal sections of a large mediastinal tumor mass showing response to treatment. Arrow indicates tumor. Note intensity bar.

Comparison of PET outcome with categorical target lesion response

We compared PET response with clinical outcome for individual target lesions (Table 2). Overall (excluding patient no. 11 and 12), there was 1 complete responder, 12 partial responders, and 5 patients with stable disease. In 2 patients (no. 6 and 14), new metastatic tumor deposits were detected outside the PET field of view during the subsequent routine clinical cross-sectional imaging. We investigated whether reductions in tumor FLT uptake early after initiating therapy were associated with target lesion size changes after 3 cycles (Fig. 2). There was a significant correlation between FLT-PET early signal change and lesion size midtherapy (Fig. 3A). Pearson correlation coefficient was $R = 0.64$ ($P = 0.004$) for $SUV_{60,av}$; this association was less significant for $SUV_{60,max}$ ($R = 0.53$; $P = 0.02$). Categorization of the target lesion size data into two RECIST (19) classes (complete + partial response vs. stable disease) and of the FLT-PET SUV data into two classes (reduction < 20% vs. reduction > 20%), confirmed a valid association (χ^2 test) when the PET measure was $SUV_{60,av}$ ($P = 0.05$) but not $SUV_{60,max}$ ($P = 0.16$). On the basis of the RECIST stratification (19), 13 of 18 tumors responded to therapy (Table 2), and the median percentage decrease in $SUV_{60,av}$ and $SUV_{60,max}$ in these tumors were 40.2% and 34.1%, respectively. FLT-PET $SUV_{60,av}$ correctly detected 11 of the 13 RECIST responders. One tumor (patient no. 9) showed a significant decrease in $SUV_{60,av}$ of 31.2%, but this was deemed borderline stable disease by tumor size measurement using ultrasonography, with tumor size reduction of 21.8%. This is clearly depicted on Bland–Altman plots devised to further explore the association between categorical target lesion size response and changes in PET semiquantitative parameters (Fig. 3B). The parameter, $SUV_{60,av}$, appeared to have better sensitivity to predict categorical lesion size response than $SUV_{60,max}$. Using the 20% change in $SUV_{60,av}$ as (prospective) cutoff point to stratify patients as PET responders (14), the sensitivity and specificity of $SUV_{60,av}$ to predict categorical tumor size response were 0.85 and 0.80, respectively. $SUV_{60,max}$ was less robust with sensitivity of 0.62 but equal specificity of 0.80. With respect to predicting no lesion response, both $SUV_{60,av}$ and $SUV_{60,max}$ conducted well except in one patient (patient no. 9).

Kinetic analysis of tumor FLT uptake

We assessed the steady-state net irreversible flux constant for transfer of FLT from plasma to tumor (K_i; Table 3) as a means of explaining the SUV data. This was done by a metabolite-corrected modified Patlak graphical approach as

Table 2. Comparison of tumor size changes, treatment duration, RECIST response, and changes in SUV

Patient no.	Percentage change in tumor size	Cycles of docetaxel given before RECIST calculation	RECIST response achieved	Percentage change of $SUV_{60,av}$	Percentage change of $SUV_{60,max}$
1	−47	3	PR	−20	−11
2	−8	3	SD	−5	−18
3[a]	−50	3	PR	−46	−18
4[a]	−57	4	PR	−12	3
5[a]	−77	4	PR	−72	−67
6	−38	4	PR (PD)	−52	−40
7	−29	3	SD	−10	−15
8	10	3	SD	2	13
9[a]	−22	4	SD	−31	−14
10	−44	4	PR	−34	−36
13	−31	3	PR	−49	−17
14	0	2	SD (PD)	18	−4
15[a]	−71	3	PR	−30	−2
16	−47	3	PR	−45	−38
17[a]	−100	3	CR	−40	−34
18	−73	3	PR	−44	−38
19	−45	4	PR	−12	−30
20	−40	3	PR	−23	−7

