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Cryotherapy protocols for metastatic breast cancer after failure of radical surgery ☆

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ABSTRACT

To retrospectively assess the effect of cryotherapy in patients with metastatic breast cancer (MBC) but without local recurrence after resection of the primary lesion, we divided 120 MBC patients into cryotherapy (91 patients) and chemotherapy (29 patients) groups. In the cryotherapy group, 37 patients with tumor recurrence received multiple cryoablations, while 54 patients received only a single cryoablation. Moreover, 62 cryotherapy-group patients underwent cryoablation immediately after the detection of metastases (timely cryotherapy); 35 patients received simultaneous immunotherapy (cryo-immunotherapy), and 29 patients underwent cryoablation in our hospital 3 months after receiving chemotherapy in other centers (chemo-cryotherapy and delayed cryotherapy). Overall survival (OS) after the diagnosis of MBC was assessed after a 10-year follow-up. The median OS was higher in the cryotherapy group (55 months) than in the chemotherapy group (27 months; $P < 0.0001$). In the cryotherapy group, longer median OS was associated with multiple (76 months) rather than single cryoablations (48 months; $P = 0.0005$) and with timely (67 months) rather than delayed cryoablation (48 months; $P = 0.0012$). The median OS was higher after cryo-immunotherapy (83 months) than after chemo-cryotherapy (48 months) or cryotherapy alone (43 months; $P < 0.0001$ for both). In conclusion, timely and multiple cryoablations, especially when combined with immunotherapy, offer significant advantages over chemotherapy in extending the OS of MBC patients.

Introduction

Breast cancer is the second leading cause of mortality among women. Breast cancer-related mortality is almost invariably due to metastasis. Between 25% and 50% of patients diagnosed with breast cancer will eventually develop deadly metastases, often decades after the diagnosis and removal of the primary tumor. The prognosis for patients with metastatic breast cancer (MBC) is generally unfavorable, with an average 5-year survival rate of only about 25% [22,27]. The therapeutic alternatives for MBC are mainly based on the systemic administration of cytotoxic chemotherapeutic agents; the long-term impact on survival, however, is only 20-months and depends heavily on the nature of the metastases and tumor biology [2,3,8,9,16].

While substantial advances have been made in the treatment of localized malignancies, metastatic disease still lacks effective treatment and remains the primary cause of mortality due to cancer, including breast cancer [4,15,18]. Thus, to increase the survival of cancer patients, it is necessary to effectively improve the prevention or treatment of metastasis. To achieve this goal, complementary strategies can be designed by adding novel modalities to current treatments, such as chemo-, cryo- or immunotherapy. In the past 20 years, accelerated approval has been granted to 35 oncology products for 47 new indications, allowing products to reach patients an average of 4 years earlier than they might have done through regular approval [11]. The good treatment effects and fewer side effects of cryoablation have been confirmed by many pilot studies, primarily, in early-stage breast cancer [13,19,20,24]. Active immunization, in the form of dendritic cell (DC) vaccines, has resulted in the delay and prevention of breast cancer recurrence. Adoptive T cell therapy has produced long-term survivors. In addition, manipulations of the immune system to enhance the immune response, prevent its down modulation and induce tumor cell death are under development [28].

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To our knowledge, there is currently no report on the long-term effects of cryotherapy, with or without chemo- or immunotherapy, in MBC patients who had no local recurrence after resection of the primary lesion. Here, we retrospectively analyzed the data of patients admitted in our cancer hospital in order to screen the best treatment method. To specifically determine the survival time of MBC patients, the overall survival (OS) after the diagnosis of metastatic disease was used as the main evaluation index.

