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TITLE

[18F]FDG PET/CT for predicting triple-negative breast cancer outcomes after neoadjuvant chemotherapy with or without pembrolizumab

RUNNING TITLE

[18F]FDG PET/CT for predicting response to NAC and NACI in TNBC

AUTHORS

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AUTHORSHIP CONTRIBUTIONS

- 1) All authors made substantial contributions to the design of the work; or the acquisition, analysis or interpretation of data;
 - 2) All authors revised it critically for important intellectual content;
 - 3) All authors approved the version to be published and,
 - 4) All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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All other authors declare that they have no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS**ETHICAL APPROVAL**

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration.

INFORMED CONSENT

For this type of study (retrospective), formal consent is not required.

ABSTRACT

Purpose

To determine if pretreatment [18F]FDG PET/CT could contribute to predicting complete pathological complete response (pCR) in patients with early-stage triple-negative breast cancer (TNBC) undergoing neoadjuvant chemotherapy with or without pembrolizumab.

Methods

In this retrospective bicentric study, we included TNBC patients who underwent [18F]FDG PET/CT before neoadjuvant chemotherapy (NAC) or chemo-immunotherapy (NACI) between March 2017 and August 2022. Clinical, biological and pathological data were collected. Tumor SUVmax and total metabolic tumor volume (TMTV) were measured from the PET images. Cut-off values were determined using ROC curves and a multivariable model was developed using logistic regression to predict pCR.

Results

N=191 patients were included. pCR rates were 53 and 70% in patients treated with NAC (N=91) and NACI (N=100), respectively ($p < 0.01$). In univariable analysis, high Ki67, high tumor SUVmax (> 12.3) and low TMTV (≤ 3.0 cm³) were predictors of pCR in the NAC cohort while tumor staging classification ($< T3$), BRCA1/2 germline mutation, high tumor SUVmax (> 17.2) and low TMTV (≤ 7.3 cm³) correlated with pCR in the NACI cohort. In multivariable analysis, only high tumor SUVmax (NAC: OR 8.8, $p < 0.01$; NACI: OR 3.7, $p = 0.02$) and low TMTV (NAC: OR 6.6, $p < 0.01$; NACI: OR 3.5, $p = 0.03$) were independent factors for pCR in both cohorts, albeit at different thresholds.

Conclusion

High tumor metabolism (SUVmax) and low tumor burden (TMTV) could predict pCR after NAC regardless of the addition of pembrolizumab. Further studies are warranted to validate such findings and determine how these biomarkers could be used to guide neoadjuvant therapy in TNBC patients.

KEYWORDS

Early triple-negative breast cancer
Neoadjuvant chemotherapy +/- pembrolizumab
Pathologic response
[18F]FDG PET/CT
Tumor metabolism
Metabolic tumor burden

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INTRODUCTION

Triple-negative breast cancer (TNBC) is an aggressive and heterogeneous form of malignant tumors that accounts for about 10-15% of all breast cancers [1]. Neoadjuvant therapy has become the standard of care for stage II-III TNBC, leading to a shrinkage of the tumor and enabling less extensive surgery [2,3]. Achieving complete pathologic response (pCR), which refers to the absence of any residual invasive cancer cells in the breast and lymph nodes following neoadjuvant therapy evaluated on the surgical specimen, has been associated with a better long-term outcome and survival compared to patients who do not achieve pCR [4].

The paradigm for neoadjuvant therapy for TNBC has evolved over the past few years [5]. Historically, the standard of care for TNBC was neoadjuvant chemotherapy (NAC), based on anthracyclines, cyclophosphamide and taxanes [6]. More recently, immunotherapy with pembrolizumab, an immune checkpoint inhibitor (ICI) targeting programmed death-1, has been added to the neoadjuvant treatment regimen containing also carboplatin for TNBC [7]. This inhibitor blocks the inhibitory signals that cancer cells use to evade the immune system, thereby enhancing the T-cell-based immune response against the tumor. The addition of ICI to chemotherapy has been shown to improve pCR rates and event-free survival in TNBC patients [8,9], pembrolizumab being now approved in this setting by FDA and EMA. Despite these major therapeutic advances (13.6% absolute increase in pCR rate with pembrolizumab), not all patients respond equally well to neoadjuvant chemotherapy combined with an ICI (NACI) [7]. To this end, several markers, such as tumor-infiltrating lymphocytes (TILs) [10] or PD-L1 expression [11] have been investigated to predict pCR. However, their predictive performances vary a lot across studies so there is a need for more accurate biomarkers to guide treatment decisions.

Moreover, this immune activation under ICI can cause a loss of immunological self-tolerance leading to immune-related adverse events (irAEs), such as inflammation in healthy tissues or organs, including the skin, gastrointestinal tract, liver, heart, lungs and endocrine glands [12]. The early identification of patients at high-risk of developing such toxicities could help to adopt effective strategies for preventing and managing irAEs. As an example, thyroid dysfunction is one of the most incident irAE, occurring in around 15% of the cases, that could lead to hypothyroidism and subsequently require permanent levothyroxine substitution [13].

