

# Identification of Biomarkers Including $^{18}\text{F}$ FDG-PET/CT for Early Prediction of Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer

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## Abstract

**Purpose:** To investigate the value of the metabolic tumor response assessed with  $^{18}\text{F}$ -fluorodeoxyglucose positron emission tomography (FDG-PET), compared with clinicobiologic markers to predict pathologic complete response (pCR) to neoadjuvant chemotherapy (NAC) in women with triple-negative breast cancer (TNBC).

**Experimental Design:** Fifty consecutive women with TNBC and an indication for NAC were prospectively included. Different pretreatment clinical, biologic, and pathologic biomarkers, including SBR grade, the Ki-67 proliferation index, androgen receptor expression, EGF receptor (EGFR), and cytokeratin 5/6 staining, were assessed. Tumor glucose metabolism at baseline and its change after the first cycle of NAC ( $\Delta\text{SUV}_{\text{max}}$ ) were assessed using FDG-PET.

**Results:** The pCR rate was 42%. High Ki-67 proliferation index ( $P = 0.016$ ), negative EGFR status ( $P = 0.042$ ), and high  $\Delta\text{SUV}_{\text{max}}$

( $P = 0.002$ ) were significantly associated with pCR. In multivariate logistic regression, both negative EGFR status (OR, 6.4;  $P = 0.043$ ) and high  $\Delta\text{SUV}_{\text{max}}$  (OR, 7.1;  $P = 0.014$ ) were independent predictors of pCR. Using a threshold at  $-50\%$ , tumor  $\Delta\text{SUV}_{\text{max}}$  predicted pCR with a negative, a positive predictive value, and an accuracy of 79%, 70%, and 75%, respectively. Combining a low  $\Delta\text{SUV}_{\text{max}}$  and positive EGFR status could predict non-pCR with an accuracy of 92%.

**Conclusions:** It is important to define the chemosensitivity of TNBC to NAC early. Combining EGFR status and the metabolic response assessed with FDG-PET can help the physician to early predict the probability of achieving pCR or not. Given these results, the interest of response-guided tailoring of the chemotherapy might be tested in multicenter trials. *Clin Cancer Res*; 21(24); 5460–8. ©2015 AACR.

## Introduction

Although neoadjuvant chemotherapy (NAC) does not improve survival when compared with adjuvant chemotherapy (1), it is increasingly used in operable breast cancer to downstage the breast tumor and to make breast-conserving surgery possible (2, 3).

Breast cancer includes several molecular entities that differ in their clinical behavior, biologic characteristics, and outcomes (4, 5). Triple-negative breast cancers (TNBC) account for roughly 15% of breast tumors (6, 7) and are defined by the absence of

hormone receptor expression and no overexpression of HER2 (8). This subtype is characterized by its higher aggressiveness and poorer outcome compared with other subtypes (6) but also by its high responsiveness to NAC, called the "triple-negative paradox" (9, 10). Indeed, a pathologic complete response (pCR) is often reached at the end of NAC and is associated with a more favorable long-term outcome. In contrast, women who do not achieve pCR have a higher risk of relapse and reduced overall survival (10–12). This explains why pCR is often used as an important endpoint in the treatment of TNBC. One other important characteristic of TNBC is the diversity within this subgroup, as it includes distinct molecular subtypes. Despite a clear need for therapeutic options for women with TNBC tumors, their heterogeneity and the absence of high-frequency molecular alterations have limited the development of targeted therapies (7). It is therefore important to identify clinical, biologic, or imaging biomarkers that can predict early the therapeutic response. The aim is to avoid the use of an ineffective treatment in nonresponding women and to give them a better chance with an alternative therapy.

Fluorine-18 fluorodeoxyglucose positron emission tomography ( $^{18}\text{F}$ -FDG-PET/CT) is the gold standard for the *in vivo* evaluation of tumor glucose metabolism. Studies on the use of PET/CT to monitor early tumor response to NAC have shown promising results in predicting the final pCR whatever the tumor

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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### Translational Relevance

The early identification of women with triple-negative breast cancers (TNBC) with low chemosensitivity to standard neoadjuvant chemotherapy (NAC) is an important issue. The aim is not only to predict the better outcome in women with responsive TNBC but also to avoid the use of an ineffective treatment in nonresponding women and to give them a better chance with an alternative therapy. The present study demonstrated the value of FDG-PET imaging compared with the usual clinicobiologic markers, such as the Ki-67 tumor proliferation index and EGFR tumor expression, in the early prediction of a pathologic complete response to NAC. Given these results, multicenter trials using PET-guided treatment strategies are now necessary to evaluate the benefit of early therapeutic changes in poorly responding women. Such a strategy should lead to enhanced personalized medicine.

subtype (13–15). Few studies, however, have evaluated the relevance of metabolic responses to predict pCR in the TNBC subtype.

This prospective trial aimed to investigate the value of metabolic tumor parameters, assessed at baseline and after the first cycle of treatment with FDG-PET studies in the early prediction of pCR, and to compare these with pretreatment biologic markers.