Patients and methods

Ethics

The study protocol received ethical approval from the Regional Ethics Committee of Guangzhou Fuda Cancer Hospital. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Patient selection

Between May 2002 and May 2012, 120 MBC patients who were admitted to our hospital and satisfied our inclusion criteria were enrolled in this study. Surgery and radiotherapy were deemed unsuitable in the case of any of the following conditions: multifocal disease, refusal to undergo the above two methods, seeking of further treatment after multiple chemotherapies and advanced age. Ideal patients for cryoablation were those who satisfied the following criteria: Karnofsky performance status score, $\geqslant 70$; platelet count, $\geqslant 80 \times 10^9$/l; white blood cell count, $\geqslant 3 \times 10^9$/l; neutrophil count, $\geqslant 2 \times 10^9$/l; hemoglobin level, $\geqslant 90$ g/l; prothrombin time ratio, $\geqslant 1.5$; absence of level 3 hypertension, severe coronary disease, myelosuppression, respiratory disease, brain metastasis and acute and chronic infections; and adequate hepatic function (bilirubin, <30 μM; aminotransferase, <60 U/l; and Child-Pugh's score, A or B) and renal function (serum creatinine, <130 μM; serum urea, <10 mM).

The diagnosis of MBC in all patients was proven by positron-emission tomography–computed tomography (PET-CT) scans and biochemical markers, such as increased carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3. Forty-six patients had only one mass in the lung (14), bone (15), liver (12) or skin (5), measuring 2.3–8 cm in diameter (average, 4.5 cm). Another 46 patients had two masses ranging in diameter from 2.5 cm to 7.4 cm, while 28 patients had three masses of diameter 2.3–9.2 cm. There were a total of 222 masses in 120 patients. All patients had received their final treatments in our hospital during 10 year of study period.

Cryoablation procedure

In accordance with the personal wishes of the patients, cryosurgeries were performed on 91 patients; any obvious masses on PET-CT were completely cryoablated. A core-needle biopsy was mandatory prior to tumor ablation, so that adequate tissue could be obtained for a histopathological diagnosis and the determination of tumor receptor (estrogen receptor, progesterone receptor, Her2-neu) status. Cryoablations were performed using an argon gas-based cryosurgical unit (Endocare, Irvine, CA, USA). Cryoprobes (3 or 5 mm in diameter) were inserted into the center of the tumor mass under ultra sonographic or CT guidance, and two freeze/thaw cycles were performed, each reaching a temperature of −180 °C at the tip of the probe. The duration of freezing was dependent on the achievement of an ice ball that extended 1 cm beyond the boundaries of the tumor. Generally, the maximal freezing time was 15 min, followed by thawing for 5 min; this cycle was then repeated. For masses larger than 5 cm in diameter, three or four cryoprobes were placed within the center and periphery of the tumor, to ensure freezing of the entire mass [14]. The tracts formed were sealed with fibrin glue immediately after the removal of the cryoprobes to ensure hemostasis.

Immunotherapy and chemotherapy

Of the 91 patients in the cryotherapy group, 35 patients opted for immunotherapy, which included the adoptive transfer of dendritic cell (DC)–cytokine-induced killer (CIK) cells for a total of four times. The cells were generated according to previously published protocols in "Good manufacturing practice" condition [12,17]. One day before blood collection, recombinant human granulocyte macrophage–colony-stimulating factor (rhGM-CSF, 150 μg; Peprotech, Rocky Hill, NJ) was injected to mobilize white blood cells into the blood; 80 ml peripheral blood was drawn before cryosurgery. Immunotherapy was commenced 3–5 days after cryosurgery.

For DC culture, peripheral blood mononuclear cells were separated and resuspended in "DC" medium (X-VIVO 15, Lonza, Basel, Switzerland; 25 ng/ml recombinant human interleukin [rhIL]-4, Peprotech; and 30 ng/ml rhGM-CSF, Peprotech), at a concentration of 1×10^6/ml to 2×10^6/ml. The cells were then allowed to adhere in two plastic flasks (T75; Corning costa, Cambridge, MA), each containing 50 ml DC medium and approximately 10^8 cells. After overnight culture at 37 °C with 5% CO_2, the suspended cells were transferred to two fresh flasks. The cells sticking to the initial two flasks were continuously cultured in DC medium, and a small amount of fresh medium was added daily to the cultures.