[18F]FDG-PET/CT imaging has been shown to be a useful tool for the prediction of pathological response in TNBC patients treated with neoadjuvant chemotherapy [14]. Several studies have investigated the use of various PET-derived features, including maximum standardized uptake value (SUVmax), total metabolic tumor volume (TMTV), and total lesion glycolysis (TLG), as potential biomarkers for predicting response to NAC in TNBC patients [15,16]. However, previous studies have not evaluated yet their predictive impact on TNBC undergoing NACI.

In this study, we first investigated the ability of biomarkers measured on pretreatment [18F]FDG PET/CT to predict complete pathological response in comparison with standard clinicopathological variables in patients with early-stage TNBC undergoing NACI, using a group of patients treated by NAC as a control group. We also evaluated the association between PET biomarkers and immunological features or genetic mutations. In addition, we studied whether pretreatment blood samples and PET imaging could predict the development of ICI-induced hypothyroidism.

METHODS

Patients

We conducted a retrospective and bicentric review of 203 consecutive patients with biopsy-proven triple-negative invasive breast adenocarcinoma of no specific type who had [18F]FDG PET/CT scans for initial staging between March 2017 and September 2020 (group of 95 patients undergoing NAC) or between June 2021 and August 2022 (group of 108 patients undergoing NACI), as part of standard care at the Institut Curie Hospitals (Paris and Saint-Cloud), France. We excluded 12 patients based on the following criteria: those with a delay between [18F]FDG PET/CT scan and the first treatment superior to 6 weeks (n = 4); those without available or interpretable (e.g. extravasation) images (n = 4); and those who did not agree to participate in medical research (n = 4). Our data collection followed our Institutional Review Board's regulations (IRB DATA230006) with a waiver of informed consent ("rule of non-opposition"), and the study was conducted according to the Declaration of Helsinki.

Treatment

Neoadjuvant chemo-immunotherapy (NACI): NACI was determined by the newly established standard of care per the pivotal KEYNOTE-522 trial treatment schedule [7,8]. NACI corresponded to pembrolizumab 200 mg Q3W given with 4 cycles of paclitaxel and carboplatin, then with 4 cycles of doxorubicin or epirubicin and cyclophosphamide. Patients were treated through the French Early Access Program for pembrolizumab.

Neoadjuvant chemotherapy (NAC): NAC corresponded to 4 cycles of doxorubicin or epirubicin (Q2W) and cyclophosphamide, then with 12 weekly paclitaxel. These regimens are commonly referred to as "dose-dense AC-T or EC-T", respectively.

Surgery: After neoadjuvant therapy, all patients underwent breast-conservative surgery and/or total mastectomy, with axillary staging (lymph node dissection and/or sentinel lymph node technique).

Clinical, pathological and biological data

Clinical data consisted of the patient's age (using the previous cutoff of 65 years in the KEYNOTE-522), body-mass index (kg/m²) and menopausal status. Pathological characteristics of the primary TNBC were obtained from biopsy samples. TNBC was defined as a lack of estrogen receptor (ER, expressed in <10% of tumor cells), progesterone receptor (PR, expressed in <10% of tumor cells), and human epidermal growth factor receptor 2 (HER2) negative according to ASCO/CAP criteria [1]. T and N stages were determined according to the 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual [17]. Nodal involvement was verified through the utilization of fine-needle aspiration, which was performed in cases of significant suspicion of cancer due to lymph node enlargement following clinical examination or imaging techniques such as mammography, ultrasound, and magnetic resonance imaging. The Ki-67 index (using the cutoff of ≥ 50 versus < 50 % [18]) and histologic grade were also analyzed.

Tumor-infiltrating lymphocytes (TILs): TILs were assessed on hematoxylin and eosin stained-sections and were analyzed in three predefined groups of low (0-10% immune cells in stromal tissue within the tumor), intermediate (11-59%), and high TILs (≥ 60 %) according to the guidelines of the International TIL working group [19].

PD-L1 expression: An exploratory biomarker analysis of PD-L1 immunohistochemistry was performed in the NACI cohort since PD-L1 positivity was not required for pembrolizumab prescription. The FDA-approved antibody clone 22C3 was used for the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) pre-treatment biopsy samples. Tissues with PD-L1 Combined Positive

Score (CPS) ≥ 10 were considered positive [20] as used in the Keynote-355 for advanced triple-negative breast cancer [21].

Blood samples: Complete blood cell counts at baseline before neoadjuvant therapy (within 28 days before the first cycle) were extracted from electronic medical records. The cutoff for neutrophil-to-lymphocyte ratio (NLR = absolute neutrophils count / absolute lymphocytes count) and hemoglobin was \leq versus > 1.7 (based on the commonly accepted cutoff [22]) and \leq versus > 12 g/dL (based on the widely applied threshold for anemia), respectively.

Genetic testing: According to NCCN guidelines [23], all patients at age 60 or younger or with a familiar history of breast cancer (n=87pts) were referred to a genetic counsellor and had genetic testing for Hereditary Breast and Ovarian Cancer syndrome (HBOC). Patients then completed testing for germline mutations, including at least BRCA1 and BRCA2.

