ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Prediction of response to neoadjuvant chemotherapy for triple-negative breast cancer using nuclear magnetic resonance-based urine metabolomics and self-organizing maps

Jinping Gu[1], Tingxiao Zou[1], Yao Gao[1], Ziyi Jiang[1], Feng Su[1], and Xiangming He[2]*

[1]College of Pharmaceutical Sciences, Key Laboratory for Green Pharmaceutical Technologies and Related Equipment of Ministry of Education, Zhejiang University of Technology, Hangzhou, Zhejiang, China

[2]Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China

Abstract

Introduction: Breast cancer constitutes the most common invasive neoplasm among women, with triple-negative breast cancer (TNBC) representing 10–20% of all cases and lacking a standardized therapeutic approach. Chemotherapy remains the cornerstone of treatment for TNBC patients.

Objective: Our research aimed to identify urinary metabolomic biomarkers capable of discerning chemotherapeutic response variability.

Methods: In this study, we utilized a nuclear magnetic resonance (NMR)-based urinary metabolomics technique to evaluate the metabolic profiles of TNBC patients exhibiting diverse chemotherapeutic responses.

Results: We found that the relative abundance of urinary metabolites effectively differentiated TNBC patients based on chemosensitivity. Multivariate receiver operating characteristic curve analysis indicated potential biomarkers indicative of chemotherapy responsiveness across three patient cohorts. Pathway analysis suggested perturbations in aminoacyl-tRNA biosynthesis; arginine, aspartate, and glutamate metabolism; and valine, leucine, and isoleucine biosynthesis in the pCR subgroup, and in arginine and proline metabolism, aminoacyl-tRNA biosynthesis, and histidine metabolism in the pathological partial response subgroup.

Conclusion: Our NMR metabolomic data suggest that urinary metabolites hold potential as predictors of chemotherapy sensitivity in TNBC patients.

Keywords: Triple-negative breast cancer; Nuclear magnetic resonance; Neoadjuvant chemotherapy; Metabolic phenotype; Metabolomics

***Corresponding author:**
Xiangming He
(merchant001@163.com)

Citation: Gu J, Zou T, Gao Y, Jiang Z, Su F, He X. Prediction of response to neoadjuvant chemotherapy for triple-negative breast cancer using nuclear magnetic resonance-based urine metabolomics and self-organizing maps. *Eurasian J Med Oncol*. 2026;10(3):025480497. doi: 10.36922/EJMO025480497

Received: November 30, 2025

Revised: March 10, 2026

Accepted: May 6, 2026

Published online: May 20, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

According to the American Cancer Society, breast cancer (BC) represents the most prevalent malignancy among newly diagnosed invasive cancers in the United States as of 2022, accounting for approximately 31% of all cases in females.[1] Triple-negative BC (TNBC) constitutes 10–20% of all BCs and lacks standardized treatment strategies due to the absence of estrogen receptor, progesterone receptor, and human epidermal growth

factor receptor-2 expression.[2] Chemotherapy remains a primary treatment for TNBC,[3] encompassing preoperative neoadjuvant chemotherapy (NAC), postoperative adjuvant chemotherapy, and salvage therapy for advanced stages. NAC is a standard regimen for advanced TNBC; however, patient responses to chemotherapeutic agents vary.[4,5] Approximately 30–40% of TNBC patients achieve a pathological complete response (pCR), while others exhibit no change in pathology, termed pathological stable disease (pSD). The remaining patients experience some improvement, described as pathological partial response (pPR).[4,5] Therefore, it is critically important to accurately describe the varying responses to chemotherapy in TNBC patients. Previously, we used nuclear magnetic resonance (NMR)-based serum metabolomics to predict response to NAC in TNBC.[6] In this study, we utilized NMR-based urine metabolomics to differentiate between TNBC patients with varying chemosensitivity.

Precision medicine for cancer hinges on stratifying patients into groups based on their likely response to treatment.[7] Advances in omics technologies have enabled such precision medicine.[8] Metabolomics, being closer to the phenotypic manifestation of disease than genomics, transcriptomics, or proteomics, offers valuable insights into metabolic alterations during treatment.[9,10] For instance, TNBC cells exhibit lower glutathione levels than normal cells,[11] and compared to Luminal BC, show elevated levels of amino acids, nucleotides, nucleotide sugars, and proliferation-associated metabolites like choline.[12,13] Our team has been dedicated to applying NMR-based metabolomics to precision medicine, having previously used it to differentiate metabolic phenotypes between colorectal cancer and polyps.[14] In TNBC, we have leveraged this technique to discern variations in chemotherapy sensitivity among patients.[6]

Therefore, based on the different chemosensitivity outcomes of TNBC in clinical practice and the background of tumor metabolic characteristics in the host, our study aims to establish NMR-based metabolomics combined with self-organizing maps (SOMs) to analyze the urinary metabolic phenotypes in TNBC patients. The objective was to identify robust urinary metabolic biomarkers capable of distinguishing patient cohorts according to chemosensitivity, thereby providing a basis for the early prediction of NAC sensitivity.

2. Materials and methods

2.1. Chemicals and reagents

Deuterated reagents, including deuterium oxide and sodium 3-(trimethylsilyl) propionate-2,2,3,3-d4 (TMSP), were sourced from Cambridge Isotope Laboratories, Inc. (Tewksbury, United States). Other common reagents ($NaH_2PO_4 \cdot 2H_2O$ and $K_2HPO_4 \cdot 3H_2O$) were purchased from J&K Scientific Ltd. (China). Ultrapure water for the experiments was produced using our laboratory's Milli-Q IQ 7000 system (Merck KGaA, Germany).