For culture of CIK cells, the non-adherent cells from the DC culture were suspended in "CIK" medium, which consisted of X-VIVO 15 (Lonza), 1000 U/ml rhIL-2 (Peprotech), 2.5 μg/ml monoclonal antibody to CD3 (OKT-3; Jansen-Kyowa, Tokyo, Japan), 25 μg/ml phytohemagglutinin (Peprotech) and 1000 U/ml recombinant human interferon-γ (Peprotech). The CIK cells were allowed to grow and then continuously passaged. At approximately 7 days of culture, the CIK cells were passaged to fourteen T225 flasks. Cells adhering to the flasks were removed with a cell spatula, centrifuged and resuspended in "DC-CIK" medium (X-VIVO 15, Lonza; 400 U/ml IL-2 and 0.5 μg/ml monoclonal antibody to CD3). All DCs were distributed evenly in the fourteen T225 flasks containing CIK cells (approximately 10^8 DCs per flask). After co-culture for 24–48 h, nearly a week after cryosurgery, the DC-CIKs were harvested and suspended in 100 ml saline for intravenous injection (cells were collected on 4 consecutive days; 6×10^9 to 10×10^9 cells were collected on each day). Flow cytometric analysis showed that the final cell products included 27% ± 5% CD3 + CD56 + cells (natural killer T [NKT] cells), 87% ± 8% CD3 + cells and 15% ± 4% CD3-CD56 + cells (NK cells). The final cell products were assessed for viability by the dye-exclusion test and checked twice for possible contamination by bacteria, fungi and endotoxins.

The remaining 56 patients in the cryotherapy group refused to undergo immunotherapy owing to its cost or therapeutic effects. Of these, 29 patients had received chemotherapy more than 3 months before the cryotherapy and formed the chemo-cryotherapy group. The remaining 29 patients received chemotherapy alone, as they refused both cryo- and immunotherapy owing to their costs or effects. Their treatment selection was guided by multiple factors, most importantly, hormone receptor or HER2 expression, treatment history and prognostic factors such as short disease-free interval, presence of visceral metastases, performance status and severity of symptoms [4,26].

Evaluation and statistical analysis

Complications were recorded and classified in accordance with the Common Terminology Criteria of Adverse Events v4.0. Local tu-

mor control and OS were also evaluated. Radiographic local tumor control was assessed using image-guided tumor ablation criteria [10]. Ultrasonography and/or CT were performed 1 day, 1 week, 1 month and then at 3–4 month intervals after the cryosurgery. The revised Response Evaluation Criteria in Solid Tumors v1.1 were used to assess the response of the tumors in the lung, bone, liver or contralateral breast [7]. Three diagnostic radiologists reviewed the CT scans of every patient to determine whether progression or recurrence had occurred. Kaplan–Meier test with log-rank analysis was used for comparison of OS between two different treatment types and protocols (single vs. multiple and timely vs. delayed cryoablations). Log-rank analysis using a Bonferroni correction for mulitple comparisons was to compare OS between the four different therapeutic combinations. Differences were indicated by $P < 0.05$, and significant differences were indicated by $P < 0.01$ and $P < 0.001$. All analyses were conducted using GraphPad software (San Diego, CA, USA).

Results

Clinical data

The 29 chemotherapy-group patients were aged 27–78 years (mean, 51 years), while the 91 cryotherapy-group patients were aged 29–71 years (mean, 52 years). Informed consent was obtained from all patients. All 120 patients had initially undergone surgery and systemic chemotherapy in other centers: 31 patients were from China, 79 patients were from Southeast Asia and 10 patients were from other countries.

The number of patients who received the different therapies has been shown in Fig. 1. In the cryotherapy group, 29 patients visited our hospital for further treatment 3–22 months after the diagnosis of metastases (delayed treatment), while 62 patients visited our hospital immediately after the detection of metastases (timely treatment). A single metastasis was found in 32 patients (12, 8, 9 and 3 patients with metastasis in the lung, bone, liver and contralateral breast, respectively), double metastases were found in 36 patients and triple metastases were found in 23 patients. In total, there were 52, 50, 41 and 30 metastases in the lung, bone, liver and contralateral breast, respectively, at the time of the first cryoablation in all 91 patients. Owing to the recurrence of metastasis after treatment, 37 patients received multiple treatments (23 patients received cryo-immunotherapy; 8, cryotherapy alone; and 6, chemo-cryotherapy), while 54 patients refused to continue treatment (12, cryo-immunotherapy; 19, cryotherapy alone; and 23, chemo-cryotherapy). In the chemotherapy group, six patients visited our hospital 3–11 months after the detection of metastases, and 23 patients visited our hospital immediately after metastases were found. A single metastasis was found in 13 patients (2, 8 and 3 patients with metastasis in the lung, bone and liver, respectively), double metastases were found in 11 patients and triple metastases were found in 5 patients.