## Materials and Methods

### Patients and study design

From November 2006 to March 2013, 240 women referred to our institution (Centre Georges-François Leclerc, Dijon, France) because of clinical stage II or III invasive breast cancer with an indication for NAC were consecutively and prospectively evaluated in this study. The clinical stage was first assessed using physical examination and conventional imaging procedures (mammogram and/or breast ultrasound, bone scan, abdominal ultrasound, chest X-rays). Only women with TNBC were included in the study. Patients with high glycemia (>9 mmol/L), unwilling to undergo the 2 PET exams or with unexpected metastasis on baseline FDG-PET were excluded. The institutional review board approved this prospective study, and the women gave their informed consent.

Patients were treated with rule-based NAC: most underwent sequential NAC with anthracyclines [FEC100: fluorouracil (FU), 500 mg/m<sup>2</sup>; epirubicin, 100 mg/m<sup>2</sup>; cyclophosphamide, 500 mg/m<sup>2</sup>, 3 courses every 3 weeks] and taxanes (docetaxel, 100 mg/m<sup>2</sup>, 3 courses every 3 weeks). Some of those ( $n = 9$ ) included at the beginning of the study underwent continuous NAC with 6 cycles of anthracyclines (FEC100) every 3 weeks.

About 1 month after the last course of NAC, the tumors were surgically removed and pCR was defined as no residual invasive cancer in the breast and nodes, although *in situ* breast residuals were allowed (ypT0/is ypN0; ref. 11). pCR was the endpoint of the study. This study followed the REMARK criteria (16).

### Histopathologic analysis

Pretreatment core biopsies from the primary tumor were used to determine the histologic type and the tumor SBR grade (17) established by evaluating the architectural differentiation, nuclear

polymorphism, and rate of mitosis. The immunohistochemical (IHC) analyses reported in this study were carried out in a single laboratory, and the slides were read by a single pathologist. The following molecular markers were examined: estrogen receptor (ER), progesterone receptor (PR), HER2 expression, Ki-67 proliferation index, androgen receptor (AR), EGF receptor (EGFR), and cytokeratin (CK) 5/6 tumor expression.

Tumor samples were quickly fixed on buffered formalin, embedded in paraffin, and cut into 4- $\mu$ m-thick sections with a microtome. IHC was performed with an indirect immunoperoxidase method using antibodies directed against HER2 oncoprotein, ER and PR (HER2: prediluted rabbit monoclonal antibody 4B5; ER: prediluted rabbit monoclonal antibody SP1; PR: prediluted rabbit monoclonal antibody 1E2; Ventana), AR (monoclonal mouse anti-human androgen receptor, clone AR441, dilution 1/10e; Dako), Ki-67 (monoclonal mouse anti-human Ki67 antigen, clone MIB-1, dilution 1/50e; Dako), EGFR (mouse anti-epidermal growth factor receptor, clone 31G7, dilution 1/30e; Invitrogen), and CK 5/6 (mouse anti-cytokeratin 5 and 6, clone D5/16B4, dilution 1/25e; Invitrogen). All immunostaining was performed on an automated immunostainer (Ventana XT). ER and PR status were considered positive if the tumor showed at least 10% of positive cells (18). HER2 status was graded according to the HercepTest scoring system modified by ASCO/CAP recommendations (0, 1+, 2+, or 3+; ref. 19). Scores of 3+ were considered positive. In cases of 2+ scores, FISH was used to confirm HER2 amplification, using the dual color HER2 and CEN17 probes (ZytoLight, SPEC HER2/CEN17 Dual Color Prob Kit, Zytovision GmbH). HER2 amplification was defined according to ASCO/CAP criteria by a ratio of HER2/CEN17 > 2.2 (19). As for ER and PR tumor status, AR status was considered positive if the tumor showed at least 10% of positive cells. No cutoff is currently recommended for the definition of EGFR and CK5/6 positivity. In previous studies, tumors with no or faint membrane staining ( $\leq 5\%$ ) were considered negative for EGFR and CK5/6 (20, 21). The basal-like subtype was defined by positive EGFR and/or CK5/6 (22); other subtypes were considered non-basal-like TNBC. Finally, the cell proliferation index was studied, using Ki-67 tumor expression. As performed in 2 previous studies, a threshold for Ki-67 tumor expression of 50% was used to distinguish between tumors with a low ( $\leq 50\%$ ) and high (>50%) proliferation fraction (20, 21).

### FDG-PET/CT procedures

A first FDG-PET/CT scan was done at baseline. Two different PET/CT imaging systems were used: a Gemini GXL PET/CT scanner from November 2006 to December 2010 and a Gemini TF PET/CT scanner from December 2010 to March 2013 (Philips Medical Systems). Patients were instructed to fast for at least 6 hours before the intravenous injection of 5 MBq/kg of <sup>18</sup>F-FDG for Gemini GXL studies and 3 MBq/kg for Gemini TF studies. Patients were asked to rest. Sixty minutes after the injection, a whole-body PET/CT scan was done from the brain to the mid-thigh, with the patient supine. Finally, 90 minutes after the injection, a PET/CT scan restricted to the chest (2 bed positions) with patients in the prone position, both arms raised, was done. Emission data were all corrected for dead time, random, and scatter coincidences and attenuation before reconstruction with the RAMLA iterative method.

A second FDG-PET/CT scan with chest-restricted acquisition was done just before the second course of NAC and 90 minutes

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after the injection. For each patient, the same imaging system, FDG activity, and time from injection to acquisition were used for both studies.

