

# Elimination of Axillary-Lymph-Node Metastases in a Patient With Invasive Lobular Breast Cancer Treated by First-line Neo-adjuvant Chemotherapy Combined With Methionine Restriction

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**Abstract.** *Background/Aim:* Invasive lobular carcinoma (ILC) of the breast has a low complete-response rate in the neoadjuvant-chemotherapy setting. The addiction to methionine is a fundamental and ubiquitous characteristic of cancer cells, termed the Hoffman effect. We have previously targeted methionine addiction of breast cancer with recombinant methioninase (rMETase) using patient-derived orthotopic xenograft (PDOX) models. The aim of the present study was to determine the efficacy of methionine restriction with rMETase and a low-methionine diet combined with first-line neo-adjuvant chemotherapy, in a patient with metastatic ILC of the breast. *Case Report:* A 62-year-old female was diagnosed with metastatic ipsilateral axillary-lymph-node recurrence of breast ILC 3 years after mastectomy. The patient underwent [<sup>11</sup>C]-methionine positron-emission tomography (METPET) which showed extensive methionine accumulation in the ipsilateral axillary lymph nodes, indicating the presence of cancer cells. The patient received standard neo-adjuvant chemotherapy, which consisted of 3 months of doxorubicin and cyclophosphamide

followed by 3 months of docetaxel from March 2022. The patient also went on a low-methionine diet and daily oral rMETase as a supplement every 6 hours concurrently with six months chemotherapy. The patient's blood carcinoembryonic antigen (CEA) level decreased gradually, and computed tomography findings showed loss of axillary lymph-node metastases in the first 3 months of neo-adjuvant chemotherapy with doxorubicin and cyclophosphamide combined with rMETase and a low-methionine diet. A complete response was demonstrated by METPET at 6 months, at conclusion of docetaxel chemotherapy. *Conclusion:* Combination therapy of doxorubicin and cyclophosphamide followed by docetaxel combined with methionine restriction led to a remarkable complete response that is expected in fewer than 10% of patients with ILC of the breast treated with neo-adjuvant chemotherapy alone. The present results suggest that methionine restriction in combination with doxorubicin and cyclophosphamide followed by docetaxel may be effective, after METPET has demonstrated the presence of methionine-addicted axillary-lymph-node metastases in ILC of the breast.

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**Key Words:** Breast cancer patient, invasive lobular carcinoma, metastasis, methionine PET, METPET, methionine addiction, Hoffman effect, methionine restriction, oral recombinant methioninase, low-methionine diet, doxorubicin, cyclophosphamide, docetaxel, combination therapy, Hoffman protocol, complete response.

Breast cancer is the most common cancer type and the second cause of death in females in the United States (1). Invasive breast cancer is a histologically heterogeneous disease with many histopathological subgroups. Invasive lobular carcinoma (ILC) is the second-most prevalent type of invasive breast cancer following invasive ductal carcinoma (IDC), accounting for 5% to 15% of all breast cancer cases (2). ILC is more likely to be hormone-positive, human epidermal-growth-factor-receptor-2 negative, and have a lower proliferative index compared to IDC. ILC has a lower complete response rate to neo-adjuvant chemotherapy but better recurrence-free and overall survival than IDC (2-4).



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The addiction to methionine is a fundamental and ubiquitous characteristic of cancer cells known as the Hoffman effect (5-8). Methionine addiction is due to excess transmethylation in cancer cells, greatly elevating their requirement for methionine compared to normal cells. Hence cancer cells cannot survive under methionine restriction (9-11).

We have produced recombinant methioninase (rMETase) (12), an enzyme that catabolizes methionine, and have demonstrated the effectiveness of rMETase in breast cancer using patient-derived orthotopic xenograft (PDOX) models (13-16).

In the present case report, a 62-year-old female with ILC of the breast was treated with first-line neo-adjuvant chemotherapy, an oral supplement of rMETase, and a strict methionine-restriction diet. The patient exhibited remarkable elimination of axillary-lymph-node metastases, determined by [<sup>11</sup>C]-methionine positron-emission tomography (METPET) and computed tomography (CT).

