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Original Article

A Single-Arm Phase II Clinical Trial of Fulvestrant Combined with Neoadjuvant Chemotherapy of ER+/HER2– Locally Advanced Breast Cancer: Integrated Analysis of ^{18}F-FES PET-CT and Metabolites with Treatment Response

Qing Shao[1], **Ningning Zhang**[1], **Xianjun Pan**[1], **Wenqi Zhou**[1], **Yali Wang**[1], **Xiaoliang Chen**[2], **Jing Wu**[1,3], **Xiaohua Zeng**[1,3]

[1]Department of Breast Cancer Center, [2]Department of Nuclear Medicine, [3]Chongqing Key Laboratory for Intelligent Oncology in Breast Cancer (iCQBC), Chongqing University Cancer Hospital, Chongqing, China

Purpose This Phase II trial was objected to evaluate the efficacy and safety of adding fulvestrant to neoadjuvant chemotherapy in patients with estrogen receptor (ER)+/human epidermal growth factor receptor 2 (HER2)– locally advanced breast cancer (LABC). Additionally, the study aimed to investigate the association of 16α-18F-fluoro-17β-fluoroestradiol (^{18}F-FES) positron emission tomography (PET)–computed tomography (CT) and metabolites with efficacy.

Materials and Methods Fulvestrant and EC-T regimen were given to ER+/HER2– LABC patients before surgery. At baseline, patients received ^{18}F-FES PET-CT scan, and plasma samples were taken for liquid chromatography–mass spectrometry analysis. The primary endpoint was objective response rate (ORR). Secondary endpoints included total pathologic complete response (tpCR) and safety.

Results Among the 36 patients enrolled, the ORR was 86.1%, the tpCR rate was 8.3%. The incidence of grade ≥ 3 treatment-emergent adverse events was 22%. The decrease in ER value in sensitive patients was larger than that in non-sensitive patients, as was Ki-67 ($p < 0.05$). The maximum standardized uptake value, mean standardized uptake values, total lesion ER expression of ^{18}F-FES PET-CT in sensitive patients were significantly higher than those in non-sensitive patients ($p < 0.05$). Moreover, these parameters were significantly correlated with Miller and Payne grade and the change in ER expression before and after treatment ($p < 0.05$). Thirteen differential expressed metabolites were identified, which were markedly enriched in 19 metabolic pathways.

Conclusion This regimen demonstrated acceptable toxicity and encouraging antitumor efficacy. ^{18}F-FES PET-CT might serve as a tool to predict the effectiveness of this therapy. Altered metabolites or metabolic pathways might be associated with treatment response.

Key words Breast neoplasms, ER+/HER2–, Fulvestrant, Neoadjuvant therapy, ^{18}F-FES PET-CT, Metabolites

Introduction

Breast cancer (BC) remains the most prevalent malignancy among women worldwide [1]. In contrary to developed countries, a greater proportion of BC patients in China are diagnosed at locally advanced stages [2]. Currently, neoadjuvant chemotherapy (NCT) is the standard treatment for locally advanced breast cancer (LABC) patients, and those who achieve a pathological complete response (pCR) are more likely to achieve proglonged progression-free survival (PFS) [3]. However, patients with hormone receptor (HR)–positive tumors exhibit suboptimal responses to NCT, achieving an objective response rate (ORR) of around 65% and a pCR rate of merely 5%-10% [4]. This is notably inferior to the response rates seen in triple-negative and human epidermal growth factor receptor 2 (HER2)–positive BCs. Hence, refining neoadjuvant treatment protocols for estrogen receptor (ER)+/HER2– LABC patients is an imperative clinical challenge.

Clinical theory posits that ER+ BC patients might derive significant benefits from a combined modality of endocrine therapy and chemotherapy. Yet, until recently, limited evidence underscoring the simultaneous administration of these treatments has been scant. Notably, such as the SOFT and TEXT trials, have illuminated the potential of this synergistic approach to enhance disease-free survival in patients with HR+ BC [5]. Furthermore, the CBCSG-036 research, a randomized controlled trial for neoadjuvant chemo-endocrine treatment, found that combining chemotherapy with an aromatase inhibitor increased ORR and PFS in patients [6].

Fulvestrant emerges as a frontrunner in endocrine therapy, lauded for its efficacy. As an ER antagonist that prompts rapid

Correspondence: Jing Wu
Department of Breast Cancer Center, Chongqing University Cancer Hospital, 181 Hanyu Road, Shapingba District, Chongqing 400030, China
Tel: 86-23-65310859 Fax: 86-23-65310859 E-mail: wxj_happiness@163.com

Co-correspondence: Xiaohua Zeng
Department of Breast Cancer Center, Chongqing University Cancer Hospital, 181 Hanyu Road, Shapingba District, Chongqing 400030, China
Tel: 86-23-65310859 Fax: 86-23-65310859 E-mail: zxiaohuacqu@126.com

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*Qing Shao and Ningning Zhang contributed equally to this work.

ER degradation through competitive inhibition [7,8], fulvestrant's ability to enhance the efficacy of various chemotherapeutics positions it as an optimal partner for combination therapy [9,10]. This single-arm Phase 2 trial was conducted to assess the effectiveness and safety of neoadjuvant EC-T (epirubicin and cyclophosphamide followed by docetaxel) chemotherapy in combination with fulvestrant in ER+/HER2– LABC patients. Moreover, we utilized 16α-^{18}F-fluoro-17β-fluoroestradiol (^{18}F-FES) positron emission tomography (PET)–computed tomography (CT) at baseline to detect the functional ER expression data. By analyzing the correlation between the maximum standardized uptake value (SUVmax), mean standardized uptake values (SUVmean), and total lesion ER expression (TL-ER) of ^{18}F-FES PET-CT against treatment efficacy, we aimed to lay the groundwork for identifying more precise and sensitive predictors for the efficacy of our combined therapy approach. Furthermore, the linkage between metabolomic alterations and therapeutic response offers additional perspectives on the utility of combination therapy.

Materials and Methods

1. Study design and participants

This single-arm, open-label Phase 2 clinical trial was conducted at the Breast Cancer Center of Chongqing University Cancer Hospital. All procedures adhered to the ethical standards of the Declaration of Helsinki and the Guidelines for Good Clinical Practice (ClinicalTrials.gov ID: ChiCTR2000041235). The study protocol received approval from the Institutional Ethics Committee of Chongqing University Cancer Hospital (CZLS2020253-A). Written informed consent was obtained from all participants prior to inclusion in the study.

Patients (ages 18-70) with pathologically confirmed ER+ (defined as ≥ 10% of tumor cells exhibiting positive nuclear staining)/HER2– (as per the 2013 breast cancer HER2 testing guidelines) invasive ductal BC (stages IIB-IIIC), who had not received prior chemotherapy or endocrine therapy for invasive BC, were eligible for enrollment. Additional inclusion criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1, presence of a measurable primary breast tumor suitable for evaluation, unilateral cancer, and adequate organ functions. Patients with cN0 operable disease were required to present additional risk factors: either a large tumor size (≥ cT2), high histologic grade (grade 3), or a high proliferative index (Ki-67 > 20%). Exclusion criteria included pregnancy or breastfeeding, evidence of distant metastatic disease, concurrent secondary malignancy, mental illness, or any condition that might impact compliance, a history of drug allergy, or other severe concomitant illness.

