



ORIGINAL ARTICLE

Adjuvant treatment for triple-negative breast cancer: a retrospective study of immunotherapy with autologous cytokine-induced killer cells in 294 patients

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ABSTRACT

Objective: To examine the efficacy and safety of a sequential combination of chemotherapy and autologous cytokine-induced killer (CIK) cell treatment in triple-negative breast cancer (TNBC) patients.

Methods: A total of 294 post-surgery TNBC patients participated in the research from January 1, 2009 to January 1, 2015. After adjuvant chemotherapy, autologous CIK cells were introduced in 147 cases (CIK group), while adjuvant chemotherapy alone was used to treat the remaining 147 cases (control group). The major endpoints of the investigation were the disease-free survival (DFS) and overall survival (OS). Additionally, the side effects of the treatment were evaluated.

Results: In the CIK group, the DFS and OS intervals of the patients were significantly longer than those of the control group (DFS: $P = 0.047$; OS: $P = 0.007$). The multivariate analysis demonstrated that the TNM (tumor-node-metastasis) stage and adjuvant CIK treatment were independent prognostic factors for both DFS [hazard ratio (HR) = 0.520, 95% confidence interval (CI): 0.271-0.998, $P = 0.049$; HR = 1.449, 95% CI: 1.118-1.877, $P = 0.005$, respectively] and OS (HR = 0.414, 95% CI: 0.190-0.903, $P = 0.027$; HR = 1.581, 95% CI: 1.204-2.077, $P = 0.001$, respectively) in patients with TNBC. Additionally, longer DFS and OS intervals were associated with increased number of CIK treatment cycles (DFS: $P = 0.020$; OS: $P = 0.040$). The majority of the patients who benefitted from CIK cell therapy were relatively early-stage TNBC patients.

Conclusion: Chemotherapy in combination with adjuvant CIK could be used to lower the relapse and metastasis rate, thus effectively extending the survival time of TNBC patients, especially those at early stages.

KEYWORDS

Immunotherapy; triple-negative breast cancer; cytokine-induced killer cell; prognosis; disease-free survival; overall survival

Introduction

As a subtype of breast cancer, triple-negative breast cancer (TNBC) is defined by the lack of estrogen receptors (ERs), progesterone receptors (PRs), and the human epidermal growth factor receptor 2 (HER2). The TNBC constitutes up to 15% to 20% of all pathological types of breast cancer, with a tendency towards aggressive behavior, clinically shown by younger onset age, higher histological grade and distant metastasis rate, as well as poorer prognosis¹. As the TNBC patients are not eligible for conventional targeted therapies for lacking the molecular target renders, chemotherapy based

on anthracyclines and taxanes is currently the main post-surgical therapeutic strategy². However, the recurrence rate in patients with TNBC remains at a high level, leading to a significant decline in survival rate in the initial 3 to 5 years after surgery³. Therefore, exploring novel therapeutic strategies is an important clinical challenge in treating TNBC.

Based on the gene expression data, TNBC was categorized by Lehmann et al.⁴ into 6 subtypes, including basal-like 1 and 2 (BL1 and BL2), mesenchymal (M), immunomodulatory (IM), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR). TNBC is a heterogeneous disease with varied sensitivity to different therapies. The IM subtype (featured by enhanced expression of immune genes) indicates that immune-based therapies might be beneficial to some of the TNBC patients⁵. Therefore, chemotherapies coupled with immunotherapies may be considered as an alternative option for treating TNBC patients.

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The principle of adoptive immunotherapy is to collect the immune cells from the human body, and then transmit them back to the human body for anti-tumor activity, after an *in vitro* transformation and expansion. Cytokine-induced killer (CIK) cells are defined as a subset of cytotoxic T lymphocytes with an immunophenotype of CD3⁺CD56⁺. The CIK cells have been proven to be ideal for use in immunotherapy as they can reproduce rapidly outside the human body and directly kill tumor cells⁶, thereby regulating and enhancing host cell immune function *in vivo*⁷. Compared with another cytotoxic effector T cells, named lymphokine-activated killer (LAK) cells, the CIK cells present enhanced tumor cell lytic activity and reproduction rate, and lowered toxicity⁸. Subsequently, CIK cell-based therapy has been broadly adopted as an adjuvant treatment combined with chemotherapy for treating multiple types of cancers, such as renal cell carcinoma⁹, gastric cancer¹⁰, non-small cell lung cancer¹¹, colon cancer¹², and liver cancer¹³, with great efficacy and safety.