2.2. Sample preparation and nuclear magnetic resonance data acquisition

Patients with TNBC who underwent NAC at Zhejiang Cancer Hospital from 2019 to 2020 were included in this study. The research adhered to the protocols approved by the Zhejiang Cancer Hospital Ethics Committee, in line with the Declaration of Helsinki principles. Patients were evaluated for efficacy after NAC, of which 8 patients had pCR, 28 patients had pPR, and 16 patients had pSD. Patient cases were statistically analyzed for age, body mass index, Ki-67, menstrual status, and tumor stage, and there were no statistically significant differences; the details of which were published in our previous study.[6] Urine samples were stored at −80 °C. Prior to data acquisition, samples were thawed at 4 °C. A 400 μL aliquot of each sample was mixed with 200 μL of phosphate buffer in deuterated water (pH 7.4). After centrifugation (4 °C, 12,000 × g, 15 min), 550 μL from each sample was transferred to an NMR tube (5 mm × 7 inches). Spectra were acquired on a BRUKER 600MHz NMR spectrometer (AVANCE III HD, BRUKER BioSpin, Germany) with a TXI probe at 300 K. The NOESY pulse sequence (NOESYPR1D [RD-90°-t_1-90°-τ_m-90°-ACQ]) with water irradiation was employed, featuring a 3 s relaxation delay and 120 ms mixing time. The acquisition parameters are detailed in the referenced literature.[15-17] Subsequently, metabolite identification and relative concentration calculations based on NMR spectra were conducted using Signature Mapping (SigMa).[18] Additionally, the Human Metabolome Database (https://hmdb.ca/) served as a reference to corroborate metabolite identification.[19]

2.3. Data processing and multivariate statistical analysis

Nuclear magnetic resonance spectra preprocessing, including phase and baseline corrections, was accomplished with Topspin 3.5.7 (BRUKER, BioSpin, Germany). The spectra were then referenced to the TMSP methyl signal ($-CH_3$) at 0 ppm using the same software. To align peaks across various samples, minor adjustments were made utilizing the icoshift algorithm in MATLAB 2019B (MathWorks, United States).[20] To improve comparability among urine samples, the metabolite data were normalized to creatinine.[21] SOMs are systems employed in unsupervised learning for cluster analysis and data structure exploration.[22] When integrated with artificial neural networks, SOMs are capable of

addressing both supervised and unsupervised learning challenges.[23,24] SOMs and their associated algorithms have been extensively applied in metabolomics research.[25-27] In this study, we implemented the Kohonen and CPANN toolbox for classifying and discriminating differential metabolites using MATLAB (http://www.michem.unimib.it/download/matlab-toolboxes/kohonen-and-cpann-toolbox-for-matlab/).[28,29] To assess the discriminative potential of differential metabolites, multi-receiver-operating characteristic (multi-ROC) curve analysis was employed,[6,30] utilizing logistic regression for patient classification and the area under the ROC curve (AUC) for evaluating metabolite prediction performance in disturbed metabolic pathways, considering an AUC exceeding 0.70 as significant.[31]

2.4. Identifying the disturbed metabolic pathways

According to the relative concentration of differential metabolites in the urine samples, the MetaboAnalyst 5.0 web server (www.metaboanalyst.ca/) was used for analysis of the disturbed metabolic pathways.[32] The pathway analysis incorporated two evaluative parameters: (i) a hypergeometric test-derived *p*-value, which matched differential metabolites with those in related pathways,[33] and (ii) a pathway impact value determined by a topological analysis utilizing the out-degree centrality algorithm.[34]

3. Results

A total of 52 patients were included in this study. In the present study, the characteristics of recruited TNBC patients were homogeneous, as detailed in our preceding publication.[6]

3.1. Metabolic profiles of urine samples

The NMR spectra of urine samples revealed 89 metabolites, whose relative concentrations were quantified using SigMa. The metabolite signals in NMR spectra, along with their functional groups and relative concentrations, are detailed in Table S1. The quantitative metabolite data were analyzed with the Kohonen and CPANN Toolbox. The genetic algorithm refined the SOM architecture, with the bubble plot indicating the optimal configuration (Figure 1). This plot highlighted two indicators of superior SOMs architecture: relative selection frequency in the genetic algorithm and the optimization criterion mean value.[35] After evaluating model performance and structural complexity, an optimized architecture of 10 × 10 neurons with 250 epochs was chosen (Figure 1A). Supervised Kohonen maps with montecarlo cross-validation (20% out) were then executed in MATLAB. The Kohonen layer analysis of the SOM model distinctly differentiated three patient groups based on chemosensitivity (Figure 1B), demonstrating distinguishable metabolic phenotypes among the pSD, pPR, and pCR groups. The pPR patients clustered in the left region, pCR in the lower right, and pSD in the upper right.

3.2. Analysis of differential metabolites among triple-negative breast cancer patients

In the SOMs model, the class profiles plot (Figure 2) and corresponding weight maps were utilized to examine differential metabolites across various TNBC patient subsets. The class profiles plot revealed 10 metabolites with higher average weights, suggesting their potential as biomarkers for identifying the pCR group. These include alanine, asparagine, citrate, glutamine, isobutyrate, isoleucine, leucine, succinate, valine, and anserine. The weight maps (Figure 2A–K) of these metabolites presented a pattern analogous to the clustering of three groups in the SOMs depicted in Figure 1B. Furthermore, four metabolites (creatine phosphate, glucose, hippurate, and trans-ferulate) emerged as potential biomarkers for the pSD group, as illustrated in Figure 3A–E. Clustering within the pPR group revealed eight additional differential metabolites, namely creatinine, guanidoacetate, isovalerate, lactate, serine, methionine, proline, and histamine (Figure 4A–I).

3.3. Potential discriminant analysis of differential metabolites

Multivariate ROC curve analysis was employed to assess the capacity of differential metabolites to act as potential biomarkers. Within the pCR group, all differential metabolites demonstrated strong discriminative abilities, as evidenced by large AUC values (Figure 5A). Notably, metabolites such as alanine, glutamine, leucine, and succinate achieved high AUC values of 0.9375, 0.9773, 0.9886, and 0.9972, respectively. Asparagine, citrate, isoleucine, valine, and anserine also exhibited substantial AUC values of 0.8295, 0.8551, 0.8636, 0.8636, and 0.8892. Isobutyrate, with an AUC value of 0.7898, displayed a discernible capacity. Moreover, eight differential metabolites in the pPR group were identified as potential biomarkers, characterized by large AUC values (Figure 5C), with creatinine, guanidoacetate, isovalerate, lactate, serine, methionine, proline, and histamine demonstrating significant values (0.9628, 0.9985, 0.9568, 0.9509, 0.9524, 0.9539, 0.9390, and 0.9003). Although the AUC values for the differential metabolites in the pSD group were not as elevated as those in the pCR and pPR groups, they still exhibited discriminative potential. The metabolites creatine phosphate, glucose, hippurate, and trans-ferulate exceeded the threshold AUC values (0.7917, 0.7743, 0.7639, and 0.7622; Figure 5B). Multi-ROC curve analysis of differential metabolites suggests that each patient