2. Treatment administration

Prior to surgery, enrolled patients were administered a combination therapy of the endocrine agent fulvestrant (Chia Tai Tianqing Pharmaceutical Group Co., Ltd., Jiangsu, China), and EC-T chemotherapy. Fulvestrant was administered intramuscularly at a dose of 500 mg, initially on days 0, 14, and 28, followed by a once-every-28-day for a total of six 4-week cycles. Chemotherapy administration involved epirubicin (E) at 100 mg/m^2 and cyclophosphamide (C) at 600 mg/m^2 on day 1, with this combination repeated every 3 weeks for four cycles, followed by four cycles of docetaxel (T) at 80-100 mg/m^2 on day 1 per 3 weeks (EC4-T4 regimen). Specifically, premenopausal patients received a 3.75-mg subcutaneous injection of leuprorelin (Takeda Pharmaceutical Company, Osaka, Japan) before starting fulvestrant therapy and were prescribed a gonadotropin-releasing hormone analogue concurrent with the study treatment for ovarian suppression. Upon the completion of neoadjuvant therapy, surgery, whether mastectomy or breast-conserving surgery, was conducted. Postoperative treatment followed contemporary clinical guidelines.

3. Clinical response evaluation

A baseline magnetic resonance imaging (MRI) was performed within 4 weeks before the first dose of neoadjuvant therapy. Subsequent MRIs were conducted at 6-week intervals and pre-surgery to monitor the response of the breast lesion as per the Response Evaluation Criteria in Solid Tumors (RECIST ver. 1.1). Clinical response assessment for the primary endpoint was performed by the investigators.

4. Adverse event monitoring

Adverse events (AEs) were tracked from the inception of informed consent to 28 days post the final neoadjuvant therapy, adhering to the National Cancer Institute Common Terminology Criteria for Adverse Events ver. 5.0. Scheduled physical exams, electrocardiograms, laboratory tests, and incidental testing were performed to assess AEs and tolerance.

5. Pathological assessment

Immunohistochemistry (IHC) techniques were employed to determine baseline pathological markers, including ER, partial response (PR), HER2, and Ki-67, via core needle biopsy. Post-treatment, these markers were reassessed in excised tissue. PR positivity was defined as ≥ 10% of tumor cells exhibiting positive nuclear staining. Ki-67 positivity was identified by any level of nuclear staining intensity. Pathological responses in excised breast tissue were rated using

Table 1. Baseline and tumor clinicopathological characteristics of patients

Characteristic	No. of patients (n=36)
Median age (yr)	
≤ 50	13 (36.1)
> 50	23 (63.9)
Menopausal status	
Premenopausal	11 (30.6)
Postmenopausal	25 (69.4)
Tumor size	
T1	1 (2.8)
T2	19 (52.7)
T3	6 (16.7)
T4[a)]	10 (27.8)
Nodal status	
N0	3 (8.3)
N1	8 (22.2)
N2	19 (52.8)
N3	6 (16.7)
Clinical stage[b)] at baseline	
IIb	6 (16.7)
IIIa	14 (38.8)
IIIb	10 (27.8)
IIIc	6 (16.7)
ER expression (%)	81±10
PR expression (%)	20±26
Ki-67 expression (%)	24±15

Values are presented as number (%) or mean±SD. ER, estrogen receptor; PR, progesterone receptor; SD, standard deviation. [a)]Inflammatory breast cancer was not included, [b)]Based on 7th American Joint Committee on Cancer staging system.

Table 2. Tumor clinical response and pathological response at the completion of neoadjuvant treatment in the population

Characteristic	No. (%) (n=36)
Clinical response (breast)	
CR	3 (8.3)
PR	28 (77.8)
SD	5 (13.9)
PD	0
Pathological response (breast)	
MP score 1	1 (2.8)
MP score 2	6 (16.7)
MP score 3	22 (61.1)
MP score 4	3 (8.3)
MP score 5	4 (11.1)
tpCR	3 (8.3)
bpCR	4 (11.1)
ypN0	9 (25.0)

bpCR, pathologic complete response in breast; CR, complete response; MP, Miller and Payne; PD, progressive disease; PR, partial response; SD, stable disease; tpCR, total pathologic complete response; ypN0, pathologic complete response in axilla.

the Miller and Payne (MP) grading system.

6. ^{18}F-FES PET-CT procedure, image interpretation, and data analysis

Prior to commencing their initial treatment, participants underwent a comprehensive ^{18}F-FES PET-CT scan. The synthesis and validation of ^{18}F-FES were meticulously carried out by our Nuclear Medicine Department. The standard administered dose was 200 MBq, adjusted for individual body weight, with a radiochemical purity exceeding 99% and a specific activity of 2-5 Ci/μmol at the time of injection. The tracer caused no significant adverse effects barring minor injection site discomfort. Scanning commenced with a low-dose CT for attenuation correction, followed by the ^{18}F-FES PET scan approximately 60 minutes post-injection. Imaging covered from the top of the skull to the mid-thigh. PET and corresponding low-dose CT images were meticulously evaluated on a dedicated multimodality workstation, where regions of interest were manually delineated around tumor areas with notable uptake compared to the surrounding tissue. Quantitative uptake values, including SUVmax, SUVmean, and TL-ER, were calculated for lesion characterization. Discrepancies in image interpretation were resolved through consensus between two seasoned nuclear medicine professionals.

7. Blood sample collection, preparation and metabolomics analyses

Baseline plasma collection was performed with morning blood samples (3 mL) drawn from fasting subjects' antecubital veins into EDTA-lined polypropylene tubes. Following a 10-minute centrifugation at 3,000 rpm and 4°C, plasma was extracted and stored at –80°C until detection by liquid chromatography–mass spectrometry (LC-MS).

The preparation of samples, along with LC-MS detection and the subsequent data processing and validation, adhered to established protocols as delineated in previous literature [11]. Metabolite identification was conducted by comparing accurate mass and tandem mass spectrometry results against databases including the Human Metabolome Database (HMDB; http://www.hmdb.ca), MassBank (http://www.massbank.jp/), the Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/), LipidMaps (http://www.lipidmaps.org), mzCloud (https://www.

Table 3. Treatment-emergent adverse events in patients (n=36)

Adverse event	All grades	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	35 (97.2)	15 (41.7)	20 (55.5)	-	-
Vomiting	29 (80.6)	-	29 (80.6)	-	-
Aspartate aminotransferase increase	25 (69.4)	25 (69.4)	-	-	-
Alanine aminotransferase increase	25 (69.4)	23 (63.9)	1 (2.8)	1 (2.8)	-
Leukopenia	20 (55.6)	7 (19.4)	10 (27.8)	3 (8.3)	-
Anemia	18 (50.0)	11 (30.6)	7 (19.4)	-	-
Neutropenia	17 (47.2)	3 (8.3)	9 (25.0)	4 (11.1)	1 (2.8)
Hypokalemia	16 (44.4)	14 (38.9)	1 (2.8)	1 (2.8)	-
Fatigue	16 (44.4)	15 (41.7)	1 (2.8)	-	-
Dysphagia	16 (44.4)	14 (38.8)	2 (5.6)	-	-
Hypoalbuminemia	11 (30.6)	10 (27.8)	1 (2.8)	-	-
Anepithymia	7 (19.4)	7 (19.4)	-	-	-
Thrombocytopenia	4 (11.1)	4 (11.1)	-	-	-
Oral mucositis	4 (11.1)	-	4 (11.1)	-	-
Insomnia	3 (8.3)	-	3 (8.3)	-	-

Values are presented as number (%).

mzcloud.org), and a proprietary database from Panomix Biomedical Tech Co., Ltd. (Suzhou, China).