[18F]FDG PET/CT imaging

[18F]FDG PET/CT scans were performed as part of staging per the applicable EANM procedure guidelines [24]. Prior to scanning, patients fasted for at least 6 hours and blood glucose levels were confirmed to be less than 10 mmol/L. Patients were injected with FDG (median activity: 194 MBq; range: 88-397) and images were acquired 60 minutes later (median: 61 min; range: 55-79). A CT scan was obtained initially, followed by a PET scan, performed from the skull base to the proximal femur. Images were converted to SUV units by normalization using the patient's body weight. Five PET/CT scanners were used and acquisition parameters for each device are provided in the supplementary material. Each de-identified and anonymized patient was analyzed by an experienced nuclear medicine physician (RDS), without any knowledge of the patient's history or pathological background, or clinical outcome.

Measurement of imaging biomarkers

Biomarkers were extracted from [18F]FDG PET/CT images in tumor regions (primary tumor and lymph nodes) and the thyroid using the PET segmentation tool in Philips IntelliSpace Portal 9.0. The primary tumor and when applicable, regional lymph nodes were delineated on the PET images as recommended by the EANM for solid tumors [24], with a semi-automatic method using 41% SUVmax thresholding, assisted by CT data for the anatomical location to extract tumor PET features. A patient's SUVmax (tumor SUVmax) was defined as the highest SUVmax among all lesions detected in the patient, including primary tumor (primary SUVmax) and metastatic lymph nodes (LN SUVmax) when applicable. Total metabolic tumor volume (TMTV) was defined as the sum of the metabolic tumor volume of all lesions (primary tumor and metastatic lymph node when applicable). Thyroid SUVmax were computed by drawing a spherical 1 cm-VOI in the thyroid [25].

Outcomes

We first investigated the association of pCR with pretreatment biomarkers, defined by the absence of invasive cancer in the breast and axillary nodes, irrespective of the presence of residual ductal carcinoma in situ (ypT0/is ypN0), as recommended by the EMA and FDA guidelines. Secondly, we analyzed the association of pretreatment immunologic biomarkers (PD-L1 expression and TILs) and genetic features (BRCA1-2 germline mutation) with PET biomarkers but also the association of pretreatment blood sample values (TSH) and thyroid SUVmax with the development of permanent ICI-induced hypothyroidism. Permanent hypothyroidism was defined as the presence of symptoms with TSH elevation on two assays or TSH > 10 mUI/L, requiring levothyroxine substitution between the start of neoadjuvant therapy to breast surgery [26].

Statistical analysis

Spearman's rank correlation coefficients (ρ) were calculated to assess the relationships between PET features. Values of biomarkers between (i) patients displaying pCR versus no-pCR; (ii) tumors with PD-L1 CPS > 10 or PD-L1 CPS ≤ 10 ; (iii) tumors with high or intermediate-low TILs and with or without BRCA1-2 germline mutation; (iv) patients with or without ICI-induced permanent hypothyroidism after NACI, were compared using the Mann-Whitney-Wilcoxon test. Areas under ROC curves (AUC) of these features for the diagnosis of (i) pCR; (ii) PD-L1 CPS > 10 ; (iii) high TILs; and (iv) ICI-induced hypothyroidism, were compared. Threshold values were defined to maximize Youden's index. Factors associated with pCR were combined using logistic regression. Multivariable analysis was performed after variable selection using the stepwise Akaike information criterion (AIC) method. The likelihood ratio test (LRT) for the added predictive value of tumor SUVmax and TMTV was obtained by comparing log-likelihoods of multivariable models (chi-square test: χ^2). All reported p values are two-sided and p values less than 0.05 were deemed significant. Analyses were performed with the R software (version 4.2.1).

RESULTS

Patient characteristics

Of 203 consecutive patients with [18F]FDG PET/CT scans before receiving neoadjuvant therapy who were screened, 191 were included (flow-chart in figure 1): 91 in the NAC cohort and 100 in the NACI cohort. Pretreatment patient characteristics are summarized in table 1. The median time between the treatment initiation and surgery was 5.5 (minimum: 2.5; maximum: 10.9) months. Correlations between biomarkers extracted from [18F]FDG PET/CT and variables of interest are presented in supplemental figure 1, and showed no significant association, except a moderate but significant correlation between primary tumor SUVmax and Ki67 ($\rho=0.39$; plot in supplemental figure 2).

Association between PET biomarkers and immunological features

PD-L1 expression (NACI cohort only)

A total of 62 patients were evaluable for PD-L1 (supplemental table 1), including 41 patients (66%) with CPS ≥ 10 and 21 patients (34%) with CPS < 10 . The high rate of missing PD-L1 status was mainly because it was not requested for pembrolizumab prescription. Patients with CPS ≥ 10 had significantly higher values of primary tumor SUVmax ($p<0.01$) and TILs ($p<0.01$) (supplemental figure 3). Primary tumor SUVmax predicts PD-L1 CPS ≥ 10 with an AUC of 0.66 (95%CI 0.52-0.79, supplemental table 2). No significant difference in the pCR rate was observed between CPS ≥ 10 and CPS < 10 (73 vs 62%, $p=0.39$).

TILs

All patients were evaluable for TILs (supplemental table 3). Thirty-nine patients had high levels of TILs while 152 patients had intermediate or low levels of TILs. Patients with high TILs had higher values of primary tumor SUVmax (15.8 vs 14.3, $p=0.06$) than patients with intermediate or low TILs. In the whole cohort (NACI and NAC), primary tumor SUVmax predicts TILs $\geq 60\%$ (high) with an AUC of 0.59 (95%CI 0.48-0.71, supplemental table 2). No significant difference was observed between patients with high TILs and patients with intermediate or low TILs for other evaluated biomarkers.