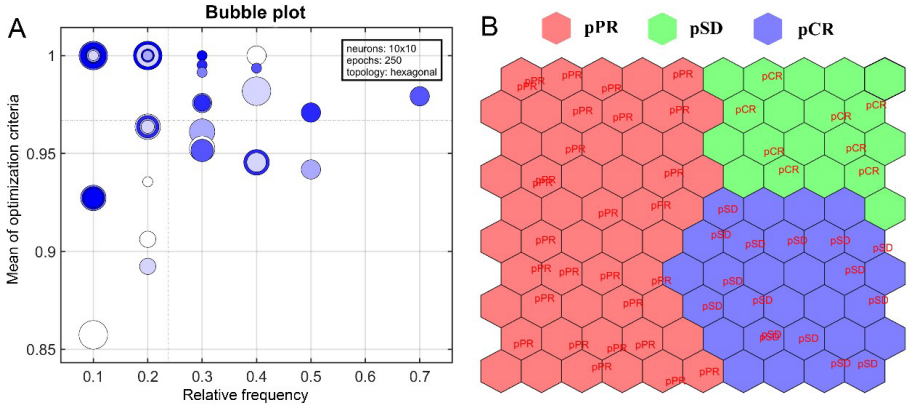

Figure 1. Development of the self-organizing map (SOM) model. (A) The bubble plot demonstrates the optimization of the SOM architecture via genetic algorithms. Each bubble represents a variant of the SOM architecture; bubble size and color correspond to the number of neurons and the quantity of epochs within the SOM, respectively. (B) Utilization of the SOM model, grounded on all 89 metabolites from the nuclear magnetic resonance-based urine metabolome, for classifying and predicting triple-negative breast cancer patients with divergent chemotherapy sensitivities: the left region is indicative of pathological partial response (pPR) patients, the upper right region is indicative of pathological stable disease (pSD) patients, and the lower right region is indicative of pathological complete response (pCR) patients.

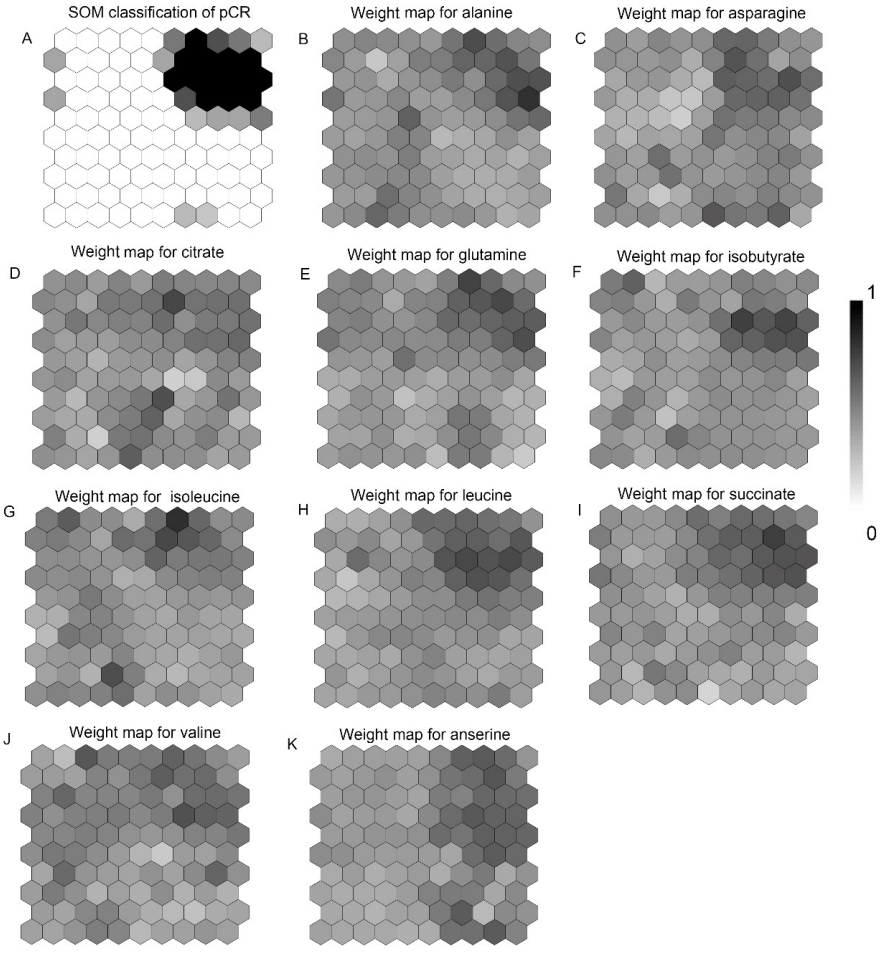

Figure 2. Weight maps for 10 metabolites in the self-organizing map (SOM) model, delineating differentiation of the pathological complete response (pCR) group. (A) SOM classification for the pCR group; weight maps for alanine (B), asparagine (C), citrate (D), glutamine (E), isobutyrate (F), isoleucine (G), leucine (H), succinate (I), valine (J), and anserine (K).