**Tumor glucose metabolism measurements.** A spheroidal VOI encompassing the primary tumor was manually drawn on the chest-restricted acquisitions obtained at 90 minutes after tracer injection to measure the standardized uptake value maximal index (SUV<sub>max</sub>) at baseline (SUV1<sub>max</sub>) and after the first course of NAC (SUV2<sub>max</sub>). Measured SUV<sub>max</sub> were systematically corrected for body surface area (BSA) and glycemia, as detailed in our previous studies (23).

The metabolic response to NAC was calculated as follows:

$$\Delta\text{SUV}_{\text{max}}(\%) = 100 \times (\text{SUV2}_{\text{max}} - \text{SUV1}_{\text{max}}) / \text{SUV1}_{\text{max}}$$

### Statistical analysis

WinSTAT software (Microsoft) and Systat software (Systat Inc.) were used for the statistical analyses. Data were described as numbers (percentages) or means and SD.

Associations between metabolic tumor parameters, clinicopathologic, molecular variables, and pCR achievement were assessed with the Mann–Whitney test.

Clinical, pathologic, molecular, and functional imaging parameters to predict pCR were identified in univariate and multivariate logistic regression. For  $\Delta\text{SUV}$  and Ki-67 tumor expression, discrimination thresholds defined in previous studies were used: 50% for Ki-67 tumor expression (20, 21) and –50% for  $\Delta\text{SUV}$  (24). Because no study was available concerning the cutoff for SUV2, its mean value was used.

Multivariate logistic regression with backward variable selection was done to identify predictive variables of independent statistical significance. To prevent colinearity, among the different PET parameters predictive of pCR in the univariate analysis, only the most predictive one ( $\Delta\text{SUV}_{\text{max}}$ ) was selected in the multivariate analysis.  $P < 0.05$  was considered significant.

## Results

### Patients' characteristics

Among the 240 women evaluated, 56 (23%) were identified as having TNBC (Table 1 and Fig. 1). Two of them were excluded because of technical problems during the baseline PET and 4 of them because NAC was no longer indicated after the first PET scan (obvious stage IV upstaging). In the remaining 50 patients included, 6 missed the second PET scan because of problems with the equipment or because they declined this second scan. The median age was 47 years (range, 26–70 years). Eighteen women (36%) were postmenopausal. The median primary tumor size, assessed with breast ultrasound and/or mammogram, was 4.0 cm (range, 1.5–7.0cm). According to axillary and subclavicular ultrasound scan, 37 of 50 women had a lymph node involvement. All of the tumors were invasive ductal carcinoma and 85% were the basal-like subtype. Mean Ki-67 tumor expression was  $67\% \pm 18\%$ . AR, EGFR and CK5/6 tumor expression were positive in 17% (8 of 46), 44% (20 of 46), and 74% (34 of 46) of tumors, respectively.

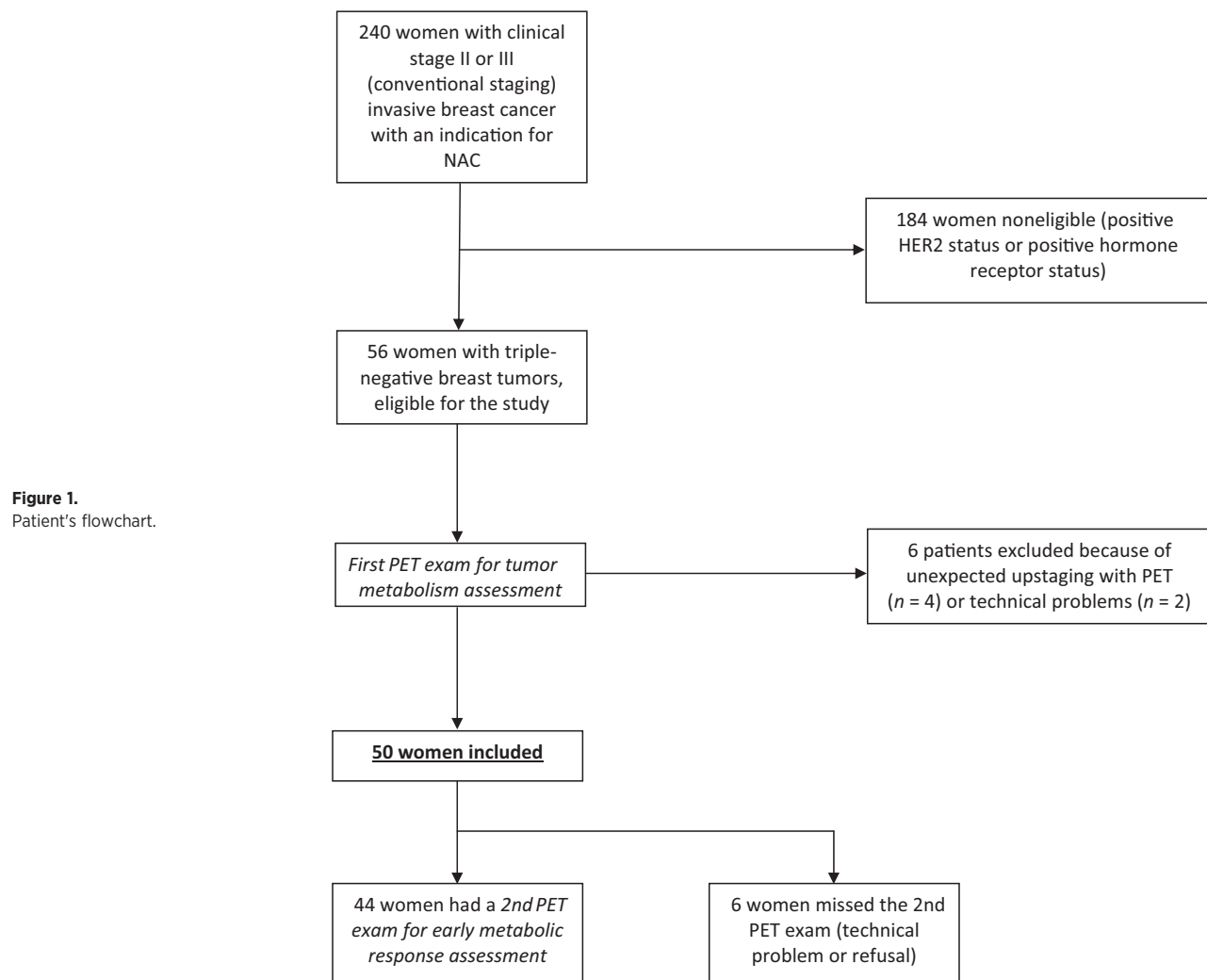
Mean tumor SUV1<sub>max</sub> ( $\pm$ SD) was  $13.4 \pm 7.6$ . After the first cycle of NAC, mean tumor SUV2<sub>max</sub> was  $6.9 \pm 4.1$  (Supplementary Fig. S1). Mean tumor  $\Delta\text{SUV}_{\text{max}}$  was  $-43.7\% \pm 25.4\%$ . Thirty-four patients of 50 had significant FDG uptake in the axillary lymph nodes, strongly suggesting lymph node involvement. Mean