Association between PET biomarkers and genetic mutations

BRCA1-2 germline mutation

A total of 158 patients were evaluable for germline mutation status (supplemental table 4). BRCA1-2

germline mutations were present in 36 patients (23%) and absent in 122 patients (77%). Patients with BRCA1-2 germline mutation were younger (40 vs 47 years, $p<0.01$) than patients without BRCA1-2 germline mutation. No significant difference was observed between patients with and without BRCA1-2 germline mutation for PET biomarkers.

Prediction of pCR

The pCR rate was significantly higher in the NACI cohort than in the NAC cohort (70 vs 53%; table 1). Associations between characteristics and pCR are provided for the NAC group (supplemental table 5) and the NACI cohort (table 2).

NAC cohort

Patients with pCR had higher tumor proliferation (Ki67: $p=0.04$), higher tumor grade ($p=0.07$) and higher value of tumor SUVmax ($p=0.06$) than patients without pCR, but the last two did not reach statistical significance (supplemental table 5). Among PET biomarkers, tumor SUVmax was the best predictor of pCR with an AUC of 0.61 (95%CI 0.50-0.73, supplemental table 2). Thresholds, obtained from ROC curves in the prediction of pCR were as follows: high tumor SUVmax if > 12.3 ; low TMTV if ≤ 3.0 cm³. In multivariable logistic regression, only high tumor SUVmax and low TMTV remained independent factors for pCR (OR 8.8, [95% CI 2.5-25.7], $p<0.01$) and OR 6.6, [95% CI 1.9-21.5], $p<0.01$) (supplemental table 6). Both provide additional predictive value to the multivariable model obtained with the stepwise AIC method (supplemental table 7). Ki67 (OR 4.3, [95% CI 1.3-16.3], $p=0.02$) only correlated with pCR in univariable logistic regression. pCR rate was 64% and 69% for patients with high tumor SUVmax and low TMTV while pCR was 28% and 46% for patients with low tumor SUVmax and high TMTV (supplemental table 8). We combined the tumor SUVmax and TMTV, each of which correlated with pCR in multivariable analysis, for risk stratification. This scoring approach enabled to calculate the probability of achieving pCR into three groups: a low-risk group (high tumor SUVmax and low TMTV) with a pCR rate of 85%; an intermediate-risk group (high tumor SUVmax or low TMTV) with a pCR rate of 58%; and a high-risk group (low tumor SUVmax and high TMTV) with a pCR rate of 6%.

NACI cohort

Patients with pCR had a significantly higher rate of BRCA1-2 germline mutations (20 vs 3%, $p<0.01$) and a higher value of tumor SUVmax (16.1 vs 12.3, $p=0.03$) than patients without pCR (table 2). Among PET biomarkers, tumor SUVmax was the best predictor of pCR with an AUC of 0.65 (95%CI 0.52-0.75, supplemental table 2). Thresholds, obtained from ROC curves in the prediction of pCR were as follows: high tumor SUVmax if > 17.2 ; low TMTV if ≤ 7.3 cm³. In multivariable logistic regression, only high tumor SUVmax and low TMTV remained independent factors for pCR (OR 3.7, [95% CI 1.3-12.4], $p=0.02$ and OR 3.5, [95% CI 1.2-11.3], $p=0.03$) (table 3). As in the NAC cohort, both provide additional predictive value to the multivariable model obtained with the stepwise AIC method (supplemental table 7). T stage (OR 2.6, [95% CI 1.0-6.5], $p=0.04$) and BRCA1-2 germline mutation (OR 5.2, [95% CI 1.5-15.3], $p=0.04$) only correlated with pCR in univariable logistic regression. There was a trend, but not statistically significant, in favour of an association between pCR and low NLR (OR 2.6, $p=0.05$) or high hemoglobin (OR 3.3, $p=0.07$). pCR rate was 84% and 81% for patients with high tumor SUVmax and low TMTV while pCR was 62% and 60% for patients with low tumor SUVmax and high TMTV (supplemental table 8). As in the NAC cohort, we combined the tumor SUVmax and TMTV, both also correlated with pCR in multivariable analysis, and used these to stratify the population into three groups: a low-risk group (high tumor SUVmax and low TMTV) with a pCR rate of 94%; an intermediate-risk group (high tumor SUVmax or low TMTV) for with a pCR rate of

76%; and a high-risk group (low tumor SUVmax and high TMTV) with a pCR rate of 48% (supplemental figure 4).

ICI-induced hypothyroidism

A total of 98 patients were evaluable using thyroid SUVmax (history of thyroidectomy for 2 patients) and 88 patients using pretreatment TSH (blood sample was taken without TSH for 12 patients). ICI-induced hypothyroidism was diagnosed in 10 patients (10%) and was the most frequent irAE observed in the whole cohort (table 4). Other irAEs, as well as myocarditis, pancreatitis, hypophysitis, pneumonitis or polyarthritis, had a much lower incidence (<10%). Thresholds to predict ICI-induced hypothyroidism obtained from ROC curves were as follows: high TSH if > 1.89 mUI/L and high thyroid SUVmax if > 1.9. Pretreatment TSH level and thyroid SUVmax, both using continuous or dichotomized values, were higher in patients who developed ICI-induced hypothyroidism compared to patients who did not but the differences were not significant ($p>0.05$) (supplemental figure 5). Thyroid SUVmax predicts ICI-induced hypothyroidism with an AUC of 0.67 (95%CI 0.49-0.85, supplemental table 2), outperforming pretreatment TSH level (AUC 0.58, 95%CI 0.34-0.81).