NOTE: Negative value implies decrease in PET parameter. Response in parentheses indicates overall response in body after docetaxel chemotherapy.Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.
[a]Cases who underwent posttreatment FLT-PET after 2 cycles of chemotherapy.

previously described (13, 20). The median (±SD) K_i at baseline was 2.95×10^{-4} ($\pm 2.44 \times 10^{-4}$) mL plasma/mL tissue/s and with good fits (low SD of fit). Drug treatment significantly reduced K_i (Wilcoxon matched pairs test: $P = 0.0009$) with a median percentage change (±SD) of 51.12% (±28.39%) mL plasma/mL tissue/s. For patient no. 9, it was not possible to calculate K_i accurately due to low uptake that decreased toward the end of the scan (possibly due to

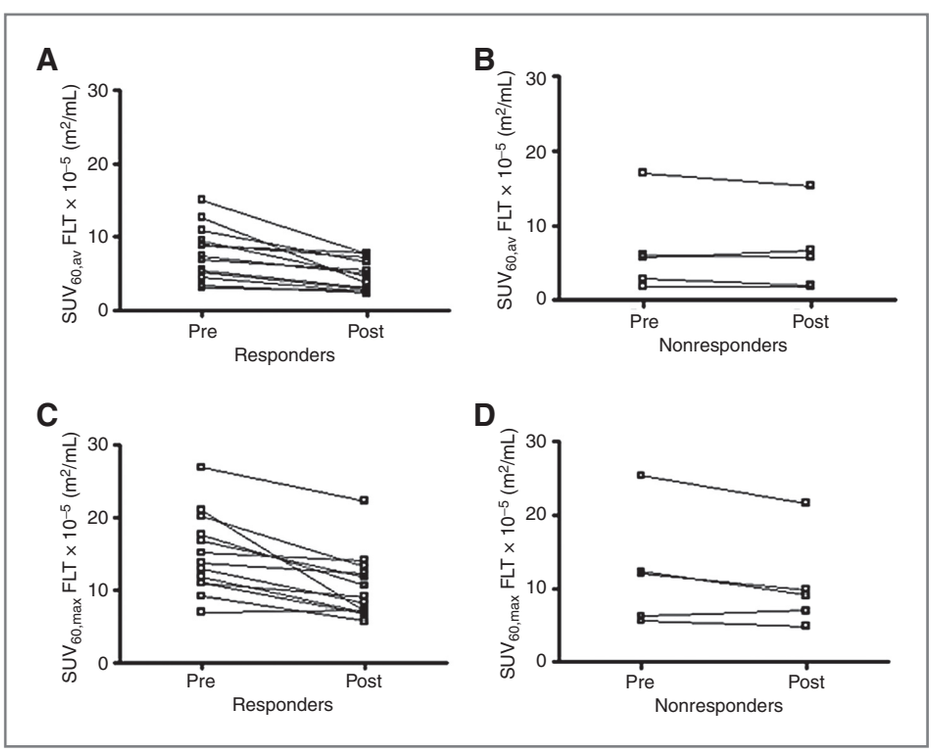

Figure 2. Graphical display of changes in FLT uptake after 1 cycle of docetaxel. Change in $SUV_{60,av}$ in (A) tumor size responders and (B) nonresponders. Changes in $SUV_{60,max}$ in (C) tumor size responders and (D) nonresponders. Classification of tumor size response was based on RECIST criteria.