However, a few studies have been conducted on the efficacy of CIK treatment in breast cancer, especially in TNBC. The existing clinical studies on treating breast cancer with CIK cells have mostly concentrated on advanced or metastatic breast cancer¹⁴⁻¹⁷. These studies have shown that CIK cell therapy can be used as a rescue therapy to facilitate the prognosis of advanced or metastatic breast cancer, and to improve the patient's quality of life. Therefore, a clinical retrospective study regarding the efficacy of CIK cell therapy on the prognosis of postoperative TNBC patients was performed.

Materials and methods

Patients

A retrospective study was conducted to examine the clinical outcomes of autologous CIK immunotherapy in TNBC patients after surgery. The patients were recruited to the study from January 1, 2009 to January 1, 2015. In the CIK group, 147 postoperative TNBC patients received autologous CIK cells after chemotherapy. Concomitantly, 147 participants (control group) were selected that received chemotherapy alone after the surgery, and also matched with age ± 1 year to the index patients. The following is the brief outline of patient enrollment procedure in the control group: Initially, between January 1, 2009 and January 1, 2015, a review of the medical records of patients diagnosed with TNBC from a computerized database in our hospital was performed; then, the matching cases were selected in

accordance with the enrollment and exclusion criteria; finally, matching patients with same age as those in CIK group were chosen; if there is no same-aged case as that of patients in CIK group, the cases with age ± 1 were selected randomly; if there are more than one same-aged cases, then one case was selected randomly by random number method.

The following were the inclusion criteria for selecting patients: 1) The selected patients must be histologically diagnosed with TNBC. TNBC is defined by the immunohistochemical staining feature of ER, PR, and HER2. The staining feature categorization is as follow: ER and PR negative is defined as ER and PR staining $< 1\%$; HER2 negative is defined as HER2 staining 0 to 2+ by, or a nonamplified HER2 by fluorescence *in situ* hybridization (FISH); 2) No occurrence of distant metastasis prior to surgery; 3) Absence of other malignant tumor; 4) Karnofsky performance status score higher than 70 %; 5) Reception of CIK treatment before disease progression. The exclusion criteria were as below: 1) absence of adjuvant chemotherapy, or inability to tolerate or complete the chemotherapy due to serious adverse reactions; 2) severe disease of heart, lung, liver or kidney, bone marrow dysfunction, autoimmune diseases; 3) pregnancy or lactation. Guided by the Declaration of Helsinki, this study has been authorized by the Ethics Committee of Tianjin Medical University Cancer Institute and Hospital (Approval No. bc2019024) and by the State Food and Drug Administration of China (No. 2006 L01023). The detailed clinical characteristics of patients in the both groups are shown in the **Supplementary Table S1**.

Treatments

All participating patients underwent modified radical mastectomy, radical mastectomy, or breast-conserving surgery. Postoperatively, these patients underwent 4 to 8 cycles of standard adjuvant chemotherapy in accordance with the NCCN Clinical Practice Guidelines in Oncology. Chemotherapy regimens involved anthracycline-based [AC (adriamycin 60 mg/m², cyclophosphamide 600 mg/m² d1, 21 days a cycle) or EC (epirubicin 90 mg/m², cyclophosphamide 600 mg/m² d1, 21 days a cycle) or CAF (5-Fu 500 mg/m², adriamycin 50 mg/m², cyclophosphamide 500 mg/m² d1, 21 days a cycle) or CEF (5-Fu 500 mg/m², epirubicin 100 mg/m², cyclophosphamide 500 mg/m² d1, 21 days a cycle)], anthracycline- and taxane-based [TAC (docetaxel 75 mg/m², adriamycin 50 mg/m², cyclophosphamide 500 mg/m² d1, 21 days a cycle) or AC-T (adriamycin 60 mg/m², cyclophosphamide 600 mg/m² d1, 4 cycles, docetaxel 80-100 mg/m² d1, 4 cycles, 21 days a cycle)], or taxane-based

[TC (docetaxel 75 mg/m², cyclophosphamide 600 mg/m² d1, 21 days a cycle)] regimens.