For pathway analysis, MetaboAnalyst software was utilized, encompassing both comprehensive pathway enrichment and topology analysis, to elucidate the role of distinctive metabolites. The KEGG pathway resource was employed to map the metabolomic profiles to their respective biological functions and systemic processes, with visual representations generated using the KEGG Mapper tool.

8. Endpoints

The primary endpoint was the ORR, defined as the proportion of patients who achieved a complete response or PR in the breast tumor as per the RECIST 1.1 criteria, measured by MRI scans performed at baseline and shortly before surgery. Secondary endpoints included the rates of total pathologic complete response (tpCR; ypT0/is, ypN0), pathologic complete response in breast (bpCR; ypT0/is), and pathological response (defined as MP grading scores of 3 to 5 in the final pathological result), the disease control rate (DCR), overall survival, and safety. The exploratory endpoints were changes in ER/PR/Ki-67 levels from baseline to post-surgery, the association of treatment efficacy with SUVmax/SUVmean/TL-ER of ^{18}F-FES PET-CT and metabolic characteristics.

9. Statistical analysis

A Simon's optimal two-stage design was used to determine sample size. Assuming a 65% ORR with standard chemotherapy, an ORR of 85% with the study treatment was considered of interest. With a one-sided α of 0.05 and power of 80%, a total of 33 patients were required. In the first stage, a minimum of 11 responses among the initial 14 participants was required to justify the continuation to recruit an additional 19 subjects. Success in the second stage, defined as at least 26 patients out of the total 33 achieving ORR, would endorse the study treatment for further study.

Evaluations of efficacy and safety were conducted on the enrolled population. The statistical analysis was conducted with R software ver. 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous data were presented as mean (±standard deviation) or median with an interquartile range as applicable. Categorical data were expressed in numbers and percentages. Using the Clopper-Pearson method, the ORR, DCR, and pCR were calculated with corresponding two-sided 95% confidence intervals. Unsupervised and supervised multivariate statistical analysis models (principle component analysis [PCA]; partial least squares discriminant analysis [PLS-DA]; orthogonal partial least squares discriminant analysis [OPLS-DA]) were used to differentiate the groups. Significance for metabolites' differential expression was set at a p-value of < 0.05 and a variable importance in projection score from OPLS-DA of > 1. The paired t-test was applied to assess changes in mean percentages of ER/PR/Ki-67–positive tumor cells from baseline to post-surgery. We examined the correlations of pre-treatment variables including SUVmax/SUVmean, TL-ER, and metabolites to treatment responses using Pearson's chi-square or Mann-Whitney U tests. A p-value of < 0.05 (two-sided) was designated as statistical significance.

Results

1. Patients and baseline characteristics

From December 2020 to September 2022, 36 patients were enrolled. All patients completed the planned cycles of study treatment and surgery, and they were included in the subsequent analysis. The baseline characteristics are shown in Table 1.

2. Clinical and pathological response in the study population

Among the initial enrolled 14 patients, objective response was obtained in 11 patients, thus satisfying the predefined ORR criterion for full accrual. Among the 36 patients enrolled, the ORR and DCR were 86.1% and 100%, respectively. Postoperative pathological results showed that 8.33% of patients achieved tpCR, 11.11% of patients achieved bpCR, 25.0% of patients achieved ypN0 status (Table 2, S1 Fig.).

3. Safety profile and grade 3 or 4 AEs

No treatment-related fatalities or life-threatening events were reported. Overall, all patients (100%) experienced at least one treatment-emergent adverse event (TEAE). Eight patients (22%) experienced ≥ grade 3 TEAEs, including neutropenia (13.9%) and leukopenia (8.3%). It is noteworthy that most AEs typically associated with endocrine therapy, such as hot flashes and musculoskeletal discomfort, were not reported in this study (Table 3).

4. Correlation analysis of ER, PR, Ki-67, and therapeutic efficacy

Out of the 36 patients enrolled, four achieved bpCR at surgery, necessitating the substitution of ER, PR, and Ki67 levels with a nominal value of 0.01 for postoperative analysis. Patients were categorized as treatment-sensitive (MP ≥ 4) or non-sensitive (MP ≤ 3) based on postoperative MP grading. No significant differences in baseline ER, PR, and Ki-67 expressions were found between the two groups. Baseline ER, PR, and Ki-67 expression did not differ significantly between the two groups ($p > 0.05$) (S2A-S2C Fig.), and they did not predict patient sensitivity to combination therapy (S2D-S2F Fig.). However, significant reductions in these markers were observed postoperatively (Fig. 1A-C): mean ER expression dropped from 81.3% to 51.3% ($p < 0.000001$), mean PR expression from 20.6% to 2.3% ($p < 0.0001$), and mean Ki-67 from 24.2% to 8.6% ($p < 0.000001$).

Analysis comparing pre- and post-treatment pathological indicators in relation to therapeutic sensitivity revealed a more pronounced decrease in ER for the treatment-sensitive group compared to the non-sensitive group (66.4% vs. 21.3%, p=0.00069) (Fig. 1D), with a similar pattern was observed in Ki-67 (29.7% vs. 12.1%, p=0.013) (Fig. 1G) but not PR (S2J Fig.). Additionally, the more pronounced the decrease in ER or Ki-67 after treatment the higher the MP grade (Fig. 1E and H, S2K Fig.). Furthermore, analysis of the relationship between changes in these pathological indicators and the regression volume of primary breast tumors (S2G-S2I Fig.), found a significant correlation only for ER (R=–0.42,

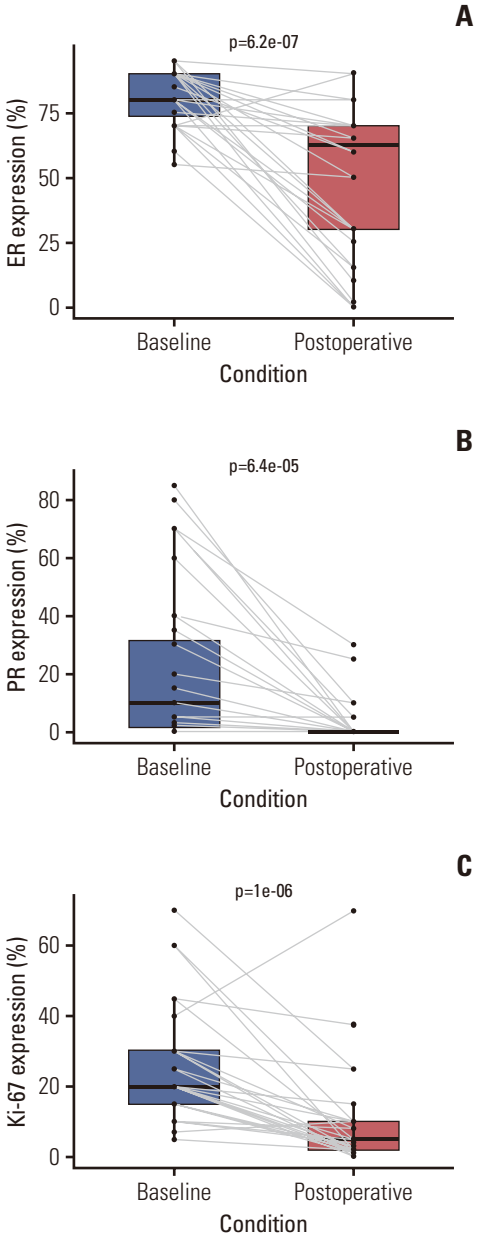

Fig. 1. Correlation analysis of estrogen receptor (ER), partial response (PR), Ki-67, and therapeutic efficacy. (A-C) ER, PR, and Ki-67 expression at baseline and postoperative (n=36). *(Continued to the next page)*