Perioperative outcomes

All percutaneous cryoablations were performed successfully. Seven patients (20%) who received immunotherapy simultaneously developed a mild fever (body temperature, <39 °C) and recovered within 3 days after the physical hypothermia. Severe complications, which are often associated with ablation of lung, bone, liver and breast tissue, such as liver rupture and failure, respiratory failure and pathological fracture, did not occur after cryoablation, and no complication-associated death occured. After the first cryosurgery in the 91 patients, many mild side effects occurred and were resolved with or without symptomatic treatment.

The following complications occurred after cryoablation of the 52 pulmonary metastases: transient hemoptysis, 14 (27%) patients and pneumothorax, 17 (33%) patients (both resolved in 2 days, after symptomatic treatment); bradycardia, 5 (10%) patients and hypotension, 6 (12%) patients (recovered spontaneously within a few hours); dull pain in the anterior chest soon after treatment, possibly due to damage of the intercostal nerves, 11 (21%) patients (usually resolved naturally within a few months); and cough with blood-streaked sputum, 16 (31%) patients (improved within 3–5 days without treatment). Cryoablation of the 50 osseous metastases was performed with meticulous fixation and care; no

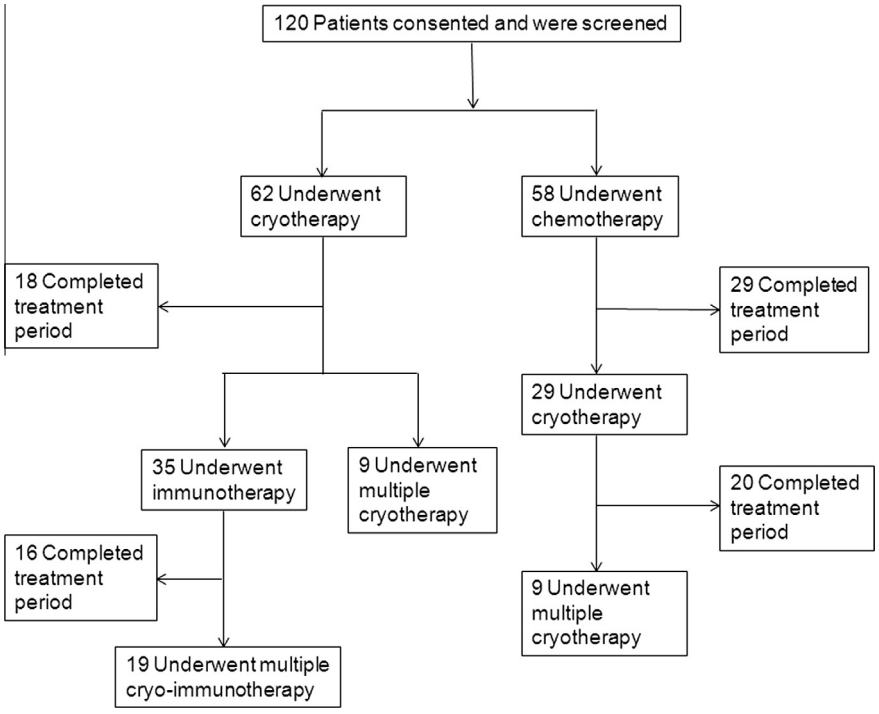

Fig. 1. Number of patients receiving different therapies.