It was observed that some patients received neoadjuvant chemotherapy or adjuvant radiotherapy based on their clinical stage and operation. For all patients who are diagnosed with invasive breast cancer and choose breast-conserving surgery, whole breast radiotherapy (RT) is recommended. In cases of adjuvant RT after radical/modified radical mastectomy, radiation should be administered mainly to the ipsilateral chest wall and supraclavicular region on the same side as the tumor in patients with four or more positive axillary nodes, or with tumor ≥ 5 cm; for the patients with negative nodes or those with tumors < 5 cm, the guidelines recommend radiation to the chest wall. Patients with small tumors and no nodal involvement do not need to undergo radiation therapy. RT is administered to the chest wall with 6 MV X-ray at a total dose of 45-50 Gy with 1.8-2.0 Gy/fraction, 5 fractions/week. Introducing a boost to the tumor bed for patients with greater risk (age < 50 and high-grade disease) using doses of 10-16 Gy at 2 Gy/fx is recommended.

CIK cells preparation and injection

At least 2 weeks after the patients completed post-mastectomy chemotherapy (with/without radiotherapy) and when routine blood count returned to normal, 50 mL of peripheral blood samples were collected for the preparation of CIK cells. The previously published research^{9, 12, 18-20} has provided the detailed method of CIK preparation. In brief, to gather the peripheral blood mononuclear cells (PBMCs) from TNBC patients, a COBE Spectra Apheresis System was used. The PBMCs were then cultured in a medium containing 1000 U/mL interferon- γ (IFN- γ), 100 U/mL recombinant human interleukin-1 α (IL-1 α), and 50 ng/mL anti-CD3 antibody, with 5% CO₂ at 37 °C for 24 h, followed by the addition of 300 U/mL of recombinant human IL-2 to the medium. This medium was constantly replaced with a fresh medium containing IFN- γ and IL-2 every 5 days. By using this approach, a cellular subset with noticeably higher CD3⁺CD56⁺ was prepared. On the 14th day, the CIK cells were harvested. Eventually, over 5×10^9 of CIK cells with $> 95\%$ viability were obtained. No fungus, mycoplasma, or bacteria were found in the reagents.

In the CIK group, on day 15 and day 16 of each chemotherapy cycle, patients received an intravenous infusion of at least 5×10^9 CIK cells. During the input, routine body indexes, such as body temperature, heart rate, respiration, blood pressure, and other basic vital signs, were monitored. Maintenance treatment was accessible to these

patients unless they refused to proceed or in case of recurrence or distant metastasis.

Follow-up and clinical assessment

From the date of surgery until May 1, 2018 or death, a follow-up was performed for all the patients. The median follow-up time was 75 months (ranging from 39-110 months). The overall survival (OS) and disease-free survival (DFS) were defined in accordance with the National Cancer Institute's Response Evaluation Criteria in Solid Tumors (RECIST)²¹. OS was measured from the date of surgery until decease and living patients were examined at the time of the last follow-up. DFS was calculated from the date of surgery until first recurrence or metastasis, or death from any cause. Patients that achieved a stable state were evaluated at the final follow-up. Besides, based on the criteria specified by the World Health Organization (WHO), adverse clinical activities were monitored and evaluated.

In the initial 2 years after the surgery, the follow-up was conducted in a 3-month cycle. The interval was extended to 6 months from year 2 through year 5, and annually thereafter. The reviewed patient records included breast ultrasound, breast tumor markers, mammography, X-ray or computed tomography (CT) on the chest, liver and abdomen ultrasound, bone scan, and head magnetic resonance imaging (MRI) if necessary. In this study, telephonic consultation was offered to each patient and no loss to follow-up was experienced.