**Table 1.** Patient characteristics

Characteristic	n (%)
Total patients	50
Menopause	
No	32
Yes	16
Unknown	2
T-stage (tumor size obtained with breast ultrasound and/or mammogram)	
T1	6
T2	34
T3	4
T4	6
AJCC clinical stage <sup>a</sup>	
IIA	13
IIB	21
IIIA	7
IIIB	2
IIIC	7
Tumor grading	
Grade I	0
Grade II	7
Grade III	39
Missing	4
Architectural differentiation	
Score I	0
Score II	7
Score III	38
Missing	5
Nuclear pleomorphism	
Score I	0
Score II	4
Score III	41
Missing	5
Number of mitoses	
Score I	3
Score II	9
Score III	33
Missing	5
Ki-67 tumor expression (%)	
Mean $\pm$ SD	67 $\pm$ 18
Median	70
Missing	7
EGFR expression	
Negative	26
Positive	20
Missing	4
AR status	
Negative	38
Positive (>10%)	8
Missing	4
CK5/6 expression	
Negative	12
Positive	34
Missing	4
Phenotype	
Basal-like	39
Non-basal-like	7
Missing	4
Chemotherapy regimen	
6 courses of FEC100	9
3 courses of FEC100 followed by 3 courses of docetaxel	41
Surgery	
Breast-conserving surgery	37
Mastectomy	13
pCR (ypT0/is ypN0) <sup>b</sup>	
Yes	21
No	29

<sup>a</sup>Clinical stage according to the seventh edition of the American Joint Committee on Cancer (AJCC) staging manual (tumor size assessed with ultrasound scan, lymph node involvement, and metastasis assessed both with ultrasound scan and with FDG-PET, metastasis status assessed with FDG-PET).

<sup>b</sup>pCR is defined as ypT0/is ypN0: no invasive residual in the breast and nodes; *in situ* breast residuals allowed.



lymph node  $SUV_{1max}$  was  $7.1 \pm 5.0$ . After the first cycle of NAC, mean lymph node  $SUV_{2max}$  was  $3.0 \pm 3.5$ . Mean nodes  $\Delta SUV_{max}$  was  $-50.1\% \pm 29.2\%$ . Nine women exhibited a complete nodal metabolic response after the first cycle of treatment (qualitative disappearance of nodal uptake).

The NAC regimens are detailed in Table 1. Conservative surgery was performed in 74% (37 of 50) of the women. The pCR rate was 42% (21 of 50).

#### Association between clinical/histopathologic parameters and FDG-PET features

Higher tumor grading, tumor architectural dedifferentiation, and inflammatory breast cancer were associated with higher tumor  $SUV_{1max}$  ( $P = 0.003$ ,  $P = 0.03$  and  $0.04$  respectively; Table 2).

Tumors with no AR expression tended to have higher  $SUV_{1max}$ , but this difference was not significant ( $SUV_{1max} = 14.3 \pm 8.0$  in AR-negative tumors vs.  $9.9 \pm 6.6$  in AR-positive tumors,  $P = 0.09$ ).