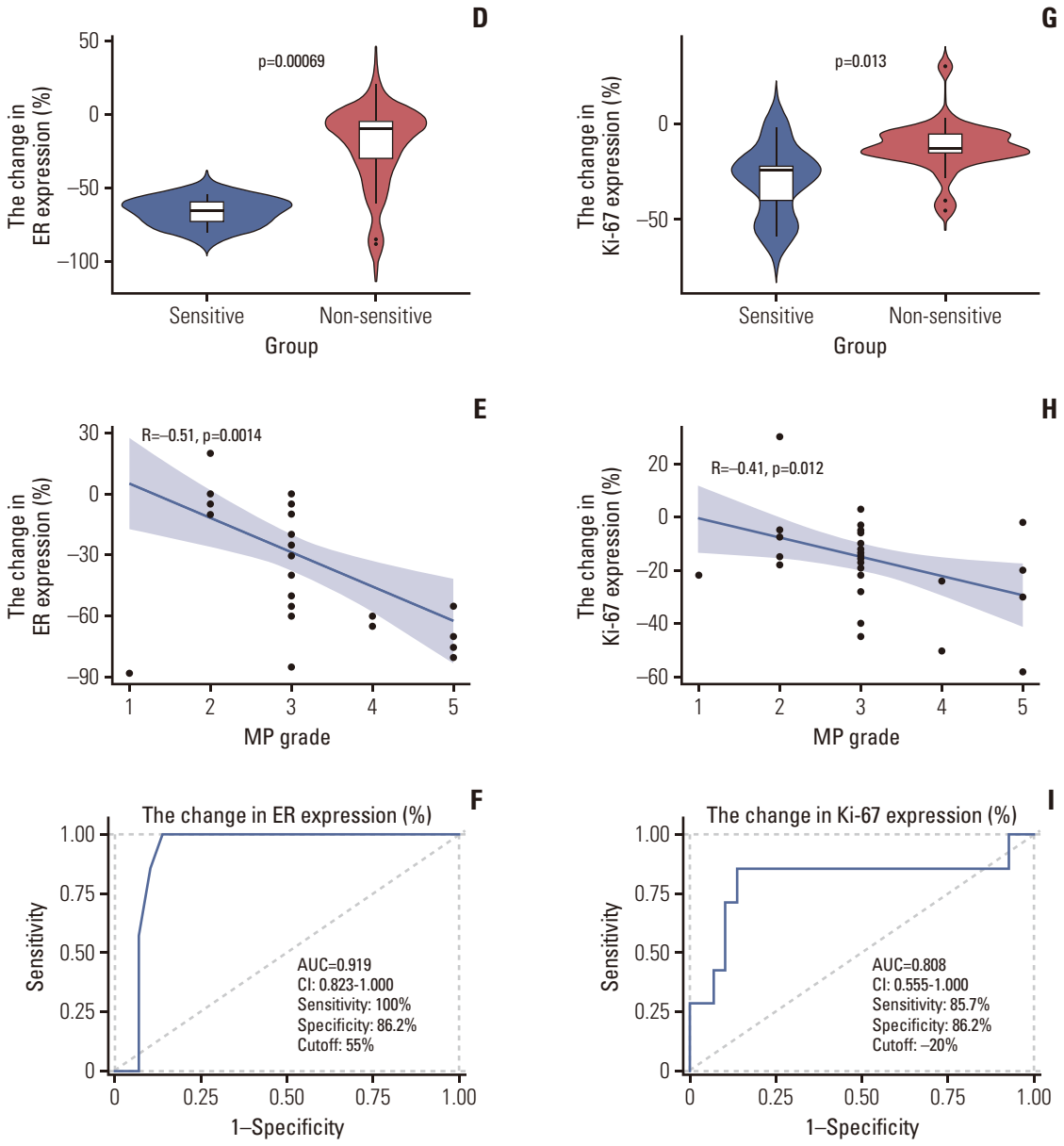

Fig. 1. *(Continued from the previous page)* (D) The change in ER expression between sensitive and non-sensitive group. (E) The correlation between the change in ER expression and Miller and Payne (MP) grading. (F) Receiver operating characteristic (ROC) curves of the change in ER expression distinguishing the sensitive and non-sensitive breast cancer patients. (G) The change in Ki-67 expression between sensitive and non-sensitive group. (H) The correlation between the change in Ki-67 expression and MP grading. (I) ROC curves of the change in Ki-67 expression distinguishing the sensitive and non-sensitive breast cancer patients.

p=0.011). Receiver operating characteristic (ROC) analysis for ER, PR, and Ki-67 changes was performed (Fig. 1F and I, S2L Fig.), indicating that a decrease in ER of more than 55% post-treatment is predictive of sensitivity to the combination therapy, while a decrease in Ki-67 of more than 20% is also indicative.

5. Correlation analysis of ^{18}F-FES PET-CT and therapeutic efficacy

Among the 36 patients, 24 underwent a ^{18}F-FES PET-CT scan before initiating fulvestrant. Quantitative parameters, including SUVmax, SUVmean and TL-ER were obtained from this analysis. For primary breast lesions, the mean SUVmax was 4.17 (range, 1.00 to 12.80), the average SUVmean