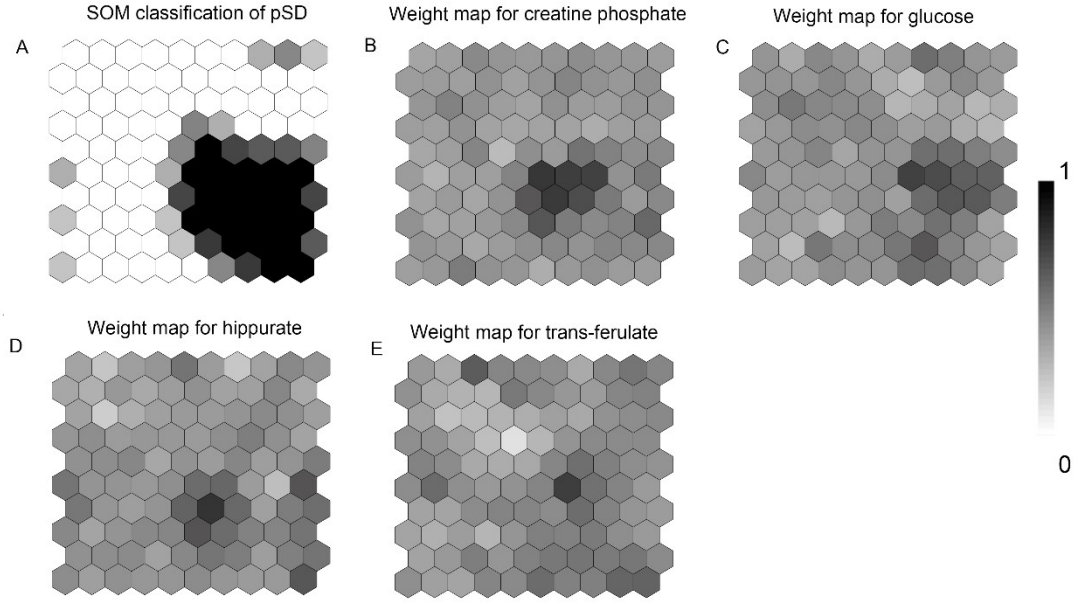

Figure 3. Weight maps for four metabolites in the self-organizing map (SOM) model, delineating differentiation of the pathological stable disease (pSD) group. (A) SOM classification for the pSD group; weight maps for creatine phosphate (B), glucose (C), hippurate (D), and trans-ferulate (E).

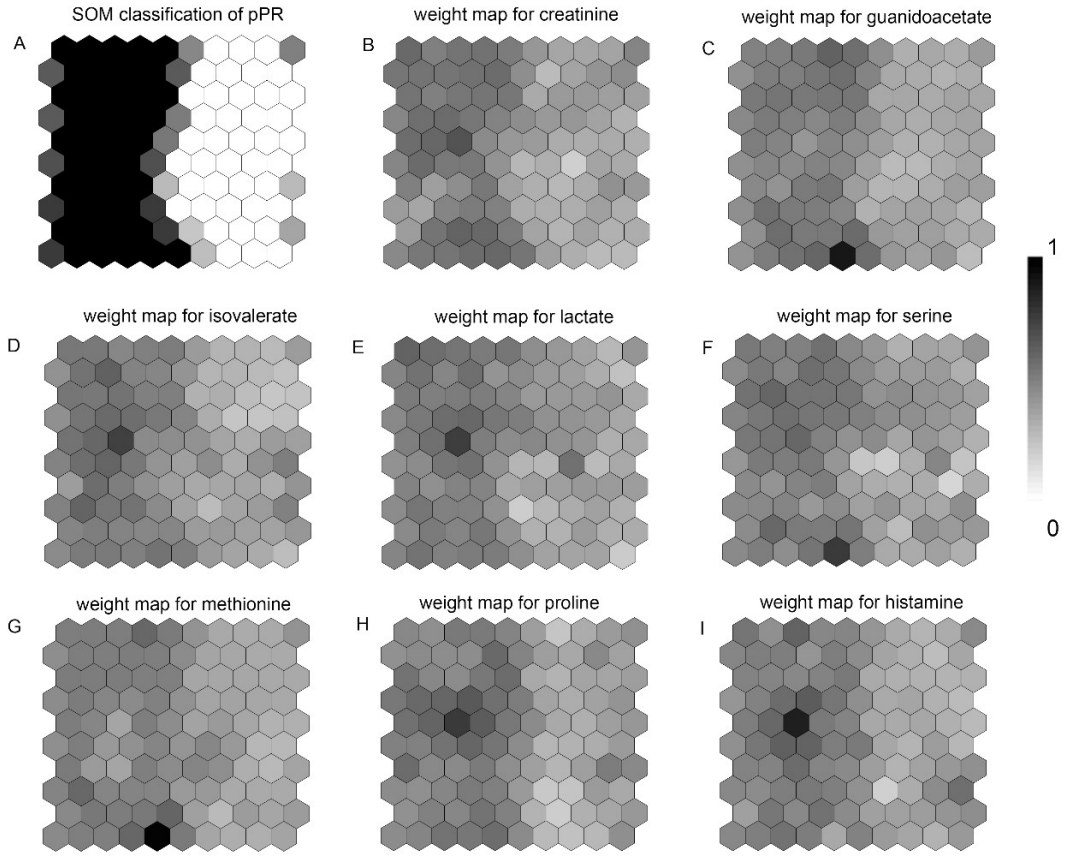

Figure 4. Weight maps for eight metabolites in the self-organizing map (SOM) model, delineating differentiation of the pathological partial response (pPR) group. (A) SOM classification for the pPR group; weight maps for creatine (B), guanidoacetate (C), isovalerate (D), lactate (E), serine (F), methionine (G), proline (H), and histamine (I).

subgroup (pCR, pPR, and pSD) could be distinguished by specific urinary biomarkers. At the same time, we also calculated the relative concentrations of metabolites in urine, and the t-test results of two-by-two comparisons between the three groups, and the results were corrected by false discovery rate adjustments (Table S2).

3.4. Disturbed metabolic pathway analysis

Disrupted metabolic pathways were identified for different patient groups (pCR, pSD, and pPR) using MetaboAnalyst 5.0. In the pCR group, three pathways were affected: aminoacyl-tRNA biosynthesis; alanine, aspartate, and glutamate metabolism; and valine, leucine, and isoleucine biosynthesis (Figure 6A). The pSD group did not exhibit any disrupted pathways (Figure 6B). The pPR group was associated with disturbances in arginine and proline metabolism, aminoacyl-tRNA biosynthesis, and histidine metabolism (Figure 6C). A comparative analysis revealed that aminoacyl-tRNA biosynthesis was disrupted in both the pCR and pPR groups. It is important to note that these pathway analyses are exploratory and enrichment-based; variations in urinary amino acid levels suggest metabolic shifts rather than definitively proving the disruption of aminoacyl-tRNA biosynthesis.