Using a threshold of 50%, a higher Ki-67 proliferation index was not associated with baseline tumor  $SUV_{max}$  but was associated with a greater metabolic response ( $\Delta SUV_{max} = -47.2\% \pm 25.3\%$  in high-proliferation tumors vs.  $-23.4\% \pm 23.0\%$  in low-proliferation tumors,  $P = 0.02$ ).

#### Association between pCR and tumor clinicopathologic, biologic, and imaging biomarkers

Using the Mann-Whitney test, pCR was significantly associated with higher Ki-67 tumor expression ( $P = 0.016$ ) and higher  $\Delta SUV_{max}$  ( $P = 0.0004$ ; Tables 3 and 4, Fig. 2). Mean  $\Delta SUV_{max}$  was  $-58.7\% \pm 19.5\%$  in women achieving pCR and  $-32.2\% \pm 23.6\%$  in those without pCR. Mean Ki-67 expression was  $77\% \pm 13\%$  in women achieving pCR and  $62\% \pm 21\%$  in those without pCR.

In univariate logistic analysis, negative EGFR status ( $P = 0.042$ ), high metabolic response (cutoff =  $-50\%$ ;  $P = 0.002$ ), and low tumor  $SUV_{2max}$  (cutoff = 6.9;  $P = 0.013$ ) correlated with pCR (Table 4). Using 50% tumor expression as a cutoff, Ki-67 tended to be, but was not, significantly correlated with pCR ( $P = 0.079$ ).

The most accurate biomarker to predict a pCR was tumor  $\Delta SUV_{max}$ : with the cutoff at  $-50\%$  previously defined by Groheux and colleagues (24), the negative predictive value (NPV), positive predictive value (PPV), sensitivity, specificity, and accuracy of a high  $\Delta SUV_{max}$  to predict pCR were 79%, 70%, 74%, 76%, and 75%, respectively (Fig. 2).

In multivariate analysis,  $\Delta SUV_{max}$  was the strongest independent predictor of pCR: High tumor  $\Delta SUV_{max}$  had a high OR of 7.1

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**Table 2.** Metabolic characteristics of the primary tumor according to the clinical, biologic, and pathologic status

	Baseline SUV <sub>max</sub>			ΔSUV <sub>max</sub>		
	n	Mean ± SD	P <sup>a</sup>	n	Mean (%) ± SD	P <sup>a</sup>
All patients	50	13.4 ± 7.6		44	-43.7 ± 25.4	
Inflammatory breast cancer			<b>0.04</b>			NS
No	46	13.0 ± 7.3		40	-44.7 ± 23.3	
Yes	3	22.2 ± 7.7		3	-52.3 ± 32.6	
Tumor grading (SBR)			<b>0.003</b>			NS
Score II	7	7.3 ± 3.5		6	-33.7 ± 12.3	
Score III	39	14.8 ± 7.5		34	-45.5 ± 27.0	
Architectural differentiation			<b>0.03</b>			NS
Score II	7	9.3 ± 3.0		7	-40.2 ± 22.3	
Score III	38	14.7 ± 7.7		33	-44.4 ± 26.5	
Ki-67 expression			NS			<b>0.020</b>
≤50%	11	11.7 ± 6.6		8	-23.4 ± 23.0	
>50%	32	13.9 ± 6.4		29	-47.2 ± 25.3	
EGFR expression			NS			NS
Negative	26	13.7 ± 7.4		24	-46.8 ± 25.5	
Positive	20	13.4 ± 8.6		16	-35.6 ± 24.4	
CK5/6 expression			0.06			NS
Negative	12	10.3 ± 6.9		12	-41.9 ± 19.6	
Positive	34	14.7 ± 8.0		28	-42.5 ± 27.8	
AR status			0.09			NS
Negative (<10%)	38	14.3 ± 8.0		33	-44.5 ± 26.2	
Positive	8	9.9 ± 6.6		7	-31.7 ± 18.6	
Phenotype			NS			NS
Basal-like	39	14.1 ± 7.7		33	-42.2 ± 26.3	
Non-basal-like	7	10.5 ± 8.8		7	-42.8 ± 21.8	
Chemotherapy regimen			NS			NS
Continuous (FEC100)	9	13.9 ± 5.9		9	-54.7 ± 22.1	
Sequential (FEC100 + taxotere)	41	13.3 ± 8.0		35	-40.8 ± 25.8	
Surgery			NS			<b>0.03</b>
Breast-conserving surgery	37	14.1 ± 7.8		33	-48.5 ± 23.0	
Mastectomy	13	11.4 ± 7.0		11	-29.0 ± 27.8	

NOTE: Age (cutoff, 50 years), menopausal status, tumor size (cutoff, 5 cm), nuclear pleomorphism, number of mitoses, phenotype (basal vs. nonbasal), CA15.3, ACE, and CA-125 values were not significantly correlated with tumor metabolic characteristics. Bold numerals correspond to statistically significant *P* values (<0.05). Abbreviation: NS, not significant (*P* < 0.1).

<sup>a</sup>Mann-Whitney test.

[95% confidence interval (CI), 1.48-33.3; *P* = 0.014]. EGFR tumor status was also an independent predictor of pCR (OR, 6.4; 95% CI, 1.05-40.0; *P* = 0.043).