DISCUSSION

Based on results from this retrospective bicentric cohort of 191 TNBC patients, biomarkers measured on pretreatment [18F]FDG PET/CT were independent factors for predicting pCR after neoadjuvant therapy. Our findings confirm previous studies that reported an association between biomarkers extracted from [18F]FDG PET/CT and the probability of achieving pCR for patients undergoing NAC [40,41]. However, the majority of studies considered BC patients regardless of the molecular subgroup (e.g. triple-negative) and none of these have yet explored if pretreatment [18F]FDG PET/CT could predict pCR in the specific population of TNBC patients undergoing NACI [16].

In our study, TNBC patients with a high tumor SUVmax and a low TMTV had a higher probability of achieving pCR independently of the neoadjuvant treatment received (NACI or NAC). Higher SUVmax indicates higher metabolic activity, which is often associated with tumor proliferation activity but also increased chemosensitivity. Lower TMTV indicates a lower tumor burden with a small metabolically active lesion within the breast +/- regional lymph node(s). These findings will need to be validated in independent cohorts. It would also be valuable to understand the specific mechanisms by which these biomarkers are associated with short-term (pCR after neoadjuvant therapy) and long-term clinical outcomes (recurrence-free survival and overall survival after the whole therapeutic sequence: neoadjuvant therapy – surgery – radiotherapy – adjuvant therapy). Ultimately, the goal would be to use these biomarkers to develop more personalized and effective treatment strategies for patients.

Research suggests that TNBC patients may experience systemic inflammation that can affect chemotherapy and immunotherapy outcomes. This inflammation process is known to trigger immune evasion and resistance to therapy [27]. There are various methods to evaluate this inflammation, such as analyzing routine blood counts to determine inflammatory biomarkers like the neutrophil-to-lymphocyte ratio (NLR) [28] and hemoglobin (Hb) levels [29]. Studies have shown that high NLR and/or anemia may be associated with poor outcomes in TNBC patients, particularly before neoadjuvant chemotherapy [30,31] and also in patients with cancer other than breast cancer undergoing pembrolizumab [32,33]. In our study, high NLR and high Hb were not associated with pCR in the two cohorts despite significant associations in univariable analysis for patients in the NACI cohort, possibly due to the relatively small sample and the resulting lack of statistical power.

Approximately 15 to 25% of all TNBC patients harbor germline BRCA1-2 mutations, which are often associated with tumor aggressiveness but also high treatment effectiveness. This “triple-negative paradox” has already been described for TNBC patients treated with NAC [34]. In our cohort, all patients displaying BRCA1-2 germline mutation except for one experienced pCR after NACI. In the NAC cohort, there were almost as many patients with germline BRCA1-2 mutations who achieved pCR and no pCR (27% versus 19%). These findings emphasize a potential link between germline BRCA1-2 mutation and immunotherapy response that should be thoroughly explored.

To better understand whether and how tumor metabolism could be triggered by the tumor microenvironment (TME), we correlated primary tumor SUVmax with immunological factors as well as PD-L1 expression or TILs. We found that patients with PD-L1 CPS ≥ 10 TNBC had significantly higher values of primary tumor SUVmax and TILs, which is consistent with the recent literature. Past research had shown that tumor SUVmax could be related to tumor and active immune cells in TME for BC patients [35]. Moreover, PD-L1+ BC had specific lymphoid features with higher TILs [36], which are one of the major components of TME, and specific metabolic patterns with higher primary tumor SUVmax [35]. In TNBC patients, two recent studies reported that the relationship between primary tumor SUVmax and TILs could be explained by glucose metabolism in the TME [37,38]. Kimura et al. illustrated such metabolic link by a positive correlation between primary tumor SUVmax and CD8/FOXP3 ratio [37]. This highlights a balance in the TME with, on one hand, CD8-positive T cells, which are the primary determinants of TILs, displaying anti-tumoral effects, and on the other hand, FOXP3-positive T cells, which are the primary determinants of T-regulatory cells, displaying pro-tumoral effects. Thus, [18F]FDG PET/CT imaging could provide metabolic information regarding the complex crosstalk between tumor and its TME, which is being recognized as a potential target for the treatment of TNBC [39].

Early detection of potential permanent irAEs is currently an area of intensive investigation in the onco-immunology community. Here, we investigated the association between pretreatment thyroid uptake and the occurrence of ICI-induced hypothyroidism in TNBC patients. Although the association was not statistically significant, pretreatment thyroid SUVmax was higher in patients who developed ICI-induced hypothyroidism compared to patients who did not. Moreover, thyroid SUVmax predicted ICI-induced hypothyroidism with a fair performance (AUC of 0.67). This result aligns with previous studies performed in other malignancies treated with an ICI, as well as melanoma or lung cancer [25,40]. From a pathophysiological point of view, few authors suggested that a high pretreatment thyroid SUVmax can be an indicator of the presence of preexisting autoimmune thyroiditis or other thyroid disorders that can be unmasked by immunotherapy [13,41]. However, the presence of thyroid antibodies does not predict hypothyroidism either and is observed in less than half of patients who develop ICI-induced hypothyroidism [41]. Further studies with higher statistical power are needed to confirm if pretreatment [18F]FDG PET/CT images displaying high thyroid SUVmax could identify patients at higher risk of developing thyroid irAEs during immunotherapy treatment.