4. Discussion

Triple-negative breast cancer is a highly heterogeneous breast malignancy[36] that, unlike other molecular subtypes of BC, lacks common receptor-based therapeutic targets, is prone to recurrence and metastasis, and is typically

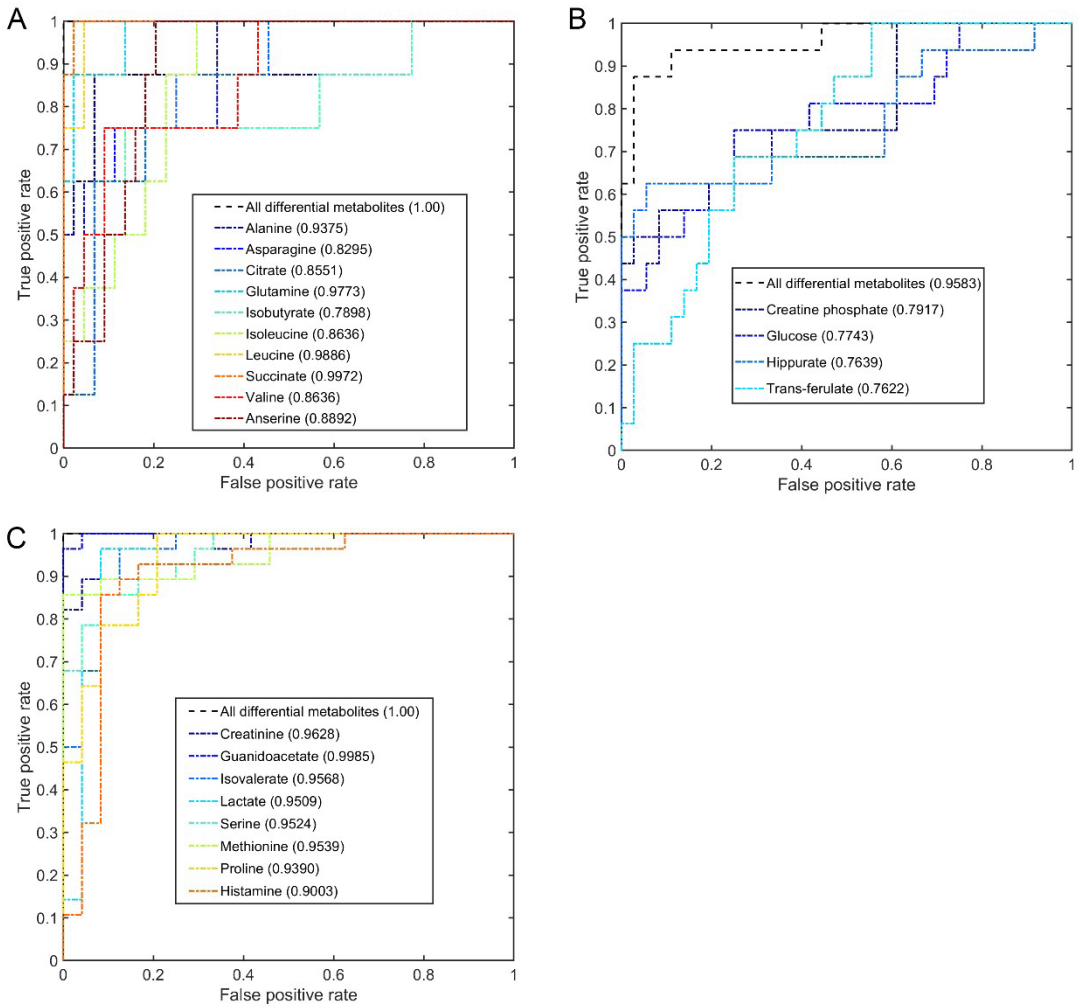

Figure 5. Multi-receiver-operating characteristic curves assessing the discriminant capabilities of significantly altered metabolites. (A) Differential metabolites in the pathological complete response group, used for distinction from other groups; (B) Differential metabolites in the pathological stable disease group, used for distinction from other groups; (C) Differential metabolites in the pathological partial response group, used for distinction from other groups.