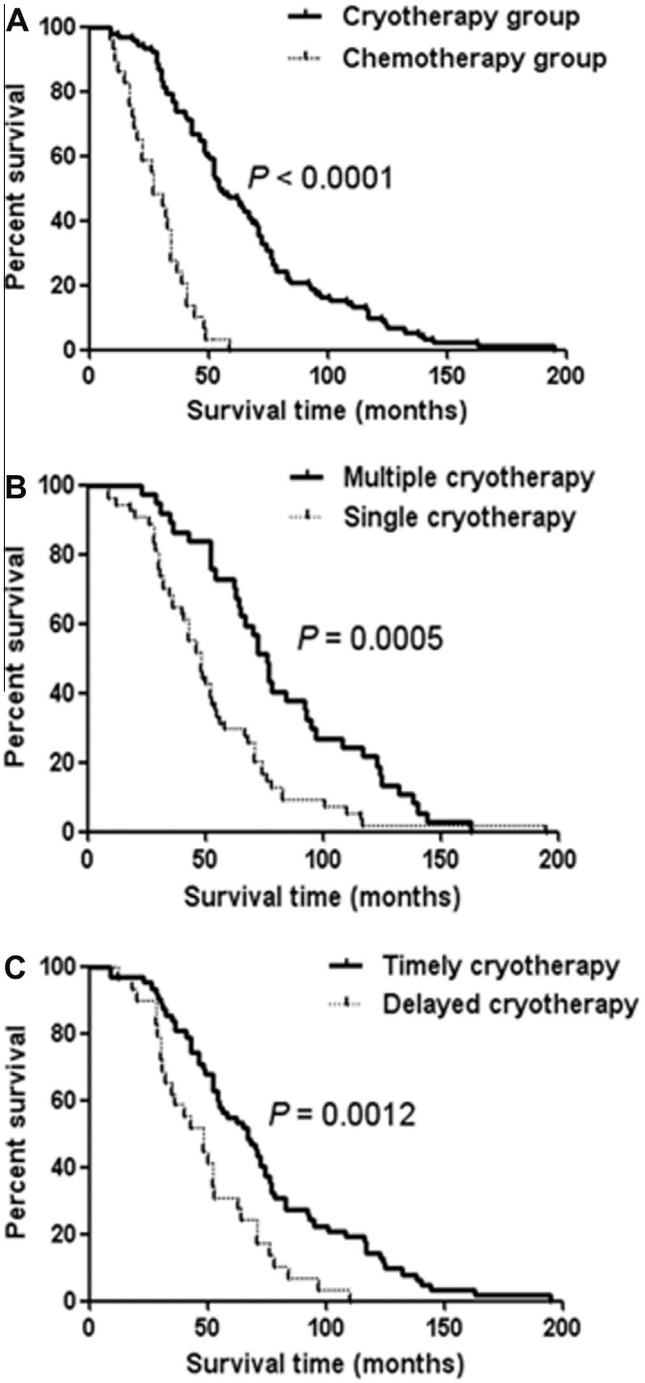

Fig. 2. Overall survival (OS) of patients who received different treatment types and protocols (number and timing of ablation). All 120 patients had MBC and died before October 2012. The Kaplan–Meier test with long-rank analysis was used to compare between-group differences. (A) Comparison of OS between different types of treatments. There were 91 patients in the cryotherapy group and 29 patients in the chemotherapy group. (B) Comparison of OS between multiple (37 patients) and single (54 patients) cryoablations. (C) Comparison of OS between timely (62 patients) and delayed (29 patients) cryoablations.

pathological fractures occurred. Cryoablation of the 41 hepatic metastases was associated with the following complications: mild liver hemorrhage, 6 (15%) patients (healed within 5 days after injection of hemostatic agents); rupture of liver capsular, 2 (5%) patients (recovered after blood transfusion); transient thrombocytopenia within 1 week after cryoablation, 7 (17%) patients (2 of these received platelet transfusion); and liver abscess at previous cryoablation site occurred 2 or 4 days after cryoablation, 3 patients