## Materials and Methods

**rMETase production and formulation.** rMETase was produced by fermenting recombinant *Escherichia coli* that had been transformed with the methioninase gene from *Pseudomonas putida*. rMETase was purified using a high-yield method that included a 60°C heat step, polyethylene glycol precipitation, and diethylaminoethyl-sepharose fast-flow chromatography. rMETase was formulated in normal saline at 5 mg/ml (17, 18).

**Methionine restriction and rMETase administration.** The patient maintained a low-methionine diet in accordance with the Nutritional Oncology Research Institute protocol, which suggested daily consumption of less than 2 mg of methionine per kg body weight (19). As a dietary supplement, 250 units of rMETase were administered four times daily orally.

**[<sup>11</sup>C]-Methionine positron-emission tomography (METPET).** METPET was carried out at the Utsunomiya Central Clinic, Tochigi, Japan.

## Case Report

A 62-year-old female was diagnosed with ILC of the left breast in September 2018. CT revealed that the patient had no lymph-node or distant metastases. Therefore, the patient underwent a mastectomy and breast reconstruction in November 2018. Pathological findings of the surgical specimen were as follows: ILC, classical type; grade 1; nuclear grade 1; tumor size: 7.5×3.8×6.0 cm; estrogen receptor 3+; progesterone receptor 3+; human epidermal-growth-factor-receptor-2 1+; Ki67 index 8.8%; T3N0M0; stage IIB. Post-surgery, the patient received adjuvant endocrine therapy (anastrozole). However, the patient noticed left-axillary masses in December 2021. CT findings in January 2022 showed multiple axillary-lymph-node metastases on the ipsilateral side as the breast cancer (Figure 2). METPET was performed to

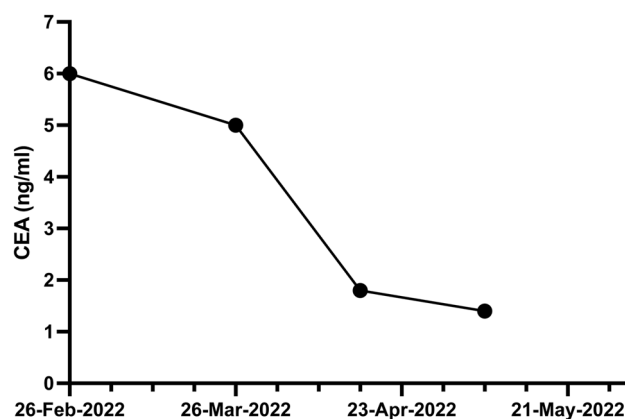


Figure 1. Time course of carcinoembryonic antigen (CEA) in the patient with metastatic lobular carcinoma of the breast. Chemotherapy with doxorubicin combined with cyclophosphamide started on March 26 2022. The patient was also on a low-methionine diet, and took oral recombinant methioninase 4 times a day at 250 units per dose.

confirm the recurrence and showed the accumulation of [<sup>11</sup>C]-methionine in the ipsilateral axillary lymph nodes. The signal from METPET was stronger and clearer in the metastasis than in the CT scan, which indicated that cancer cells were methionine-addicted (data not shown). Because metastases were limited to axillary lymph nodes, it was decided that the treatment strategy would be to administer neo-adjuvant chemotherapy, which consisted of 3 months of doxorubicin and cyclophosphamide followed by 3 months of docetaxel, started on March 26, 2022. The patient also went on a low-methionine diet (19) and oral rMETase as a supplement 4 times daily at 250 units/dose for the entire 6 months of chemotherapy. During the therapy, the blood CEA level decreased gradually (Figure 1), and CT findings showed loss of the axillary lymph-node metastases after 3 months of doxorubicin and cyclophosphamide therapy and methionine restriction (Figure 2). After additional taxane treatment for 3 months, along with methionine restriction, the patient's CEA level continued to decrease, and the final evaluation at 6 months, demonstrated by METPET, was complete response. Except for the mild adverse effects of chemotherapy, the patient had no rMETase-related side-effects at any time during this period.

## Discussion

Oral METase has shown clinical promise in patients with prostate cancer (20-22), pancreatic cancer (23) and rectal cancer (24). No adverse effects have been reported, demonstrating its administration is acceptable to patients.