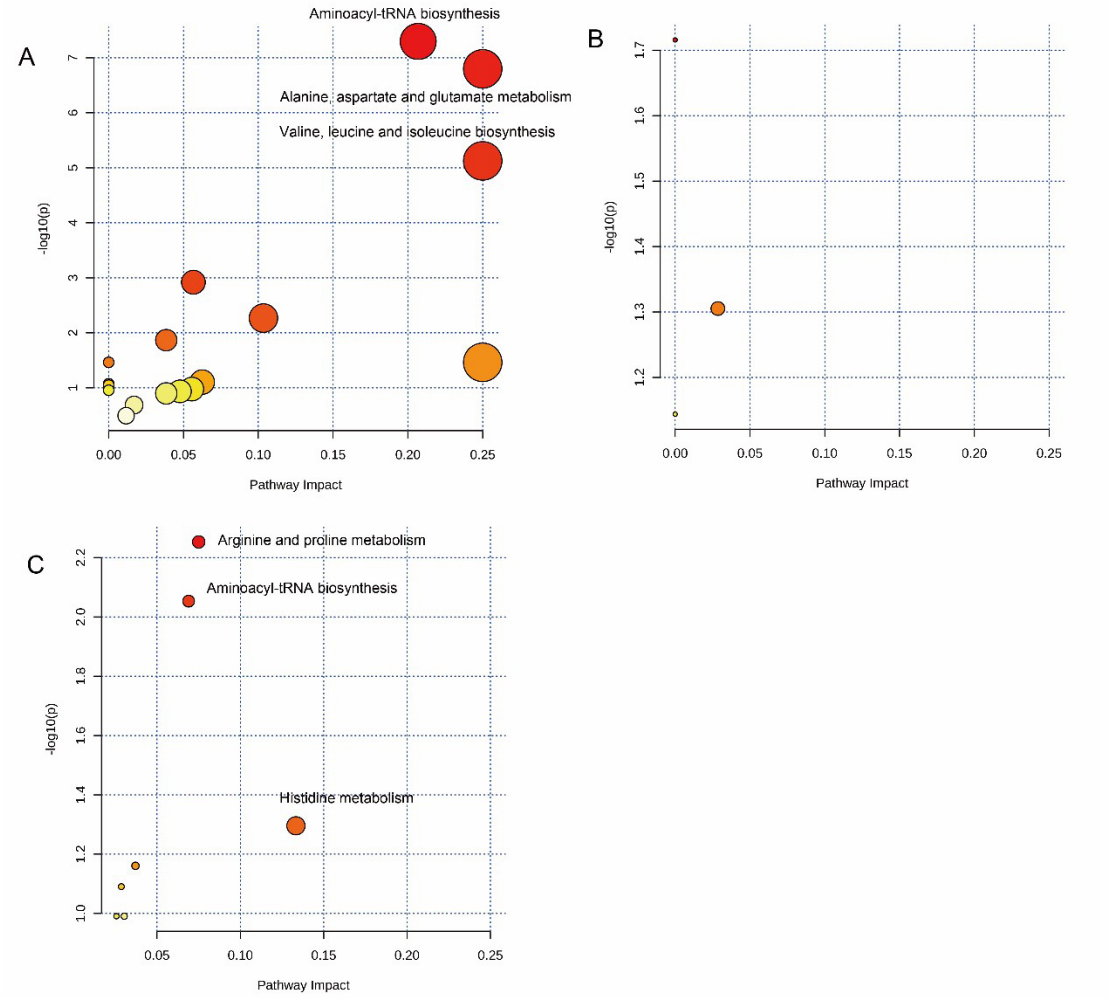

Figure 6. Analysis of significantly perturbed metabolic pathways among the three triple-negative breast cancer patient groups. (A) Metabolic pathway perturbations in pathological complete response patients versus others; (B) Perturbations in pathological stable disease patients versus others; (C) Perturbations in pathological partial response patients versus others.

associated with a poor prognosis. Chemotherapy remains a principal treatment, particularly NAC, with current guidelines recommending various intensive treatments based on NAC efficacy. Early prediction of tumor response to chemotherapy, to tailor individualized treatment, is a current research challenge. Therefore, our study sought metabolic markers for predicting TNBC NAC sensitivity via NMR-based urinary metabolomics profiles. Although the clinicopathological characteristics did not differ significantly among the three patient groups ($p > 0.05$), these indices appeared limited in their ability to distinguish chemotherapy sensitivity in this cohort. Our analysis identified distinct urinary metabolic phenotypes across the three response groups, based on NMR metabolomics of 52 TNBC patient urine samples with varying chemotherapy sensitivities.

Based on the relative concentration of 89 urinary metabolites, the SOM map successfully differentiated the three patient groups. Subsequent analysis with the SOMs model's weight maps identified 10 differential metabolites in the pCR group, 4 in the pSD group, and 8 in the pPR group. The multivariate ROC curve analysis revealed that the differential metabolites in the three groups could serve as potential biomarkers for assessing chemotherapy sensitivity among diverse patients.

The potential biomarkers in the pCR group, including alanine, asparagine, isoleucine, leucine, and valine, are implicated in this pathway. Our study suggests that these metabolic processes and metabolites could also serve as indicators for NAC sensitivity in patients with TNBC in

the pCR and pPR groups. Moreover, disruptions were observed in the metabolic pathways involving alanine, aspartate, glutamate metabolism, and the biosynthesis of valine, leucine, and isoleucine in pCR patients. These differential metabolites are also associated with the latter two pathways. Abnormalities in amino acid metabolism are well-documented across various tumor types,[37,38] and our findings corroborate the link between amino acid metabolism and chemosensitivity. Specifically, the enrichment of branched-chain amino acids (BCAAs) such as valine, leucine, and isoleucine may be linked to tumor proliferation and redox balance. BCAAs are known to activate mTOR signaling, driving cancer cell growth,[39] and their catabolism can contribute to the maintenance of the cellular antioxidant pool, potentially conferring resistance to chemotherapeutic agents.

In addition to the aforementioned metabolic aberrations, our study identified disturbances in the metabolic pathways of arginine and proline, as well as histidine, in the pPR group. This observation suggests that these pathways are also modulated in patients who exhibit partial responses to chemotherapy. Jasbi *et al.*[40] reported alterations in arginine and proline metabolism in the plasma of patients with early BC. Willmann *et al.*[41] discovered that histidine metabolism could influence cellular turnover and DNA/RNA modifications, leading to increased excretion of modified nucleosides in BC cells. Our findings reveal that histidine metabolism is altered in the urine samples of pPR patients.

The convenience of using urine samples for metabolomics analysis is well-recognized. Lo *et al.*[42] found that levels of acetylspermine, spermidine, norepinephrine, and dopamine in urine were significantly higher in patients with favorable chemotherapy outcomes compared to non-responders, as determined by untargeted metabolomics in the urine samples of BC patients. Although urine samples can be calibrated, their accuracy can be compromised due to the variability in urine concentration among individuals, which can be influenced by factors such as diet, fluid intake, and time of collection. Moreover, signal fluctuations in urine samples can arise from matrix effects.[43] Since urine undergoes glomerular filtration, there is a risk that many significant metabolites may be filtered out, potentially leading to the non-detection of meaningful data.

In our study, pre-chemotherapy first morning urine samples were utilized, and patients with metabolic diseases were excluded to minimize confounding effects on urinary metabolite profiles. While the results of this study can predict NAC sensitivity through urine modeling and differential metabolites, the applicability of these findings warrants further validation. The study has limitations, including its reliance on NMR metabolomics to analyze the relative concentration of urinary metabolites associated with varying chemotherapy sensitivities without quantifying their absolute concentrations in urine. Such a limitation presents challenges in standardizing data for broader clinical application. Furthermore, the clinical patient samples collected for this project were limited in number, underscoring the need for more extensive clinical urine sample collections in future research. In summary, NMR metabolomic profiling of urinary metabolites suggests distinct metabolic phenotypes in TNBC with varying chemosensitivities, and specific metabolic panels, prominently including succinate, guanidoacetate, leucine, and glutamine, may serve as potential biomarkers to differentiate these phenotypes. This provides a foundation for the early prediction of chemosensitivity in TNBC.