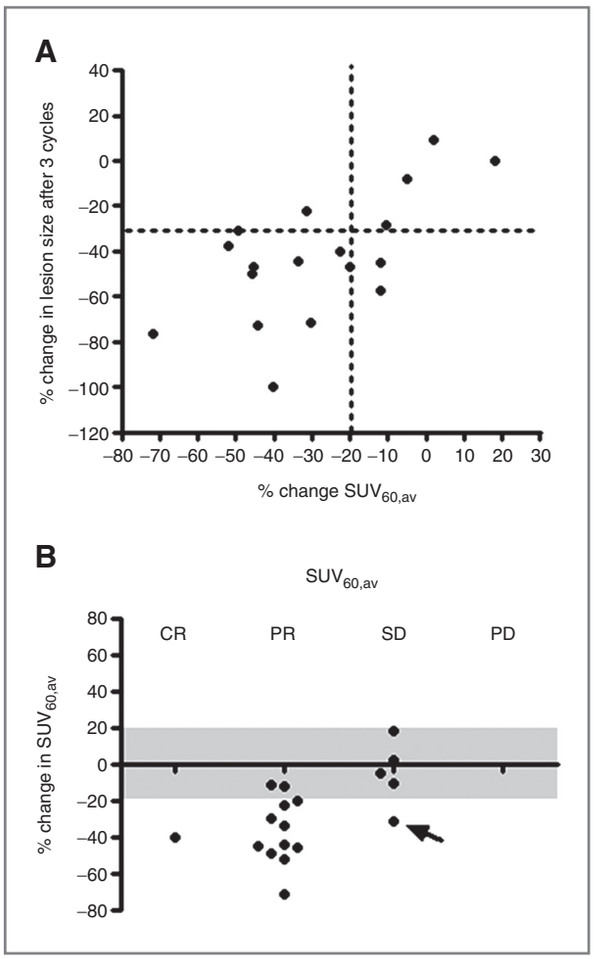

Figure 3. A, comparison of tumor FLT uptake 2 weeks after 1 or 2 cycles of docetaxel with "continuous" target lesion size changes measured by cross-sectional imaging after 3 or 4 cycles. Quadrants depict categorization of FLT $SUV_{60,av}$ (<20% vs. >20%) and tumor size [complete response (CR) + partial response (PR) vs. stable disease (SD)]. The figure illustrates true positives (left bottom), true negatives (right top), false positives (right bottom), and false negatives (top left) B, Bland–Altman plots showing percentage changes in FLT $SUV_{60,av}$ 2 weeks after 1 or 2 cycles of docetaxel in patients compared with target lesion response (categorical). Target lesion response was based on RECIST criteria after 3 to 4 cycles of docetaxel. Arrow indicates 1 tumor that showed 21% reduction in tumor size. Shaded area indicates the 95% normal range of spontaneous fluctuation during serial FLT-PET scan.

dephosphorylation or contribution of labeled metabolites). The drug did not affect FLT metabolism. The median (±SD) percentage of parent FLT in plasma at 60 minutes was 77.6 (±0.07)% and 76.5 (±0.07)%, respectively, before and after docetaxel. Like $SUV_{60,av}$, K_i also showed a significant association with midtreatment target lesion size change (Pearson $R = 0.56$; $P = 0.02$; Table 3). With the exception of patients 6 and 13, K_i was found on lesion-by-lesion comparison to be low (>31%) in tumors that had low $SUV_{60,av}$ (of >20%). This signifies that changes in tumor uptake could be explained largely by net irreversible retention of the radiotracer.

Discussion

The future of taxane therapy includes defining optimal combinations and dosing frequency and strategies to reduce toxicity, as well as trials of novel second-generation taxanes. We showed that early in the course of therapy, an FLT-PET reduction may predict response to docetaxel. FLT-PET has been shown to image proliferation in breast and lung tumors with high reproducibility and accuracy (14, 21, 22). Furthermore, decreases in FLT uptake depicting reductions in cell proliferation within 1 to 2 weeks have been reported in breast cancer (14) and non–Hodgkin lymphoma (23). Most patients in this present trial showed an early reduction in FLT-PET 2 weeks after commencing the first or second cycle of docetaxel. This FLT-PET response study of taxanes in patients are in keeping with intrinsic reductions in FLT uptake reported by Dittmann and colleagues in breast cancer cells (24) and hormone-refractory prostate cancer xenografts (25). This suggests that the activity of docetaxel is detectable by FLT-PET.