The main limitation of the study concerns the retrospective nature and relatively small sample size. The PET images were taken using five different devices, possibly introducing detrimental variability in PET feature values. Moreover, threshold values determined from ROC curves for each imaging biomarker may have led to an inflated perception of the prediction performance, and should certainly be refined based on additional cohorts. Another limitation is that patients did not receive the same neoadjuvant chemotherapy regimen in the two cohorts. In the NAC cohort, patients were treated with a conventional regimen (composed of doxorubicin or epirubicin and cyclophosphamide then paclitaxel); while in the NACI cohort, patients received a carboplatin-containing regimen (composed of paclitaxel and carboplatin, then doxorubicin or epirubicin and cyclophosphamide). The

differences in results observed between the two cohorts might thus also be due to that change in chemotherapy regimen, especially given that carboplatin-containing regimens demonstrated superior pCR rates (improving pCR by 10 to 15%) when compared with conventional regimens [3].

CONCLUSION

In this cohort, we demonstrated that patients with a low tumor burden or a high tumor uptake on pretreatment [18F]FDG PET/CT had more frequent pCR after NAC regardless of the addition of pembrolizumab. In addition, our data suggest that high thyroid uptake on pretreatment [18F]FDG PET/CT may help in the early identification of patients at higher risk of developing hypothyroidism under pembrolizumab. Further studies using [18F]FDG PET/CT are warranted to guide neoadjuvant therapy in TNBC patients and decipher immunological processes.

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TABLE 1. Patients' characteristics	NACI cohort (n=100)	NAC cohort (n=91)
n = 191 pts	Median [range], n	
CLINICAL CHARACTERISTICS		
<i>Demographic parameters</i>		
Age (years)	48 [22-68]	48 [24-78]
Body mass index (kg/m ²)	23.9 [16.6-39.5]	24.4 [17.1-38.1]
Post-menopausal status (yes/no/unk)	36 (36) / 51 (51) / 13 (7)	29 (32) / 56 (61) / 6 (7)
<i>TNM stage (AJCC classification)</i>		
T1-2 / T3-4	71 (71) / 29 (29)	67 (74) / 24 (26)
N+*	51 (51)	35 (38)
<i>Histological parameters</i>		
Grade		
I-II	16 (16)	18 (20)
III	84 (84)	73 (80)
Ki67 (%)	70 [20-90]	70 [5-98]
TILs (%)	20 [0-100]	30 [0-80]
Necrosis	40 (40)	34 (37)
<i>PD-L1 expression:</i>		
CPS ≥ 10	41 (41)	na
CPS < 10	21 (21)	na
Unk	38 (38)	na
<i>BRCA1-2 germline mutations</i>		
Yes/no/unk	15 (15) / 73 (73) / 12 (12)	21 (23) / 49 (53) / 21 (23)
<i>Biology</i>		
Neutrophils (G/L)	3.7 [1.2-8.8]	4.5 [1.3-12.5]
Lymphocytes (G/L)	2.1 [0.6-4.0]	1.8 [0.5-11.1]
Hemoglobin (g/dL)	13.4 [10.8-15.3]	13.1 [10.4-16.3]
NLR	1.9 [0.6-12.4]	2.3 [0.4-19.0]
PET IMAGING PARAMETERS		
<i>Tumor glucose metabolism</i>		
Tumor SUVmax (PT or LN)	15.2 [4.6-47.5]	14.4 [2.8-38.9]
PT SUVmax	13.5 [3.0-47.5]	14.2 [2.8-38.9]
LN SUVmax**	12.0 [2.1-28.9]	11.6 [1.8-27.4]
<i>Metabolic Tumor Burden</i>		
TMTV	7.4 [0.6-284.6]	7.6 [0.8-284.6]
PT volume	5.5 [0.6-244.7]	5.5 [0.8-73.1]
LN volume**	3.3 [0.6-135.4]	3.8 [0.5-60.0]
PATHOLOGICAL RESPONSE		
RCB class		
Class 0 (=pCR)	70 (70)	48 (53)
Class I	9 (9)	14 (15)
Class II	16 (16)	24 (26)
Class III	5 (5)	5 (6)

Abbreviations: unknown (unk), tumor (T), node involvement (N), tumor-infiltrating lymphocytes (TILs), programmed death-ligand 1 (PD-L1), combined positive score (CPS), NLR (neutrophil-to-lymphocyte ratio), maximum standardized uptake value (SUVmax), primary tumor (PT), lymph node(s) (LN), total metabolic tumor volume (TMTV), pathological complete response (pCR), residual cancer burden (RCB).