While these findings are promising, this exploratory study has several critical limitations. First, the cohort size is small (n = 52), particularly the pCR subgroup (n = 8). Consequently, the high AUC values observed in our multi-ROC analysis may be subject to overfitting, necessitating future validation utilizing permutation testing and independent external cohorts. Second, the study relies on relative metabolite concentrations; absolute quantification with internal standards is required to establish clinical thresholds. Third, only baseline, pre-chemotherapy first-morning urine samples were collected, precluding the assessment of dynamic metabolic adaptations during treatment. Finally, while creatinine normalization is standard for mitigating hydration variability, future studies should consider comparing this with probabilistic quotient normalization (PQN) and strictly controlling dietary intake.

5. Conclusion

This study used NMR-based urine metabolomics combined with self-organizing maps to distinguish distinct metabolic phenotypes among patients with triple-negative breast cancer according to their response to neoadjuvant chemotherapy. Specific panels of differential urinary metabolites, including succinate, guanidoacetate, leucine, and glutamine, showed potential as biomarkers for differentiating between pCR, pPR, and pSD. Perturbations in amino acid metabolism were associated with treatment response, including alanine, aspartate, glutamate, and branched-chain amino acid metabolism in the pCR group, and arginine, proline, and histidine metabolism in the pPR group.

Acknowledgments

This study was carried out under the laboratory support

of Pharmaceutical Public Analytical Platform from the College of Pharmaceutical Sciences, Zhejiang University of Technology.

Funding

This work was supported by the Zhejiang Provincial Medicine and Health Science Fund (2020KY482 and 2022KY632).

Conflict of interest

The authors declare no competing financial interest.

Author contributions

Conceptualization: Jinping Gu, Xiangming He
Formal analysis: Tingxiao Zou
Investigation: Yao Gao, Ziyi Jiang, Feng Su
Methodology: Jinping Gu, Xiangming He
Supervision: Jinping Gu, Xiangming He
Writing – original draft: Jinping Gu, Xiangming He
Writing – review & editing: All authors

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Zhejiang Cancer Hospital (Ethical approval No.: IRB-2023-137 (Research)). The patients/participants provided their written informed consent to participate in this study.

Consent for publication

All patients provided their written informed consent for the publication of their data.

Availability of data

The NMR spectroscopy of urine has been released into the public domain and can be downloaded from the Mendeley Data repository (https://data.mendeley.com/datasets/fsjz6gf4xv/1).

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7-33.

   doi: 10.3322/caac.21708

2. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*. 2020;22(1).

   doi: 10.1186/s13058-020-01296-5

3. Masuda N, Lee SJ, Ohtani S, *et al*. Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. *N Engl J Med*. 2017;376(22):2147-2159.

   doi: 10.1056/nejmoa1612645

4. Liedtke C, Mazouni C, Hess KR, *et al*. Response to Neoadjuvant Therapy and Long-Term Survival in Patients With Triple-Negative Breast Cancer. *J Clin Oncol*. 2008;26(8):1275-1281.

   doi: 10.1200/jco.2007.14.4147

5. Glück S, Ross JS, Royce M, *et al*. TP53 genomics predict higher clinical and pathologic tumor response in operable early-stage breast cancer treated with docetaxel-capecitabine ± trastuzumab. *Breast Cancer Res Treat*. 2011;132(3):781-791.

   doi: 10.1007/s10549-011-1412-7

6. He X, Gu J, Zou D, *et al*. NMR-Based Metabolomics Analysis Predicts Response to Neoadjuvant Chemotherapy for Triple-Negative Breast Cancer. *Front Mol Biosci*. 2021;8.

   doi: 10.3389/fmolb.2021.708052

7. Zou J, Wang E. Cancer Biomarker Discovery for Precision Medicine: New Progress. *Curr Med Chem*. 2020;26(42):7655-7671.

   doi: 10.2174/0929867325666180718164712

8. Olivier M, Asmis R, Hawkins GA, Howard TD, Cox LA. The Need for Multi-Omics Biomarker Signatures in Precision Medicine. *Int J Mol Sci*. 2019;20(19):4781.

   doi: 10.3390/ijms20194781

9. Jacob M, Lopata AL, Dasouki M, Abdel Rahman AM. Metabolomics toward personalized medicine. *Mass Spectrom Rev*. 2019;38(3):221-238.

   doi: 10.1002/mas.21548

10. Yang H, Pawitan Y, Fang F, Czene K, Ye W. Biomarkers and Disease Trajectories Influencing Women's Health: Results from the UK Biobank Cohort. *Phenomics*. 2022;2(3):184-193.

    doi: 10.1007/s43657-022-00054-1

11. Beatty A, Fink L, Strigun A, *et al*. Abstract A73: Metabolite profiling reveals the glutathione biosynthetic pathway as a therapeutic target in triple negative breast cancers. *Mol Cancer Res*. 2016;14(1_Supplement):A73-A73.

    doi: 10.1158/1557-3125.metca15-a73

12. Stewart DA, Winnike JH, McRitchie SL, Clark RF, Pathmasiri WW, Sumner SJ. Metabolomics Analysis of Hormone-Responsive and Triple-Negative Breast Cancer Cell Responses to Paclitaxel Identify Key Metabolic Differences. *J Proteome Res*. 2016;15(9):3225-3240.

    doi: 10.1021/acs.jproteome.6b00430

13. Huang DP, Ye XH, Jin C. Successful use of bevacizumb and paclitaxel in a male breast cancer with liver metastases. *Int J Clin Exp Med*. 2014;7(9):3076-3079. PMID: 25356184

14. Gu J, Xiao Y, Shu D, *et al*. Metabolomics Analysis in Serum

from Patients with Colorectal Polyp and Colorectal Cancer by 1H-NMR Spectrometry. *Dis Markers*. 2019;2019:1-14.

doi: 10.1155/2019/3491852

15. Beckonert O, Keun HC, Ebbels TMD, *et al*. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat Protoc*. 2007;2(11):2692-2703.

doi: 10.1038/nprot.2007.376

16. Gu J, Su F, Hong P, Zhang Q, Zhao M. 1H NMR-based metabolomic analysis of nine organophosphate flame retardants metabolic disturbance in Hep G2 cell line. *Sci Total Environ*. 2019;665:162-170.

doi: 10.1016/j.scitotenv.2019.02.055

17. Shao W, Gu J, Huang C, *et al*. Malignancy-associated metabolic profiling of human glioma cell lines using 1H NMR spectroscopy. *Mol Cancer*. 2014;13(1).