The PET technique estimates phosphorylation of FLT to FLT-phosphate. The wide range of pretreatment FLT uptake values may be explained by the wide variation in proliferation (13, 26, 27). Other mechanisms including relative use of salvage versus *de novo* precursors for DNA synthesis could also influence FLT uptake (28, 29). We used the SUV at 60 minutes as a semiquantitative measure of this biochemical activity. In most patients, tumor FLT $SUV_{60,av}$ was higher than the 95% confidence limit of normal breast (1.9×10^{-5} m^2/mL). In patient 8 who received 5 cycles of FEC before entering the study, baseline tumor FLT uptake although detectable above background, was only 1.73×10^{-5} m^2/mL. In general, however, baseline FLT uptake did not appear to be confounded by prior treatment. We used a prospective threshold (14) to define statistically significant FLT response. Overall, the magnitude of tumor FLT reduction was as expected more in clinical responders than nonresponders. Changes in tumor FLT SUV did not differ between patients who had a posttreatment scan after 1 or 2 cycles or between primary and metastatic disease (Mann–Whitney test: $P = 0.6$).

We found a good association between changes in FLT uptake and changes in lesion size. This was confirmed when the data were categorized (Fig. 3). In this study, 2 tumors showed significant reductions in $SUV_{60,av}$ but were classified borderline stable disease (Fig. 3). Despite these important differences, changes in $SUV_{60,av}$ (after 1 or 2 cycles) overall predicted changes in tumor size after 3 cycles with high sensitivity and specificity (0.85 and 0.80, respectively). Analysis of receiver operating characteristics indicated that the optimal sensitivity and specificity (100% and 57.1%, respectively; data not shown) would be achieved when the cutoff value was 11.8%. Notably, using that cutoff point, the high sensitivity was linked to a much lower specificity. Of interest, FLT-PET test–retest reproducibility studies by de Langen and colleagues (30) in non–small cell lung and head and neck cancers suggested that a cutoff value of more than 15% qualified as response. Similarly, Shields and

Table 3. Effect of docetaxel therapy on K_i and SUV in responders and nonresponders

Patient no.	Pre $K_i \times 10^{-4}$ (mL plasma/mL tissue/s)	Post $K_i \times 10^{-4}$ (mL plasma/mL tissue/s)	Percentage change	
			K_i	$SUV_{60,av}$
Responders				
1	6.12	1.33	−78	−20
3[a]	3.79	0.62	−84	−46
4[a]	0.75	0.27	−64	−12
5[a]	5.42	0.77	−86	−72
6	3.09	2.53	−18	−52
10	2.61	0.76	−71	−34
13	9.14	7.12	−22	−49
15[a]	1.64	0.71	−57	−30
16	2.82	1.38	−51	−45
17[a]	4.14	2.73	−34	−40
18	2.18	0.81	−63	−44
19	6.18	5.41	−12	−12
20	4.87	2.23	−54	−23
Nonresponders				
2	2.20	1.62	−26	−5
7	8.65	8.64	0	−10
8	0.79	0.62	−21	2
9[a]	0.50	PF	NP	−31
14	3.38	3.46	3	18

Abbreviations: NP, modeling not possible; PF, poor fit to data.

[a]Cases who underwent posttreatment FLT-PET after 2 cycles of chemotherapy.

colleagues reported a cutoff point of 21% as FLT-PET response in lung cancer (31). We did not consider new lesions in the determination of sensitivity and specificity, as only target lesions in the PET field of view were assessed. Future studies should consider whole body PET imaging to permit additional lesions to be detected. Lack of a significant change in FLT-PET will indicate futile treatment and signify a change to alternative treatment. In contrast, an FLT-PET response will indicate reduction in proliferation that probably warrants another assessment (say after 1 additional cycle) to confirm efficacy.