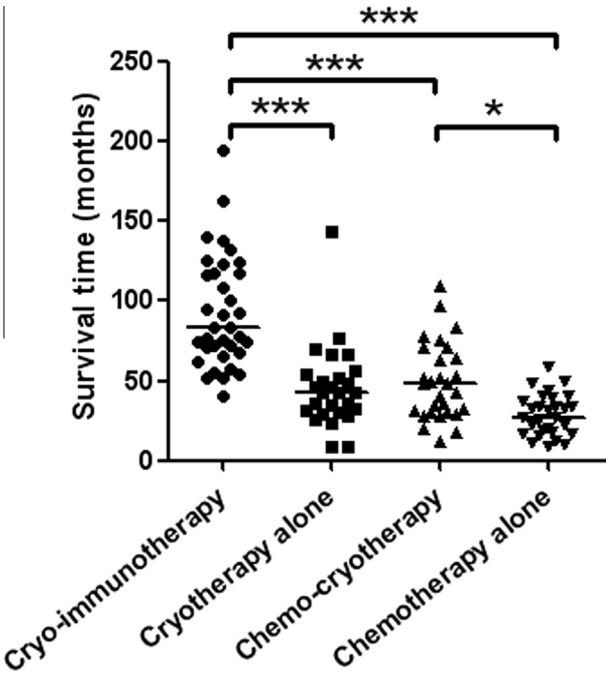

Fig. 3. Comparison of overall survival (OS) of patients receiving different combinations of therapies (three of which included cryoablation, and the fourth consisted of chemotherapy alone). The Tukey multiple comparison test was used to compare OS between different therapy combinations. The horizontal lines in the symbols represent median OS; *represents $P < 0.05$, and ***represents $P < 0.001$.

(7%) (recovered after antibiotic and drainage treatment). Cryoablation of the 30 mammary metastases was complicated by the following: ecchymosis and hematoma, 6 (20%) patients (gradually resolved within 2 weeks); pain and tenderness at the ablation site, nearly all patients (relieved within 1 week to 1 month); and post-procedural breast edema, nearly all patients (relieved within 1 week).

Influence of treatment types and protocols on OS

In our hospital, all metastases, whether single, double or treble, were often treated in the same session. The median OS was significantly longer in the cryotherapy group (55 months; 95% CI of the mean: 57.27–72.48 months) than in the chemotherapy group (27 months; 95% CI of the mean: 26.64–37.36 months) ($P < 0.0001$; Fig. 2A). Thus, cryotherapy may be associated with better survival time, and in our study, prolonged the median OS by 28 months.

Owing to disease progression, tumor recurrence and individual patients' wishes, 37 members of the cryotherapy group underwent repeat treatment after reexamination. The median OS was significantly longer among patients who underwent multiple treatments (76 months; 95% CI of the mean: 69.65–93.87 months) than among those who underwent a single treatment (48 months; 95% CI of the mean: 44.54–62.09 months) ($P = 0.0005$; Fig. 2B). Thus, multiple cryoablations extended the median OS by 28 months. In the cryotherapy group, 62 patients received timely cryotherapy soon after the metastases were found, while 29 patients first underwent chemotherapy and received cryotherapy (chemo-cryotherapy group) after a delay of 3–22 months. The median OS of patients who received timely treatment was 67 months (95% CI of the mean: 62.25–82.1 months), while that of the patients who received delayed treatment was 48 months (95% CI of the mean: 40.04–58.51 months) ($P = 0.0012$; Fig. 2C). Thus, timely cryoablation significantly prolonged patient survival time (median OS was extended by 19 months).

Of the patients who received timely cryotherapy, 35 patients simultaneously received immunotherapy; the remaining 27 patients received cryotherapy only. The median OS in the cryo-immunotherapy group was 83 months (95% CI of the mean: 80.73–104.8 months), while that in the cryotherapy-alone group was only 43 months (95% CI of the mean: 35.25–55.79 months); the survival time was significantly longer in the cryo-immunotherapy group than in the chemo-cryotherapy, cryotherapy-alone and chemotherapy-alone groups ($P < 0.001$ for all; Fig. 3). The survival time of patients who underwent chemo-cryotherapy was better than that of those who underwent chemotherapy alone ($P < 0.05$). Timely and multiple cryo-immunotherapy sessions were associated with the best survival time in MBC patients.