Statistical analysis

The Chi-square and Fisher's exact tests were used to analyze the differences in variables of the two groups, in terms of both demographic and clinical characteristics. The Kaplan-Meier method was used to evaluate the survival time and rate distribution. The log-rank test univariate analyses were used to assess the relationship between survival and the potential prognostic factors. This was further verified by the multivariate analysis of Cox proportional hazards regression. Further, SPSS 20.0 software was used as a tool to analyze all the calculations. Statistical significance was considered at two-tailed $P < 0.05$ for all the calculations.

Results

Patients' characteristics

This retrospective analysis involved total 294 patients, with

147 members in each group (CIK and control group). Each participant was compared to the matching patient from the other group for the time of diagnosis, age at onset of disease, pathological type, tumor size, TNM stage and regional lymph node metastasis at the first visit, operation and treatment, and subsequent therapies. It was found that there were no statistically significant differences between the two groups ($P > 0.05$). **Table 1** shows the data for all the patients.

Survival analysis

It was observed that the patients in the CIK group experienced significantly longer DFS intervals than their counterparts in the control group ($P = 0.047$, **Figure 1A**). DFS rates of the CIK and control group after 1-, 3-, and 5-year intervals were 99.3% vs. 95.9%, 91.8% vs. 83.7%, and 88.1% vs. 81.3%, respectively. Similarly, the OS interval of the CIK group was significantly longer than that of the control group ($P = 0.007$, **Figure 1B**), and the 1-, 3-, and 5-year OS rates of the CIK and control group were 99.3% vs. 98.0%, 96.6% vs. 91.8%, and 93.4% vs. 84.1%, respectively. Therefore, compared to the control group patients treated with adjuvant chemotherapy (with or without radiotherapy), post-mastectomy TNBC patients, who received additional sequential CIK treatment, had significantly improved DFS and OS rates. In the CIK group, the median courses of CIK treatment were 6 cycles (range 1-26 cycles). Patients undergoing ≥ 6 cycles of CIK cell therapy had greater DFS ($P = 0.020$, **Figure 2A**) and OS ($P = 0.040$, **Figure 2B**) rates than those treated with < 6 cycles. Therefore, it can be inferred that longer CIK treatment courses are associated with better prognosis.

Until the completion of follow-up, recurrence or metastasis was observed in 16 patients in the CIK group, and 29 patients in the control group. Statistically, the two groups had no difference in the metastatic sites or the number of sites. It was found that the most common sites of distant metastases were the bone, lung, liver, and brain (**Table 2**).

Subgroup analysis

Further study was conducted to analyze the TNM stages of the patients that received better benefits from the CIK cell treatment. For this, all 294 patients were divided into an early-stage group (I, IIa stage) and a late-stage group (IIb, III stage), and a survival analysis of each subgroup was conducted. It was observed that the OS of TNBC patients in the early-stage group was extended by CIK treatment ($P = 0.018$, **Figure 3B**). However, such results were not obtained

for the DFS ($P = 0.081$, **Figure 3A**; $P = 0.114$, **Figure 3C**) or the OS of late-stage TNBC patients ($P = 0.054$, **Figure 3D**).

Prognosis analysis

In the univariate and multivariate analysis, the impact of CIK treatment on the prognosis of post-surgery patients with TNBC was further evaluated. It was revealed by the log-rank test univariate analysis that the size of a tumor, TNM stage, lymph node metastasis, histological grade, radiotherapy, and CIK treatment were the prognostic factors influencing DFS and OS in TNBC patients. Additional Cox multivariate analysis showed that for TNBC patients, the adjuvant CIK treatment and TNM stage remained independent prognostic factors for both DFS (CIK treatment: HR = 0.520, 95% CI:0.271-0.998, $P = 0.049$; TNM stage: HR = 1.449, 95% CI:1.118-1.877, $P = 0.005$, respectively) and OS (CIK treatment: HR = 0.414, 95% CI:0.190-0.903, $P = 0.027$; TNM stage: HR = 1.581, 95% CI:1.204-2.077, $P = 0.001$, respectively, **Table 3**).