The patient's age, menopausal status, tumor size, UICC staging, lymph node involvement, tumor inflammation, tumor histologic grading, CA15.3 values, ACE values, and tumor baseline metabolism showed no correlation with the pCR rate.

Combining a low metabolic response ( $\Delta\text{SUV}_{\text{max}} \leq -50\%$ ) with positive EGFR status provided the opportunity to predict non-

pCR with an accuracy of 92% (11 women with non-pCR of a total of 12 women). Conversely, the association of a good metabolic response and negative tumor EGFR status predicted pCR with an accuracy of 77% (10 of 13 women).

Concerning the axillary tumor response, none of the 9 women with a complete lymph node response on interim PET had residual axillary disease at the end of NAC.

## Discussion

Among the various breast cancer subtypes, the TNBC subtype remains a challenge as it has a poor prognosis and as no specific targeted therapy is currently available (6, 7). Indeed, TNBC is associated with higher risk of distant recurrence and death, especially within the first 3 years after diagnosis (8, 25). It is thus extremely important to identify the clinicobiologic, molecular, or imaging biomarkers that can early predict which TNBC tumors will respond to NAC. The ultimate goal is to better tailor neoadjuvant treatment in poorly responding TNBC.

### Biologic biomarkers of tumor response

The present prospective study was undertaken to determine which biomarkers could predict a pCR in TNBCs. No pretherapy clinical factors, including UICC staging, are able to predict pCR. Among the biologic markers, negative EGFR status correlated with the achievement of pCR (Table 4), which is consistent with previous studies (20, 21).

**Table 3.** Tumor biologic and imaging characteristics according to achievement of pCR

	N	Mean ± SD	P <sup>a</sup>
SUV1 <sub>max</sub>			
pCR	21	14.3 ± 6.1	NS
No pCR	29	12.8 ± 8.6	
SUV2 <sub>max</sub>			
pCR	19	5.6 ± 3.0	0.09
No pCR	25	7.9 ± 4.6	
ΔSUV <sub>max</sub> (%)			
pCR	19	-58.7 ± 19.5	<b>0.0004</b>
No pCR	25	-32.2 ± 23.6	
Ki-67 expression (%)			
pCR	18	77 ± 13	<b>0.016</b>
No pCR	25	62 ± 21	

NOTE: CA15.3, ACE, and CA-125 values were not significantly correlated with pCR. Bold numerals correspond to statistically significant *P* values (<0.05).

<sup>a</sup>Mann-Whitney test.

**Table 4.** Univariate and multivariate logistic analysis of significant predictive factors for pCR

	Patients		Univariate analysis		Multivariate analysis		
	Overall (n)	pCR (%)	OR	P	OR	(95% CI)	P
SUV2 <sub>max</sub>							
≥6.9	18	3 (17)	1				
<6.9	26	16 (61)	5.6	<b>0.013</b>			
Missing	6	2					
ΔSUV <sub>max</sub>							
>−50%	24	5 (21%)	1		1		
≤−50%	20	14 (70%)	8.8	<b>0.002</b>	7.09	(1.48–33.3)	<b>0.014</b>
Missing	6	2					
Number of mitosis							
Score I and II	12	3 (25)	1				
Score III	33	18 (54)	3.6	0.089			
Missing	5	0					
Ki-67 expression							
≤50%	11	2 (18%)	1				
>50%	32	16 (50%)	4.5	0.079			
Missing	7	3					
EGFR							
Positive	20	4 (20)	1		1		
Negative	26	13 (50)	4.0	<b>0.042</b>	6.45	(1.05–40.0)	<b>0.043</b>
Missing	4	4					

NOTE: Age (cutoff, 50 years), menopausal status, pregnancy history, UICC staging (2 vs. 3), SBR grade, architectural differentiation, nuclear pleomorphism, AR status, CA15.3 (cutoff, 30 kU/L), CA-125 (cutoff, 35 kU/L), ACE (cutoff, 0.8 μg/L), SUV1<sub>max</sub>, tumor size, tumor inflammation, CK, tumor phenotype (basal and non-basal) were not significant predictive factors of pCR. Among the significant imaging parameters in univariate analysis, only the most predictive one was used for multivariate analysis. Bold numerals correspond to statistically significant *P* values (<0.05).

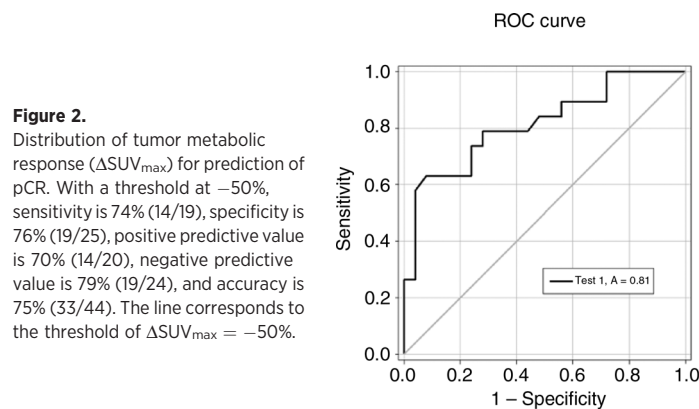
As the biology of TNBC is better understood today, it is clear that TNBC does not directly correspond to a single molecular entity but rather to a heterogeneous subgroup that includes various genomic entities (26, 27). The basal-like subtype, defined by gene expression analysis, is the most frequent one. Using IHC, the basal-like subtype can be approached as CK5/6-positive and/or EGFR-positive (22, 28). We found that a negative EGFR status, mainly observed in non-basal-like tumors, was independently associated with a higher pCR rate (50% in EGFR-negative tumors vs. 20% in EGFR-positive tumors). This result is consistent with 2 previous reports, which showed a higher pCR rate in EGFR-negative and non-basal-like tumors, defined by IHC (20, 21) but not with the results of Rouzier and colleagues, who reported that the basal-like subtype, defined using gene expression profiling, was more sensitive to NAC than were normal-like cancers (29). The reason may be that the normal-like subtype, classified according to the gene expression profile, included 60% of ER-positive tumors and thus did not match the non-basal-like phenotype based on IHC. Moreover, Lehmann and colleagues