Discussion

In this study, we performed a retrospective review of our hospital's database to evaluate the survival time of MBC patients after the metastases had occurred. These patients had received several types of therapies in different medical centers before the metastases were detected, and our treatment program directly determined their survival time after the occurrence of metastasis. Cryoablation of multiple organs might be a valuable option to protect the function of the organs containing metastatic tumors. Skillful treatment and stringent patient selection can effectively (a) prevent the occurrence of severe complications (i.e., liver rupture and failure, acute renal failure with myoglobinuria, respiratory failure and pathological fracture), (b) reduce the probability of side effects (i.e., bleeding in the ablated tissue, rupture of liver capsular, thrombocytopenia, nerve damage, hemoptysis, ecchymosis and hematoma, and pain and tenderness at the ablation site) and (c) ensure the extension of OS. Since most side effects resolved spontaneously or after symptomatic treatment, within 2 weeks, our multi-organ cryotherapy protocol for MBC patients was found to be safe during the follow-up period. Moreover, in comparison with chemotherapy, cryotherapy extended the median survival period by 21 months (Fig. 2A), proving the effectiveness of cryotherapy.

As the timing and number of cryoablations had significant effects on patient outcomes, the effects of cryotherapy were assessed by dividing the patients according to the cryotherapy protocol. Multiple cytoreductive cryoablations were more therapeutically valuable than single treatments, and extended the median OS by 28 months (Fig. 2B). Similarly, timely cryotherapy was better than delayed cryotherapy and extended the median OS by 19 months (Fig. 2C). The above results indicated that cryotherapy performed immediately after the detection of MBC may improve prognosis; chemotherapy might reduce therapeutic efficacy by delaying cryotherapy.

Since many patients in the cryotherapy group had received other treatments (preoperative chemotherapy, 29 patients; postoperative immunotherapy, 35 patients), we compared the effects of different combinations of these therapies. The survival time of patients who had received timely cryo-immunotherapy (median OS: 83 months) was significantly longer than that of patients who received chemo-cryotherapy, cryotherapy alone or chemotherapy alone (median OS: 48, 43 and 27 months, respectively; Fig. 3). Based on these results, the following conclusions can be drawn regarding cryotherapy: (a) in the case of local growths, cryotherapy can minimize tumor load and maximize organ function, (b) cryotherapy alone, without systemic treatments, can achieve better outcomes than chemotherapy; (c) postoperative immunotherapy, as a systemic treatment, can extend survival time; and (d) locoregional cryotherapy, even when delayed due to preoperative chemotherapy, had a major impact on OS.

Of the different cryotherapy combinations assessed, cryo-immunotherapy was the most efficacious, possibly due to the dual therapeutic and preventive effects of DC-CIK immunotherapy. The organ-like structural environment of tumors is very hostile toward immune cells, and can result in local immune tolerance, due to which the tumor cells escape immune surveillance [21]. After cytoreductive cryosurgery, numerous breast cancer-associated antigens, including CEA, human epidermal growth factor receptor (EGFR)-2/neuregulin (HER-2/neu), mucin1 (MUC1), carbohydrate antigens (Tn, TF, sialyl Tn) and telomerase reverse transcriptase [5] are gradually released into the blood over a few months [23]. These tumor antigen can act as in vivo targets for DCs [29]. Activated DCs after cryoablation have been found to be potent stimulators of both CD4 + and CD8 + T cells in experimental [6] and human studies [1,25]. It has been observed that temporary immunotherapy reduced tumor recurrence; the level of tumor-specific lymphocytes decreased prior to tumor recurrence and increased again with booster immunizations [28]. Thus, it seems plausible that booster immunizations prevent tumor recurrence.

In summary, we have retrospectively analyzed the therapeutic effects of cryotherapy in association with other therapies among MBC patients after failure of radical surgery. The various cryoablation protocols assessed yielded significantly better results than chemotherapy in terms of median OS. We believe that timely, multiple cryoablations combined with simultaneous immunotherapy will greatly benefit MBC patients.

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