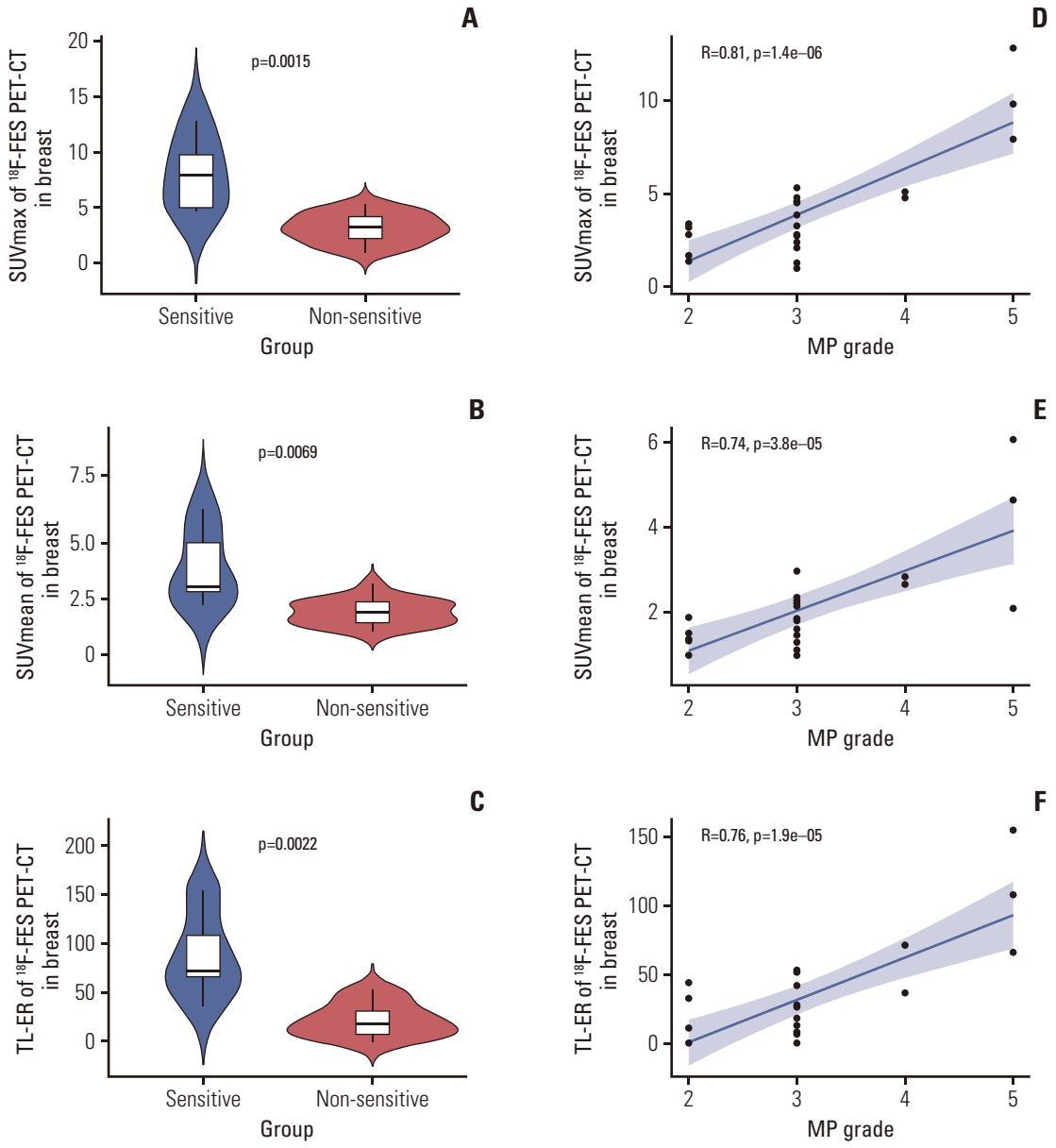

Fig. 2. Correlation analysis of 16α-[18]F-fluoro-17β-fluoroestradiol ([18]F-FES) positron emission tomography (PET)–computed tomography (CT) and therapeutic efficacy. (A-C) The maximum standardized uptake value (SUVmax), mean standardized uptake values (SUVmean), total lesion estrogen receptor (TL-ER) expression of [18]F-FES PET-CT in breast lesions between sensitive and non-sensitive group. (D-F) The correlation between the SUVmax, SUVmean, TL-ER of [18]F-FES PET-CT in breast lesions and Miller and Payne (MP) grading. *(Continued to the next page)*

was 2.16 (range, 1.00 to 6.07), and the average TL-ER was 35.39 (range, 0.77 to 154.58). Analysis showed that these parameters were all significantly associated with sensitivity to combination therapy. The SUVmax in non-sensitive group patients was significantly lower than that in sensitive group (average 3.14 vs 8.08, p=0.0015) (Fig. 2A), with similar trends observed for SUVmean (1.77 vs. 3.65, p=0.0069) (Fig. 2B) and TL-ER (21.77 vs. 87.5, p=0.0022) (Fig. 2C). Higher values of SUVmax, SUVmean, and TL-ER corresponded to a greater MP classification (Fig. 2D-F, S3 Fig.), and these parameters also correlated strongly with changes in ER expression (Fig. 2G-I). In addition, as can be seen by the ROC (Fig. 2J-L), SUVmax ≥ 4.6, SUVmean ≥ 2.65, and TL-ER ≥ 66.44 can be considered as sensitive to this combined treatment.