reported a 7-subtype molecular classification of TNBC, in which they distinguished between 2 basal-like subtypes: BL1 and BL2 (30). Masuda and colleagues recently demonstrated that among the 7 TNBC subtypes, the BL1 subtype had the highest chemosensitivity (pCR rate = 52%) whereas the BL2 subtype had the lowest one (pCR rate = 0%; ref. 31). Thus, clinically speaking, basal tumors cannot be considered a single good-response entity.

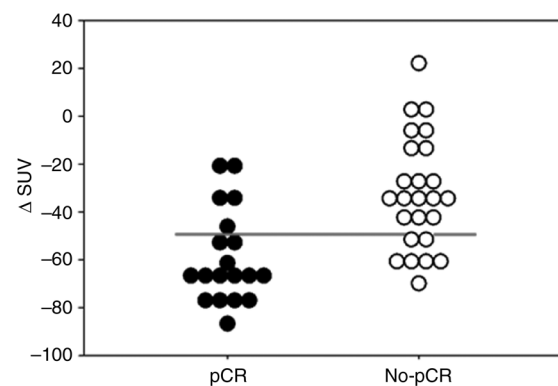
Classification based on IHC is easier to use in clinical practice and less expensive than gene expression. Nevertheless, one limit is the absence of consensus regarding the optimal threshold to define EGFR, CK5/6-positive tumors or the Ki-67 optimal cutoff.

pCR achievement was also associated with higher Ki-67 tumor expression (*P* = 0.016, Table 3) but, in contrast with previous studies (20, 21, 32), Ki-67 expression could not significantly predict pCR in the logistic regression analysis (*P* = 0.08 with a 50% tumor expression threshold).

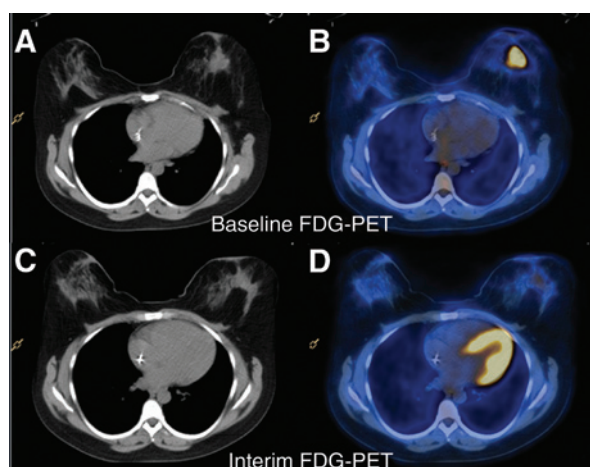
Our results found that IHC can help define a TNBC subgroup with a higher likelihood of achieving pCR, but IHC is still not accurate enough to reliably identify, at baseline, patients with no

**Figure 2.**

Distribution of tumor metabolic response ( $\Delta\text{SUV}_{\text{max}}$ ) for prediction of pCR. With a threshold at  $-50\%$ , sensitivity is 74% (14/19), specificity is 76% (19/25), positive predictive value is 70% (14/20), negative predictive value is 79% (19/24), and accuracy is 75% (33/44). The line corresponds to the threshold of  $\Delta\text{SUV}_{\text{max}} = -50\%$ .



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**Figure 3.** Assessment of tumor metabolic response with baseline and interim  $^{18}\text{F}$ -FDG-PET exams. CT (A and C) and fusion (B and D) imaging at baseline and after the first cycle of neoadjuvant chemotherapy of a woman with a good-responding triple-negative tumor in the left breast ( $\Delta\text{SUV} = -79\%$ ). This woman achieved a pCR at the end of NAC.

benefit to a conventional NAC regimen. Other early biomarkers of tumor response are needed to better tailor the treatment.