**Confirmed by fine needle aspiration; **Data for 86 pts (n=51/ n=35 in the NACI/NAC cohort) with lymph node involvement.*

TABLE 2. Association with pCR in the NACI cohort

n = 100 pts	pCR n=70pts	No pCR n=30pts	Statistical significance
Median [range], n (%)			p value
CLINICAL CHARACTERISTICS			
<i>Demographic parameters</i>			
Age (years)	48 [22-66]	48 [28-68]	0.60
Body mass index (kg/m ²)	23.8 [16.7-39.5]	24.1 [17.7-37.6]	0.42
Post-menopausal status (yes/no/unk)	25 (36) / 34 (48) / 11 (16)	11 (36) / 17 (57) / 2 (7)	0.93
<i>TNM stage (AJCC classification)</i>			
T1-2 / T3-4	53 (76) / 17 (24)	17 (57) / 13 (43)	0.08
N+*	32 (46)	19 (63)	0.11
<i>Histological parameters</i>			
Grade			
I-II	10 (14)	6 (20)	0.51
III	60 (86)	24 (80)	0.51
Ki67 (%)	70 [20-90]	60 [20-90]	0.02
TILs (%)	20 [0-100]	20 [2-90]	0.98
Necrosis	30 (43)	10 (33)	0.37
<i>PD-L1 expression</i>			
CPS ≥ 10	30 (43)	11 (37)	0.57
CPS < 10	13 (19)	8 (27)	0.40
Unk	27 (39)	11 (37)	0.76
<i>BRCA 1-2 germline mutations</i>			
Yes/no/unk	14 (20) / 46 (66) / 10 (14)	1 (3) / 27 (90) / 2 (7)	<0.01
<i>Biology</i>			
Neutrophils (G/L)	3.6 [1.6-8.8]	4.2 [1.2-7.3]	0.37
Lymphocytes (G/L)	2.0 [0.6-3.3]	2.2 [0.8-4.0]	0.29
Hemoglobin (g/dL)	13.3 [11.0-15.3]	13.6 [10.8-15.2]	0.80
NLR	1.84 [0.93-12.38]	1.88 [0.55-6.01]	0.67
TSH	1.44 [0.25-3.80]	1.59 [0.75-4.11]	0.08
PET IMAGING PARAMETERS			
<i>Tumor glucose metabolism</i>			
Tumor SUVmax	16.1 [4.8-47.5]	12.5 [4.6-37.8]	0.03
PT SUVmax	15.2 [3.3-47.5]	12.2 [3.0-37.8]	0.09
LN SUVmax**	12.2 [2.9-28.9]	7.8 [2.1-23.8]	0.22
<i>Metabolic Tumor Burden</i>			
TMTV	6.4 [0.6-284.6]	12.3 [1.9-70.1]	0.15
PT volume	5.2 [0.6-244.7]	10.3 [1.0-64.1]	0.34
LN volume**	3.9 [0.7-135.4]	1.8 [0.6-11.6]	0.04
<i>Thyroid glucose metabolism</i>			
Thyroid SUVmax	1.89 [1.14-3.83]	1.69 [1.12-4.54]	0.89

Abbreviations: pathological complete response (pCR), unknown (unk), tumor (T), node involvement (N), tumor-infiltrating lymphocytes (TILs), programmed death-ligand 1 (PD-L1), combined positive score (CPS), NLR (neutrophil-to-lymphocyte ratio), thyroid-stimulating hormone (TSH), maximum standardized uptake value (SUVmax), primary tumor (PT), lymph node(s) (LN), total metabolic tumor volume (TMTV).

*Confirmed by fine needle aspiration; **Data for 51pts with lymph node involvement.

TABLE 3. Prediction of pCR in the NACI cohort		LOGISTIC REGRESSION		
n = 100 pts	Univariable		Multivariable	
	OR (95% CI)	p value	OR (95% CI)	p value
Age < 40 years (vs ≥ 40)	0.7 (0.3-1.6)	0.37	-	-
T stage 1-2 (vs 3-4)	2.6 (1.0-6.5)	0.04	1.3 (0.4-3.9)	0.66
N- (vs N+)	2.1 (0.9-5.1)	0.11	-	-
Normal Hb level (vs low)	3.3 (0.9-12.2)	0.07	3.7 (0.8-17.8)	0.09
Low NLR (vs high)	2.6 (1.0-7.3)	0.05	1.8 (0.6-5.6)	0.33
High Ki67 (vs low)	2.1 (0.7-5.6)	0.15	-	-
High TILs (vs intermediate/low)	0.8 (0.3-2.4)	0.60	-	-
BRCA 1-2 germline mutation (vs no)	5.2 (1.5-15.3)	0.04	4.2 (0.9-20.9)	0.07
PD-L1 CPS > 10 (vs CPS < 10)	1.7 (0.5-5.2)	0.36	-	-
High tumor SUVmax (vs low)	3.2 (1.2-9.4)	0.01	3.7 (1.3-12.4)	0.02
Low TMTV (vs high)	3.0 (1.2-7.6)	0.02	3.5 (1.2-11.3)	0.03

Abbreviations: odds ratio (OR), confidence interval (CI), tumor (T), node involvement (N), hemoglobin (Hb), NLR (neutrophil-to-lymphocyte ratio), tumor-infiltrating lymphocytes (TILs), programmed death-ligand 1 (PD-L1), combined positive score (CPS), maximum standardized uptake value (SUVmax), total metabolic tumor volume (TMTV).