Toxic and side effects

Adverse reactions during the treatment in both groups of patients were observed. Both groups experienced common adverse reactions, including myelosuppression, fever, nausea and vomiting, liver dysfunction, kidney dysfunction, and the peripheral nerve toxicity. The main adverse reactions were I to II degrees. In the III-IV-degree myelosuppression group, 11 were in the CIK group and 12 in the control group; the side effects of the digestive tract were within the III degree; fever, renal impairment, and neurotoxicity were of I-II degrees. No intolerable adverse reactions were observed in both the groups, and no statistical difference was observed on comparing the adverse events between two groups (**Table 4**). There were no obvious adverse reactions observed during the injection of CIK cells. In the CIK group, 11 patients had a transient fever reaction (temperature $< 38.5^{\circ}\text{C}$) that returned to normal condition within 24 h after symptomatic treatment. Moreover, during the course of CIK cell treatment, no patient quit midway due to intolerant side effects.

Discussion

Compared to the non-triple-negative breast cancers, TNBC shows more biological aggression. It is also associated with poorer prognosis and shorter survival time¹. On account of the stronger antigenicity owing to genomic instability and

Table 1 Clinical characteristics of patients in the two groups

Characteristics	CIK group	Control group	<i>P</i>
Patients, <i>n</i>	147	147	
Age (years)			1.000
< 35	9	9	
≥ 35	138	138	
Tumor size (cm)			0.644
≤ 2	64	68	
> 2, ≤ 5	65	58	
> 5	11	14	
Unable to value	7	7	
Lymph node metastasis			0.338
Yes	53	61	
No	94	86	
TNM stage			0.064
I	46	43	
IIa	56	56	
IIb	23	11	
IIIa	5	14	
IIIb	1	2	
IIIc	9	15	
Unable to value	7	6	
Histological grade			0.365
1	0	2	
2	89	88	
3	58	57	
Pathological type			0.069
Invasive ductal carcinoma	138	129	
Others	9	18	
Surgery			0.469
Radical mastectomy	24	17	
Modified radical mastectomy	110	118	
Breast-conserving surgery	13	12	
Radiotherapy			0.162
Yes	38	28	
No	109	119	
Neoadjuvant chemotherapy			1.000
Yes	21	21	
No	126	126	
Adjuvant chemotherapy regimens			0.319
Anthracycline-based	17	10	
Anthracycline-and taxane-based	113	122	
Taxane-based	17	15	

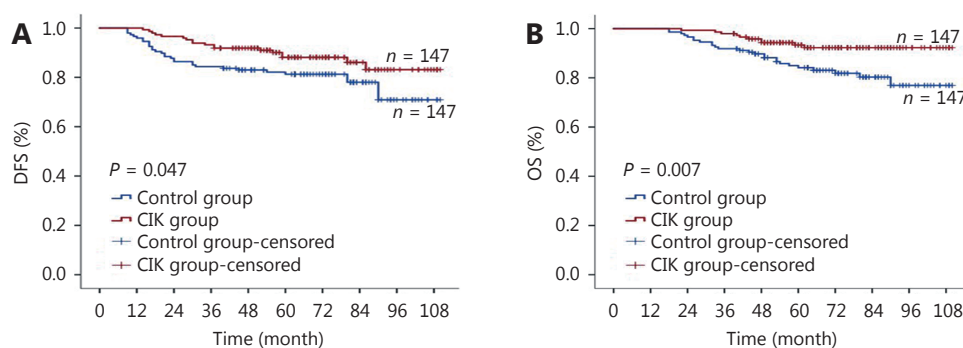


Figure 1 Survival analysis of patients in cytokine-induced killer (CIK) group and control group. (A) Disease-free disease-free survival (DFS) curves. (B) Overall survival (OS) curves. The Kaplan-Meier method was used to compare the DFS and OS between the CIK group and control group.