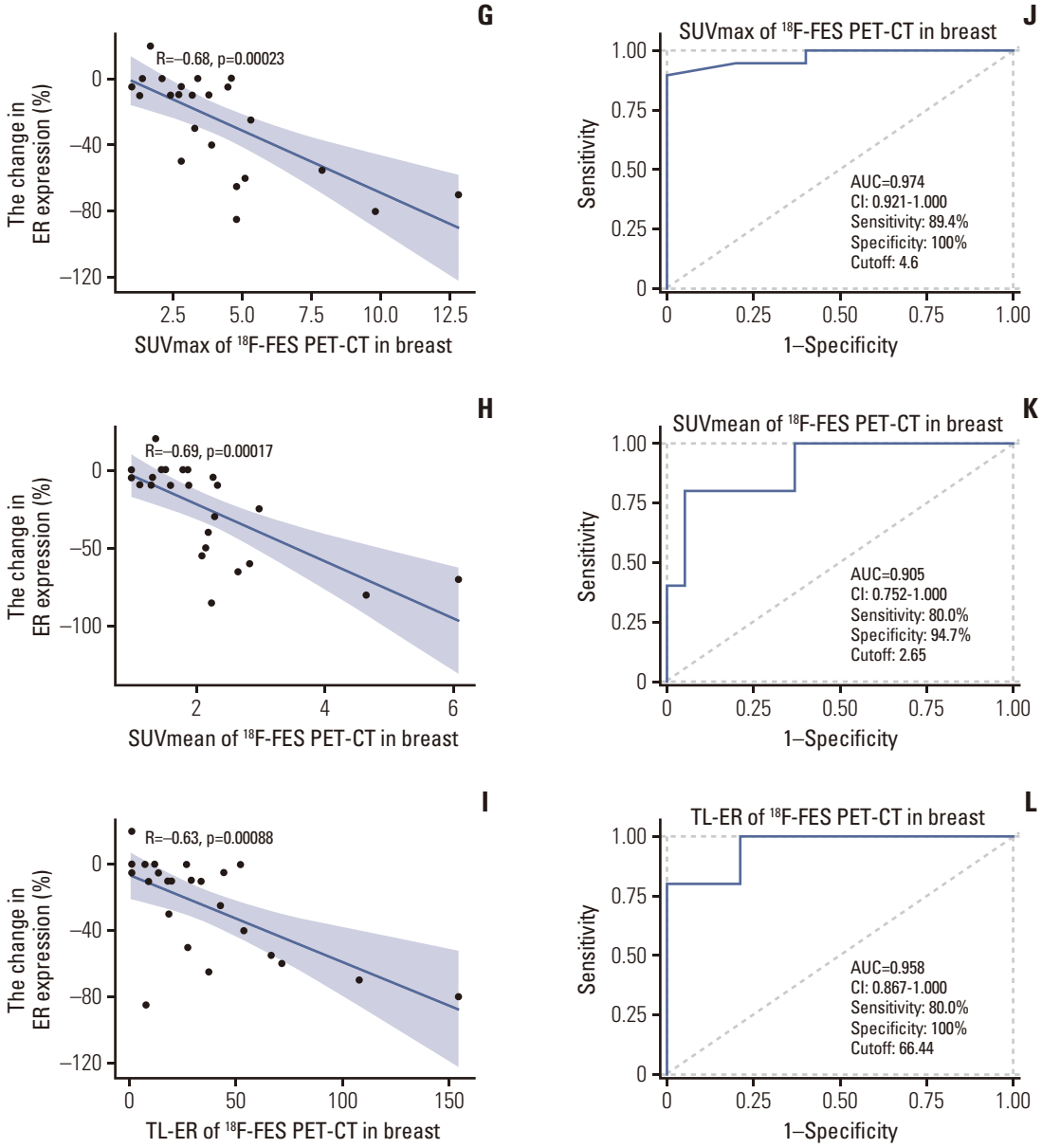

Fig. 2. *(Continued from the previous page)* (G-I) The correlation between the SUVmax, SUVmean, TL-ER of ^{18}F-FES PET-CT in breast lesions and the change in ER expression. (J-L) Receiver operating characteristic curves of the SUVmax, SUVmean, TL-ER of ^{18}F-FES PET-CT in breast lesions distinguishing the sensitive and non-sensitive breast cancer patients. AUC, area under curve; CI, confidence interval.

6. Association analysis of metabolites and efficacy

Twenty-five plasma samples were collected from 25 patients at baseline for LC-MS determination. The results of OPLS-DA revealed distinct metabolic features separating the treatment-sensitive group from the non-sensitive group (Fig. 3A and B). Volcano plot analysis highlighted 13 metabolites, including succinic acid semialdehyde, 5,6-dihydro-5-fluorouracil, 2-oxo-4-methylthiobutanoic acid, uric acid, limonene-1,2-diol, D-alanyl-D-serine, 5′-methylthioadenosine, vitamin D3, taurine, 3-hydroxyphenylacetic acid, 12-hydroxydodecanoic acid, uridine, and hexadecanedioate, that might be linked to treatment response (Fig. 3C). Cluster analysis (Fig. 3D) further differentiated the metabolomic profiles of 19 non-sensitive patients from those of six sensitive patients (S4 Table). As illustrated in S5 Fig., the levels of taurine and vitamin D3 were elevated in non-sensitive group, while remaining 11 metabolites were higher in sensitive patients. Meanwhile, a significant negative correlation was observed

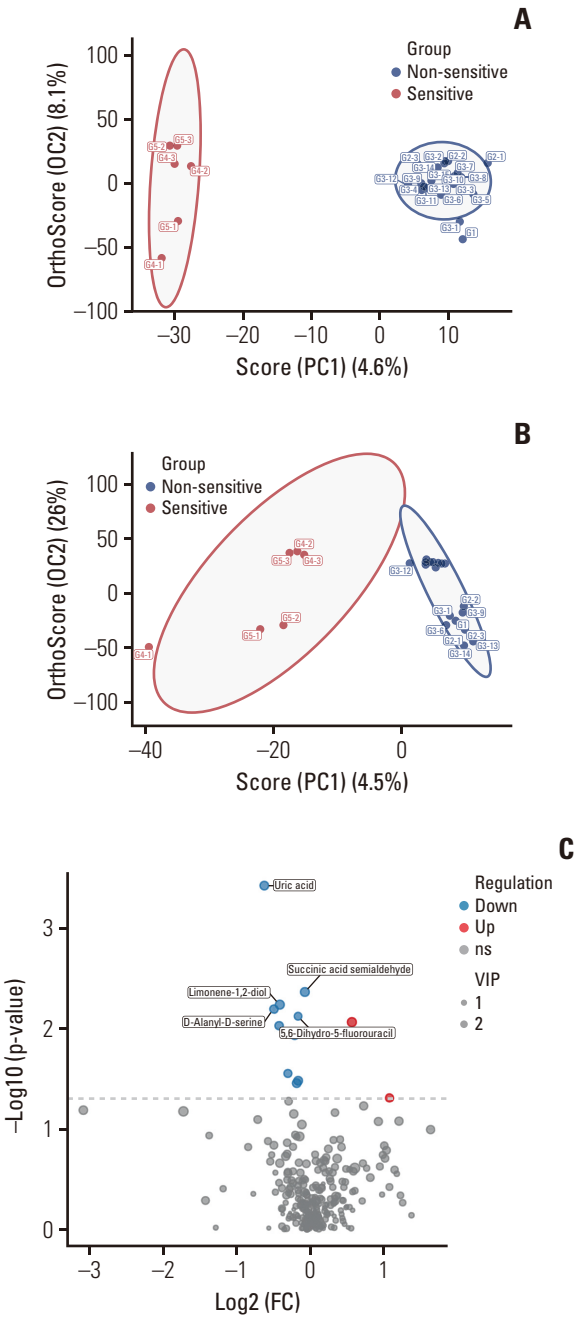

Fig. 3. Analysis of serum metabolomics of patients with ER+/HER2– breast cancer patients from liquid chromatography–mass spectrometry data. (A) Partial least squares discriminant analysis (PLS-DA) score plot for discriminating non-sensitive and sensitive to neoadjuvant therapy (NAT) in the electrospray ionization negative (ESI–) mode. (B) PLS-DA score plot for discriminating non-sensitive and sensitive to NAT in the ESI+ mode. (C) The volcano diagram depicting the differentially expressed metabolites in the non-sensitive and sensitive groups. ER, estrogen receptor; FC, fold change; HER2, human epidermal growth factor receptor 2; ns, not significant; VIP, variable importance in the projection.
(Continued to the next page)

between vitamin D3 levels and MP grade, while succinic acid semialdehyde and limonene-1,2-diol showed a significant positive correlation with MP grade (S5 Fig.).

Pathway enrichment analysis revealed that the 13 metabolites under investigation were associated with 19 distinct metabolic pathways. Notably, pathways involved in rheumatoid arthritis (p=0.012, involving vitamin D3), cysteine and methionine metabolism (p=0.024, with 5′-methylthioadenosine and 2-oxo-4-methylthiobutanoic acid), and tyrosine metabolism (p=0.036, with succinic acid semialdehyde and 3-hydroxyphenylacetic acid) were significantly impacted ($p < 0.05$) (Fig. 3E). The network of metabolites and their enriched pathways were showed in Fig. 3F. Subsequent ROC curve analysis (S6 Fig.) indicated that some metabolites achieved area under curve values above 0.7, suggesting they possess moderate predictive power for treatment response.

Discussion

ER plays a central role in the oncogenesis of BC. Thus, patients with ER+ BC who are treated with chemotherapy alone may not have a better outcome without endocrine therapy. The combination of endocrine drugs with chemotherapy, while historically questioned due to potential antagonistic effects, is gaining support for its synergistic potential [12,13].

Fulvestrant is markedly superior to other endocrine therapeutic agents in the first-line treatment of advanced BC. In addition to degrading ER, it remarkably downregulates PR expression [8]. Several studies have shown that fulvestrant significantly prolongs PFS with minimal side effects [14], and can synergize with a variety of chemotherapeutic agents, including taxanes and anthracyclines [15]. Therefore, we envision that combining fulvestrant with NCT may be more effective in patients with ER+/HER2– LABC who require neoadjuvant therapy (NAT) while ensuring safety.