Muzi and colleagues have suggested that, unlike labeled thymidine, FLT phosphorylation may be reversible in somatic tumors making simple variables such as $SUV_{60,av}$ liable to error (32). Hence, we evaluated the net unidirectional flux (constant, K_i) of FLT from plasma into the intracellular space (via transport and phosphorylation) at steady state. A linear phase was discernible in the graphical plots for most patients indicating net retention. Noisy fits were obtained for tumors (patient no 4, 8, and 9) with low proliferation ($SUV_{60,av} < 3 \times 10^{-5}$ m^2/mL; $K_i < 1$ mL plasma/mL tissue/s). Patient 9 had an uptake profile characterized by an initial positive linear slope followed by a downward slope, consistent with dephosphorylation of FLT-phosphate. Further compartmental analysis supported this assertion; there was a reasonable association between K_i and k_3 ($R = 0.42$, $P = 0.02$; data not shown). These data indicate that although simple measures of FLT phosphorylation may have value for widespread clinical use, analysis may be limited in low proliferative tumors, in this case, tumors with $SUV_{60,av}$ values of 3×10^{-5} m^2/mL (1.2 when normalized to body weight) or less.

There were limitations of this study that are worthy of discussion. Both primary and metastatic patients, who may respond differently to docetaxel (4, 5, 8), were included in this study potentially impacting on heterogeneity of response. Notably, however, target lesion changes measured after 3 cycles did not appear to differ between the 2 groups (Mann–Whitney test: $P > 0.05$). While the overall sample size was small (sample size powered to detect a 20%–31% change in PET variables), this represents a single tumor type group of patients and with the same treatment and allowed detailed assessment of both simple measures of uptake, as well as more detailed analysis of radiotracer kinetics. In this study, tumor size reduction after 9 weeks was used for comparison with changes in tumor FLT uptake at 2 weeks after cycle 1 or 2. Given that FLT-PET changes occur rapidly after treatment (14), a potential limitation of the study was the assessment of PET response after 2 cycles in 6 patients (compared with one cycle in the rest). The reduction in tumor SUV was, however, not statistically different between the 2 groups (Supplementary Table S3, and, therefore, reported separately but combined for analysis). For these patients who had FLT studies after 2 cycles,

the benefit of using FLT to predict response would be less obvious. Another limitation is the use of RECIST rather than histopathology for comparison. RECIST response was determined at the physician's discretion earlier after cycle 2 in one patient (patient 14) due to suspicion of clinical disease progression. Though this could be seen as a limitation of the study, it did not affect the overall prediction of response with FLT-PET. RECIST after 3 cycles is often used to assess whether a drug has biologic activity, though its correlation with survival is imperfect (18). It will be interesting in future studies to investigate histologic markers of proliferation (primary disease; this will involve repeat biopsies) and/or progression-free and overall survival as endpoints in a larger more homogeneous setting. Finally, on the basis of our prospective cutoff value, two patients would potentially have been denied further docetaxel treatment on the basis of early PET response. This highlights the ethical challenge of defining the ideal cutoff value for response, that is, when sensitivity is not high enough. Decreasing the cutoff value should improve sensitivity but at the cost of specificity.

In aggregate, FLT-PET is a promising imaging biomarker to detect early response to docetaxel in breast tumors and could potentially enable tailoring of therapy to individual patients warranting prospective trials to assess the ability to stop therapy in the event of non–FLT-PET response. As a next step, the promising results should be confirmed in larger trials in a more homogeneous setting and with longer follow-up, with disease-free survival and perhaps overall survival as additional endpoints. $SUV_{60,av}$ reductions were due, in part, to inhibition of tracer retention and correlated with target lesion size response after 3 cycles of chemotherapy. The outcome of this study may have general application for testing of new taxanes or new taxane regimes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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