TABLE 4. irAE prediction	ICI-induced hypothyroidism		
	Yes	No	Statistical significance
	Median [range], n (%)		p value
Biology (n=88pts)*	n = 10 (11%)	n = 78 (89%)	
TSH (mUI/L)	2.01 [0.29-3.80]	1.45 [0.25-4.11]	0.40
TSH > 1.89 mUI/L	6 (60)	21 (27)	0.08
PET imaging (n=98pts)**	n = 10 (10%)	n = 88 (90%)	
Thyroid SUVmax	1.99 [1.42-4.54]	1.74 [1.11-3.83]	0.13
Thyroid SUVmax > 1.9	7 (70)	37 (42)	0.11
Pathologic response (n=98pts)**			
pCR	6 (60)	63 (74)	0.51

Abbreviations: immune-related adverse event (irAE), Thyroid-stimulating hormone (TSH), maximum standardized uptake value (SUVmax), pathological complete response (pCR).

**Data for TSH for available for 88pts. **Two patients had a history of thyroidectomy.*

FIGURE 1. Flow-chart.

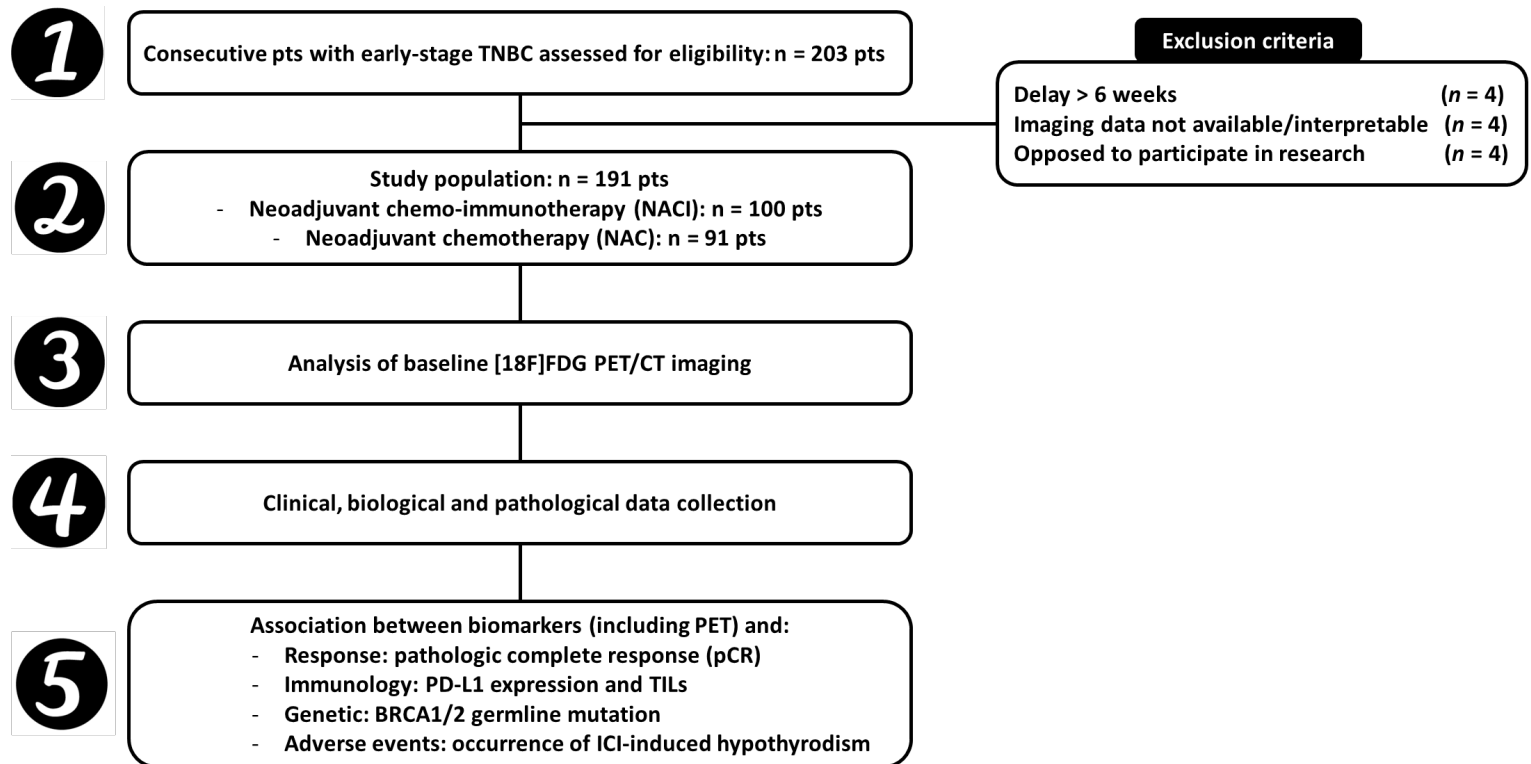


FIGURE 2. Graphical abstract.

Legend: Reported percentages correspond to the probability of pCR.

Abbreviations: maximum standardized uptake value (SUVmax), total metabolic tumor volume (TMTV), neoadjuvant chemotherapy (NAC), neoadjuvant chemo-immunotherapy (NACI), complete pathologic response (pCR).