In ER+/HER2– BC, there is no evidence that prognosis is associated with pCR after NAT. Because higher ORRs with neoadjuvant endocrine therapy are associated with improved survival outcomes [16], ORR is the more favored endpoint in clinical trials to assess the efficacy of combination therapy. Data synthesis from previous NCT indicates an ORR of approximately 60% to 70% in patients with ER+/HER2– BC. In our study, the ORR reached 86.1% when fulvestrant was added to NCT.

This study found that the concurrent use of fulvestrant and chemotherapy was tolerated, with no serious AEs or treatment-related deaths. There were no unexpected TEAEs. The most common grade 3 or 4 AEs were neutropenia and leukopenia, both were manageable. It is not possible to distinguish

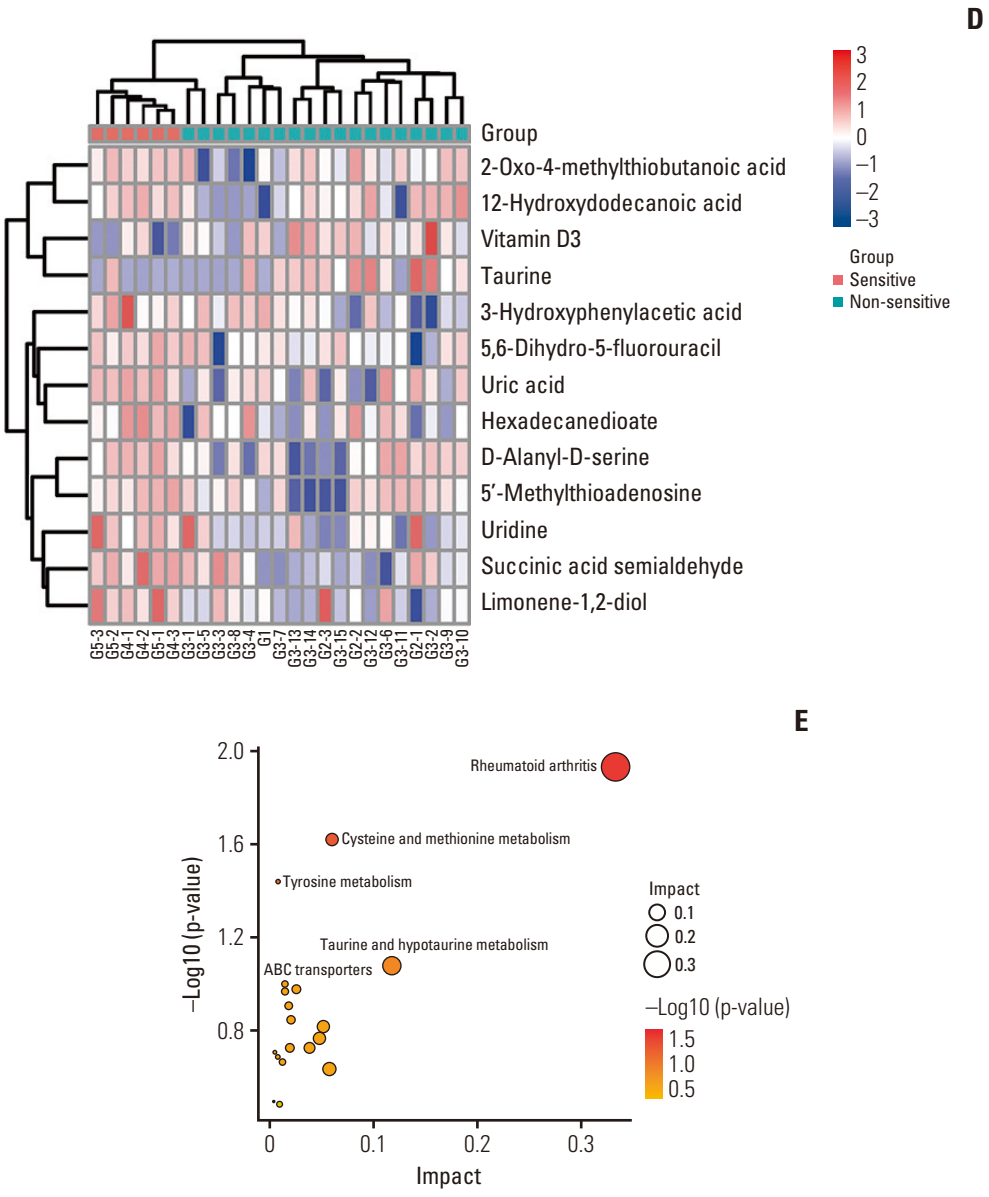

Fig. 3. *(Continued from the previous page)* (D) Heatmap depicting the differences in metabolites between non-sensitive and sensitive patients depending on metabolite class. Each column represents a subject and each row represents a metabolite. (E) The scatter plot of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched for differential expression metabolites. *(Continued to the next page)*

whether AEs were due to chemotherapy or endocrine therapy, as such events can occur with both chemotherapy agents and fulvestrant, but combination therapy did not increase the incidence or severity of AEs, such as nausea, vomiting, fatigue, or hepatic function abnormalities. Common endocrine therapy-related AEs, like hot flashes and musculoskeletal pain, were also not found because of the limited number of cases.

For HR-positive BC, ER and PR are the indications and targets of endocrine therapy. They serve as both prognostic and predictive biomarkers in HR+/HER2– BC. Moreover, the expression of ER and PR was significantly reduced in surgical samples after combination therapy. This reduction indicates a decrease in ER expression and an inhibition of the ER signaling pathway, demonstrating the effectiveness of this treatment approach. Further analysis revealed that having a greater pathologic remission was related with a 55% drop in ER expression following therapy. In addition, changes in Ki-67 before and after treatment are more effective than pCR in predicting treatment benefit and long-term outcomes

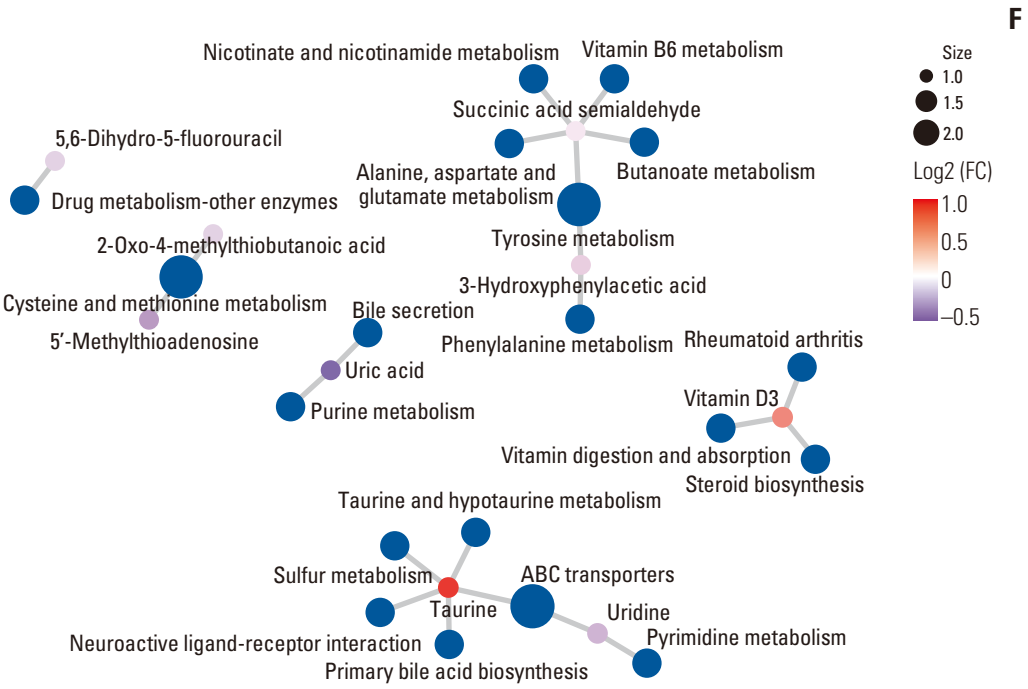

Fig. 3. *(Continued from the previous page)* (F) The network of metabolites and their enriched pathways.