doi: 10.1186/1476-4598-13-197

18. Khakimov B, Mobaraki N, Trimigno A, Aru V, Engelsen SB. Signature Mapping (SigMa): An efficient approach for processing complex human urine 1H NMR metabolomics data. *Anal Chim Acta*. 2020;1108:142-151.

doi: 10.1016/j.aca.2020.02.025

19. Wishart DS, Feunang YD, Marcu A, *et al*. HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res*. 2017;46(D1):D608-D617.

doi: 10.1093/nar/gkx1089

20. Savorani F, Tomasi G, Engelsen SB. icoshift: A versatile tool for the rapid alignment of 1D NMR spectra. *J Magn Reson*. 2010;202(2):190-202.

doi: 10.1016/j.jmr.2009.11.012

21. Cassiède M, Nair S, Dueck M, *et al*. Assessment of 1H NMR-based metabolomics analysis for normalization of urinary metals against creatinine. *Clin Chim Acta*. 2017;464:37-43.

doi: 10.1016/j.cca.2016.10.037

22. Kohonen T. Self-organized formation of topologically correct feature maps. *Biol Cybern*. 1982;43:59-69.

doi: 10.1007/BF00337288

23. Zupan J, Novič M, Ruisánchez I. Kohonen and counterpropagation artificial neural networks in analytical chemistry. *Chemom Intell Lab Syst*. 1997;38(1):1-23.

doi: 10.1016/s0169-7439(97)00030-0

24. Melssen W, Wehrens R, Buydens L. Supervised Kohonen networks for classification problems. *Chemom Intell Lab Syst*. 2006;83(2):99-113.

doi: 10.1016/j.chemolab.2006.02.003

25. Wongravee K, Lloyd GR, Silwood CJ, Grootveld M, Brereton RG. Supervised Self Organizing Maps for Classification and Determination of Potentially Discriminatory Variables: Illustrated by Application to Nuclear Magnetic Resonance Metabolomic Profiling. *Anal Chem*. 2009;82(2):628-638.

doi: 10.1021/ac9020566

26. Kumpula LS, Mäkelä SM, Mäkinen VP, *et al*. Characterization of metabolic interrelationships and in silico phenotyping of lipoprotein particles using self-organizing maps. *J Lipid Res*. 2010;51(2):431-439.

doi: 10.1194/jlr.d000760

27. Mäkinen VP, Forsblom C, Thorn LM, *et al*. Metabolic Phenotypes, Vascular Complications, and Premature Deaths in a Population of 4,197 Patients With Type 1 Diabetes. *Diabetes*. 2008;57(9):2480-2487.

doi: 10.2337/db08-0332

28. Ballabio D, Consonni V, Todeschini R. The Kohonen and CP-ANN toolbox: A collection of MATLAB modules for Self Organizing Maps and Counterpropagation Artificial Neural Networks. *Chemom Intell Lab Syst*. 2009;98(2):115-122.

doi: 10.1016/j.chemolab.2009.05.007

29. Ballabio D, Vasighi M. A MATLAB toolbox for Self Organizing Maps and supervised neural network learning strategies. *Chemom Intell Lab Syst*. 2012;118:24-32.

doi: 10.1016/j.chemolab.2012.07.005

30. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem*. 1993;39(4):561-577.

doi: 10.1093/clinchem/39.4.561

31. Mandrekar JN. Receiver Operating Characteristic Curve in Diagnostic Test Assessment. *J Thorac Oncol*. 2010;5(9):1315-1316.

doi: 10.1097/jto.0b013e3181ec173d

32. Pang Z, Chong J, Zhou G, *et al*. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res*. 2021;49(W1):W388-W396.

doi: 10.1093/nar/gkab382

33. Goeman JJ, Bühlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics*. 2007;23(8):980-987.

doi: 10.1093/bioinformatics/btm051

34. Chong J, Soufan O, Li C, *et al*. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res*. 2018;46(W1):W486-W494.

doi: 10.1093/nar/gky310

35. Ballabio D, Vasighi M, Consonni V, Kompany-Zareh M. Genetic Algorithms for architecture optimisation of Counter-Propagation Artificial Neural Networks. *Chemom Intell Lab Syst*. 2011;105(1):56-64.

doi: 10.1016/j.chemolab.2010.10.010

36. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. *Nat Rev Clin Oncol*. 2016;13(11):674-690.

doi: 10.1038/nrclinonc.2016.66

37. Lieu EL, Nguyen T, Rhyne S, Kim J. Amino acids in cancer. *Exp Mol Med*. 2020;52(1):15-30.

doi: 10.1038/s12276-020-0375-3

38. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in cancer. *Br J Cancer*. 2019;122(2):150-156.

doi: 10.1038/s41416-019-0620-5

39. Sivanand S, Vander Heiden MG. Emerging Roles for Branched-Chain Amino Acid Metabolism in Cancer. *Cancer Cell*. 2020;37(2):147-156.

doi: 10.1016/j.ccell.2019.12.011

40. Jasbi P, Wang D, Cheng SL, *et al*. Breast cancer detection using targeted plasma metabolomics. *J Chromatogr B*. 2019;1105:26-37.

doi: 10.1016/j.jchromb.2018.11.029

41. Willmann L, Erbes T, Halbach S, *et al*. Exometabolom analysis of breast cancer cell lines: Metabolic signature. *Sci Rep*. 2015;5(1).

doi: 10.1038/srep13374

42. Lo C, Hsu YL, Cheng CN, *et al*. Investigating the Association of the Biogenic Amine Profile in Urine with Therapeutic Response to Neoadjuvant Chemotherapy in Breast Cancer Patients. *J Proteome Res*. 2020;19(10):4061-4070.

doi: 10.1021/acs.jproteome.0c00362

43. Thomas E, Holmes FA, Smith TL, *et al*. The Use of Alternate, Non–Cross-Resistant Adjuvant Chemotherapy on the Basis of Pathologic Response to a Neoadjuvant Doxorubicin-Based Regimen in Women With Operable Breast Cancer: Long-Term Results From a Prospective Randomized Trial. *J Clin Oncol*. 2004;22(12):2294-2302.

doi: 10.1200/jco.2004.05.207