In operable breast cancer, neo-adjuvant chemotherapy using doxorubicin and cyclophosphamide followed by docetaxel is

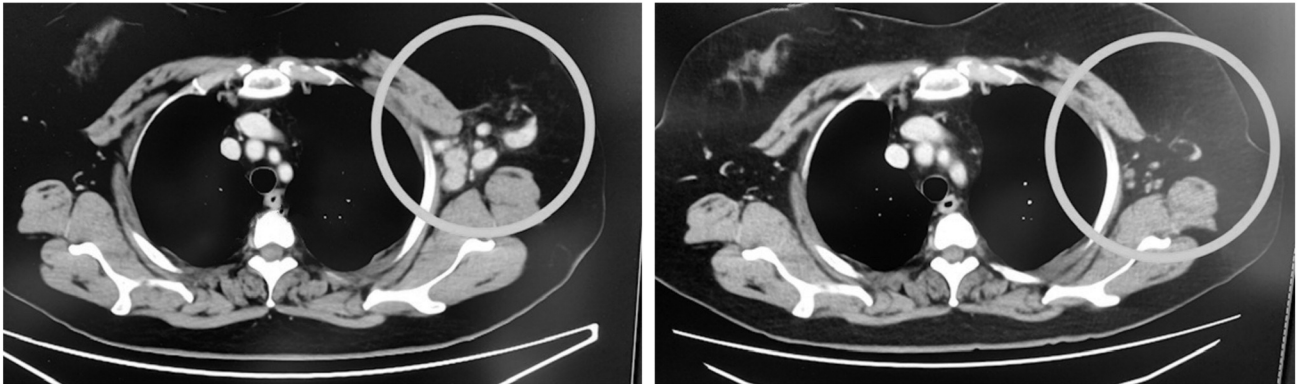


Figure 2. Computed tomographic findings of axillary-lymph-node metastases from invasive lobular breast cancer before (A) and 3 months after (B) starting treatment with doxorubicin, cyclophosphamide, a low-methionine diet and oral recombinant methioninase.

the standard of care. The efficacy of this combination therapy was investigated by the National Surgical Adjuvant Breast and Bowel Project protocol B-27 trial, which reported that the pathological complete response rate was 26.1% (25, 26). It is estimated that around 90% of the study cases would have been IDC based on its frequency in the general population. When limited to ILC, the complete response rate would be expected to be lower. A retrospective study by Cristofanilli *et al.* (2), comparing IDC with ILC, showed that the pathological chemotherapy-complete-response rate in ILC was lower than in IDC. In their retrospective study, all patients received anthracycline-based chemotherapy, and 33.5% of the patients also received a taxane. The pathological complete response rate of the ILC group was only 3% (2). Furthermore, another study, which was a meta-analysis of 1,764 ILC cases, reported a 5.9% complete response rate to preoperative adjuvant chemotherapy (27).

The present case of recurrent disease was limited to ipsilateral axillary lymph nodes. The patient had a remarkable response after doxorubicin and cyclophosphamide therapy, and methionine restriction for 3 months and a complete response at 6 months after docetaxel and methionine restriction. We were unable to determine the patient's pathological response since she did not undergo surgery, but the CT and METPET results are meaningful. This response can be attributed to two factors: One is that there was a significant accumulation of methionine in the cancer cells shown by METPET, which indicated the methionine addiction of these cancer cells. Another is that alkylating agents tend to exhibit synergistic effects in conjunction with methionine restriction (28-30) because methionine-restricted cancer cells are selectively arrested in the late S/G<sub>2</sub> phases of the cell cycle (31).

The results suggest that methionine restriction, in combination with doxorubicin and cyclophosphamide, followed by docetaxel is effective for metastatic ILC of the breast. Further studies will be needed to confirm this.

We originally discovered that methionine restriction is highly synergistic with chemotherapy in 1986 (28). Since that original publication, many studies have shown synergy of methionine restriction with chemotherapy (32, 33), including methionine restriction with rMETase (15, 29-44). The combination of methionine restriction and chemotherapy is termed the Hoffman protocol (45). Previous clinical case reports of other cancer types have shown the promise of oral rMETase (20-24). The present study suggests that methionine restriction is promising in combination with first-line chemotherapy for metastatic breast cancer and further clinical studies are necessary.

### Conflicts of Interest

The Authors declared no competing interests for this work

### Authors' Contributions

YK, TT, and RMH wrote the article. QH and NM produced methioninase. KH, YA, KO, and CH reviewed the article.

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