in patients with ER+ BC [17-20]. This study showed that patients with more than a 20% decrease in Ki-67 after treatment tended to have a better pathologic response.

However, due to sampling error and tumor heterogeneity, IHC results from a single puncture biopsy can only precisely predict ER expression in 50%-60% of patients [21,22]. More importantly, IHC-positive ER may not be functional for estrogen binding. As observed in the patients enrolled in this study, all ER expressions detected by IHC were above 50%, but there were significant differences in efficacy, suggesting that the presence of ER does not necessarily indicate its involvement in tumor development or tumorigenesis. Therefore, functional ER may be the best predictor of success in endocrine therapy. PET with ^{18}F-FES has a very high positive predictive value in reflecting ER status, with an overall sensitivity and specificity of 84% and 98%, respectively [23-25]. Patients had higher baseline ^{18}F-FES uptake, suggesting greater sensitivity to endocrine therapy [26-28]. In this study, patients with higher baseline SUVmax levels responded better to fulvestrant combined with chemotherapy, had more evident tumor lesion regression, higher clinical response rates, and pathological MP grades, especially those with an SUVmax greater than 4.6.

Aberrant metabolism is a major hallmark of cancer. In the current investigation, our data revealed that sensitive patients with ER+/HER2– BC manifested distinct metabolic characteristics at baseline of NAT compared to non-sensitive patients. These differentially expressed metabolites are highly predictive of NAT response. These metabolic alterations were most likely caused by the dysregulation of numerous metabolic pathways, which both might be related to neoadjuvant efficacy.

Among these elevated metabolites in sensitive patients, the specific roles of succinic acid semialdehyde, limonene-1,2-diol, D-alanyl-D-serine, 3-hydroxyphenylacetic acid, and hexadecanedioate in cancer are currently limited. At present, only one recent study indicated that 12-hydroxydodecanoic acid was decreased in the intestines of esophageal squamous cell carcinoma (ESCC) patients compared with healthy controls, and might be a diagnostic and predictive marker of ESCC [29]. 2-Oxo-4-methylthiobutanoic acid is a metabolite associated with cysteine and methionine metabolism and synthesized by the acireductone dioxygenase 1 (ADI1) enzyme [30]. It was reported that it was up-regulated in serous carcinoma tumors compared to endometrial endometrioid carcinomas [30]. Conversely, it was found to be decreased in patients with hepatocellular carcinoma (HCC) compared to those with liver cirrhosis, and was identified as a prognostic metabolite for HCC [31]. Whether 2-oxo-4-methylthiobutanoic acid and its synthetic enzyme ADI1 expression could be an indicator for efficacy assessment should be further evaluated. 5,6-dihydro-5-fluorouracil, uric acid, 5'-methylthioadenosine, and uridine have been relatively extensively studied in oncology, demonstrating considerable potential as

biomarkers in tumor research. But further investigation and exploration are warranted to fully elucidate their roles and potentials in BC biomarker research, especially for the prediction of NAT efficacy.

In the current study, taurine and vitamin D3 were higher in non-sensitive group compared with sensitive group. Our previous study also found that taurine was up-regulated in non-pCR patients compared with pCR patients with HER2-positive BC who underwent NAT with TCbHP (taxane, carboplatin, trastuzumab, and pertuzumab) regimen [32]. These findings suggest that taurine holds promise as a predictive biomarker for the efficacy of NAT in both HR+HER2– and HER2-positive BC. Further validation in larger sample sizes or external cohorts is warranted to corroborate these observations. Although a deficiency in vitamin D3 is associated with an elevated risk of BC [33], supplementation studies have not consistently shown a reduction in BC incidence. Notably, the bioactive form of vitamin D3, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), has been reported to augment the efficacy of cancer therapies [34,35]. Contrary to expectations, our findings showed higher Vitamin D3 levels in the non-sensitive patient group. This raises intriguing questions about potential impediments in the conversion of vitamin D3 to its active form among non-sensitive patients, a prospect that merits further exploration.

The current study had several limitations, including the relatively small sample size, the absence of a control group, and the lack of long-term follow-up. Given the exploratory nature of this study, further randomized controlled trials are required to substantiate the findings. Specifically, a larger cohort and a more extended period of follow-up are necessary to confirm whether a SUVmax threshold of 4.6 is indeed indicative of a greater benefit for patients initially presenting with high levels, as well as to assess any potential improvements in long-term survival outcomes. Additionally, the clinical relevance of the metabolites identified warrants deeper investigation to validate their utility in practice.

In conclusion, combining fulvestrant with chemotherapy for neoadjuvant treatment shows acceptable safety profile and promise in improving responses in ER+/HER2– LABC patients, particularly for those with higher baseline SUVmax on ^{18}F-FES PET scans. While this combination appears to enhance tumor shrinkage and aid surgical outcomes, long-term survival impacts require further evaluation. This therapeutic approach could be a valuable addition to neoadjuvant strategies, with baseline PET scans serving as a tool to pinpoint ideal candidates. The study also highlights the potential role of metabolic profiling in predicting treatment response, reinforcing the move towards more personalized cancer care.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Ethical Statement

The study protocol, any amendments, and informed consent were approved by the Institutional Ethics Committee of Chongqing University Cancer Hospital (CZLS2020253-A). The study was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and local applicable regulatory requirements (ClinicalTrials.gov identifier: ChiCTR2000041235). All participants provided written informed consent. The privacy rights of the participants always be observed.

Author Contributions

Conceived and designed the analysis: Shao Q, Wu J, Zeng X.
Collected the data: Shao Q, Zhang N, Pan X, Zhou W, Wang Y, Wu J.
Contributed data or analysis tools: Pan X, Zhou W, Wang Y, Chen X, Wu J.
Performed the analysis: Shao Q, Zhang N, Chen X, Zeng X.
Wrote the paper: Shao Q, Zhang N, Zeng X.

ORCID iDs

Qing Shao : https://orcid.org/0000-0002-1048-1325
Ningning Zhang : https://orcid.org/0000-0002-2919-7770
Jing Wu : https://orcid.org/0000-0002-6416-005X
Xiaohua Zeng : https://orcid.org/0000-0001-5810-8055

Conflicts of Interest

Fulvestrant was provided by Chia Tai Tianqing Pharmaceutical Group Co., Ltd.

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