#### Tumor glucose metabolism as a biomarker of response

One originality of the present study was to include FDG-PET (Fig. 3). Tumor metabolic behavior was assessed at baseline and after the first cycle of NAC. Different metabolic parameters were evaluated. Among them, the tumor metabolic response assessed using the percentage decrease in  $\text{SUV}_{\text{max}}$  on interim PET offered the greatest accuracy in predicting pCR: a decrease in tumor FDG uptake greater than 50% predicted pCR with a PPV of 70% and an NPV of 79%. In multivariate analysis, the metabolic response was an independent predictor of pCR. Few studies have evaluated the predictive value of FDG-PET in TNBC treated with NAC. Most of these found a good predictive value of PET in this subtype, after both 1 and 2 cycles of NAC (24, 33, 34). In the study of Groheux and colleagues, a cutoff of  $-50\%$  of  $\Delta\text{SUV}_{\text{max}}$  offered the best accuracy in predicting pCR, whereas a  $-42\%$  cutoff was optimal to predict relapse (24). Although our own optimal cutoff was found at  $-60\%$ , the 50% threshold previously defined was used in the present study to validate it on our independent dataset. Compared with Groheux's results, we found a lower accuracy of  $\Delta\text{SUV}_{\text{max}}$  to predict pCR (75% vs. 80%), a lower NPV (79% vs. 96%) but higher PPV (70% vs. 59%). One of the reasons may be the different timing of the interim PET examination, performed after 2 cycles in Groheux's study (24). In keeping with the previous study by Groheux and colleagues, we found that measuring the response in axillary lymph nodes provided no predictive benefit over metabolic assessment of the breast tumor alone.

As most studies that have previously assessed PET-based tumor response in breast cancer (24, 34), and in accordance with the recommendations of the European Organization of Research and Treatment of cancer (EORTC; ref. 35), the relative change in tumor  $\text{SUV}_{\text{max}}$  was measured to assess tumor response. Because  $\text{SUV}_{\text{max}}$  and  $\text{SUV}_{\text{peak}}$  have both demonstrated lower interobserver variability than other quantitative PET parameters (36),  $\text{SUV}_{\text{peak}}$  could also have been used, as suggested by Wahl and colleagues

(37). Indeed,  $\text{SUV}_{\text{peak}}$  is less sensitive than  $\text{SUV}_{\text{max}}$  to the image noise and voxel size (38). However, the region of interest ( $\text{ROI}_{\text{peak}}$ ) used to measure  $\text{SUV}_{\text{peak}}$  is not uniquely defined (39, 40). This inconsistent definition of  $\text{ROI}_{\text{peak}}$  results in substantial variation of individual PET response using  $\text{SUV}_{\text{peak}}$ , particularly in heterogeneous tumor (40).

Inflammatory breast cancer (IBC) is usually distinguished from non-IBC by specific morphologic, phenotypic, biologic properties, and a poorer outcome (41). We found higher baseline tumor glucidic metabolism ( $\text{SUV}_{\text{max}}$ ) in IBC than in non-IBC. It may be explained by 2 reasons: glucose metabolism is increased in inflammatory tissue and inflammatory breast tumors are usually highly proliferative. We did not assess significant differences of metabolic response between IBC and non-IBC. Tumor inflammation may affect the breast tumor metabolic behavior and response to treatment but larger studies are needed to clarify this point.

Among all the clinical, biologic, and imaging biomarkers evaluated, 2 of them were independent predictors of tumor response. Combining these biomarkers is an interesting approach to define probability groups for pCR and to develop accurate predictive model (nomogram). In the present study, combining a poor metabolic response with positive EGFR status predicted non-pCR with a high value of 92%.

#### Limitations of the study

One limitation of our study is the use of different therapeutic protocols and the frequent switch to docetaxel after the first 3 cycles of FEC100. Several phase III trials have demonstrated that this systematic switch at mid-course improves the pCR rate (42, 43): one meta-analysis found an absolute difference of 2.4% ( $P = 0.013$ ; ref. 44). The survival benefit of this switch is more controversial (42, 43). In the present study and others (33), the final pathologic response to the sequential regimen strongly depended on the tumor response assessed after the first cycle of FEC or EC and was independent of any further change in the chemotherapy regimen. This suggests that the systematic switch to docetaxel has little benefit.

In the neoadjuvant setting, pCR is a strong prognostic marker, particularly in the TNBC subtype (10–12). Nevertheless, it is still not clear whether an increased pCR rate, due to a novel treatment, translates into improved survival (12). This is a crucial issue for the design of future PET-guided therapeutic trials to evaluate the benefit of an early therapeutic switch in nonresponding women (45).

#### Conclusion

The early identification of TNBC tumors with low chemosensitivity to NAC is an important issue. Negative tumor EGFR status, mostly observed in non-basal-like TNBC, is an independent predictor of pCR after NAC. However, the benefit of routinely identifying basal-like and non-basal-like cancers is unclear and the distinction is not currently used for treatment decision making. The metabolic response after the first cycle of NAC can more accurately predict pCR. Moreover, the tumor metabolic response and EGFR status can be combined to improve the early identification of women unlikely to achieve pCR at the end of NAC. Given these results, multicenter trials using PET-guided treatment strategies are now necessary to evaluate the benefit of early therapeutic changes in poorly

responding women. Such a strategy should lead to enhanced personalized medicine. Further studies are also needed to evaluate the link between the metabolic behavior of TNBC and the expression of different gene signatures. These will lead to better understanding of the biology of poorly responding TNBC.

### Disclosure of Potential Conflicts of Interest

P. Fumoleau is a consultant/advisory board member for Novartis and Roche. No potential conflicts of interest were disclosed by the other authors.

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## Identification of Biomarkers Including $^{18}\text{F}$ FDG-PET/CT for Early Prediction of Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer

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