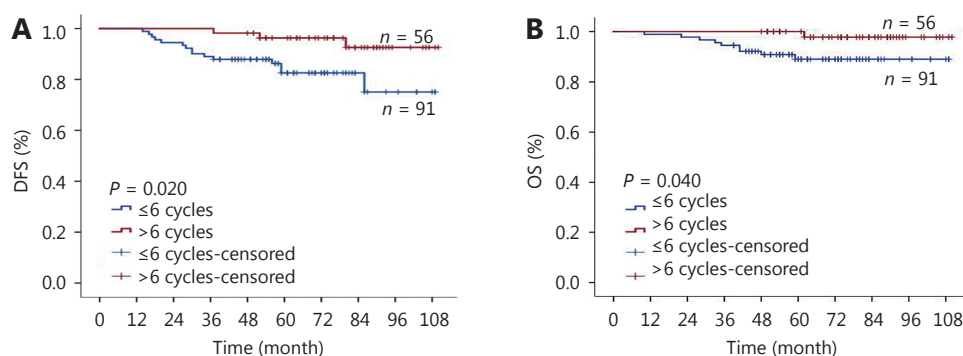


Figure 2 Prognostic impact of the frequency of cytokine-induced killer (CIK) treatment on patients in the CIK group. (A) Disease disease-free survival (DFS) curves. (B) Overall survival (OS) curves. The Kaplan-Meier method was used to compare the survival rates between the patients in the CIK group underwent ≤ 6 cycles CIK cells injection and the patients in the CIK group underwent > 6 cycles CIK cells injection.

tumor mutation load, as well as higher expression of tumor infiltrating lymphocytes (TILs)²², and programmed death-ligand 1 (PD-L1)²³ in TNBC make them a suitable target for immunotherapy, in contrast to the other subtypes of breast cancer. As the immunotherapy is non-organ-specific or non-tumor-specific, it is important to find the proper patient and treatment time, while combining it with existing treatment to achieve maximum efficacy. A breakthrough was achieved recently as immune checkpoint inhibitors (anti-PD-1 and anti-PD-L1 antibodies^{24, 25}) became clinically effective. These previous significant studies have encouraged us to conduct the retrospective research on the effectiveness and safety of autologous CIK cell therapy coupled with chemotherapy in TNBC patients.

We found that CIK treatment combined with chemotherapy could effectively reduce the recurrence and metastasis in TNBC patients, thereby prolonging overall survival, and it had a stronger effect on patients at relatively early-stage of the disease. The conclusion that the patients in the early stages are the ones most benefited from CIK

treatment was in line with some of the results from available studies on the treatment of other early-stage tumors by CIK immune cells^{26, 27}. Several mechanisms could further contribute to the observed phenomenon. On one hand, the immune function of patients with late-stage cancer could be suppressed by the heavy tumor burden, which also influences the activity of infused CIK cells²⁸. Immune system suppression related to tumor stages may hinder the initial expansion of CIK cells²⁹. On the other hand, to evade the immune surveillance or immunotherapy, late-stage cells of metastatic cancer may evolve at molecular level³⁰. Relevant information that could contribute to preventing recurrence and metastasis in early-stage TNBC patients using the new immunotherapy protocol is provided in the study.

Sequential CIK cell therapy after adjuvant chemotherapy resulted in dramatic lengthening of both DFS and OS intervals compared to those after chemotherapy alone, with a median DFS of 59 versus 55 months, and a median OS of 60 versus 59 months, respectively. The therapeutic model of cytotoxic chemotherapy combined with immunotherapy has

Table 2 The details of recurrence and metastasis between the two groups

	CIK group (<i>n</i> = 16)	Control group (<i>n</i> = 29)	<i>P</i>
Sites			0.581
Chest wall	3/16 (18.8%)	6/29 (20.7%)	
Regional lymph node	4/16 (25.0%)	5/29 (17.2%)	
Lung	6/16 (37.5%)	12/29 (41.4%)	
Bone	6/16 (37.5%)	9/29 (31.0%)	
Liver	3/16 (18.8%)	5/29 (17.2%)	
Brain	3/16 (18.8%)	4/29 (13.8%)	
Other sites	1/16 (6.3%)	2/29 (6.9%)	
Numbers of metastatic sites			0.628
1	7/16 (43.7%)	14/29 (48.3%)	
2	1/16 (6.3%)	4/29 (13.8%)	
≥ 3	8/16 (50.0%)	11/29 (37.9%)	

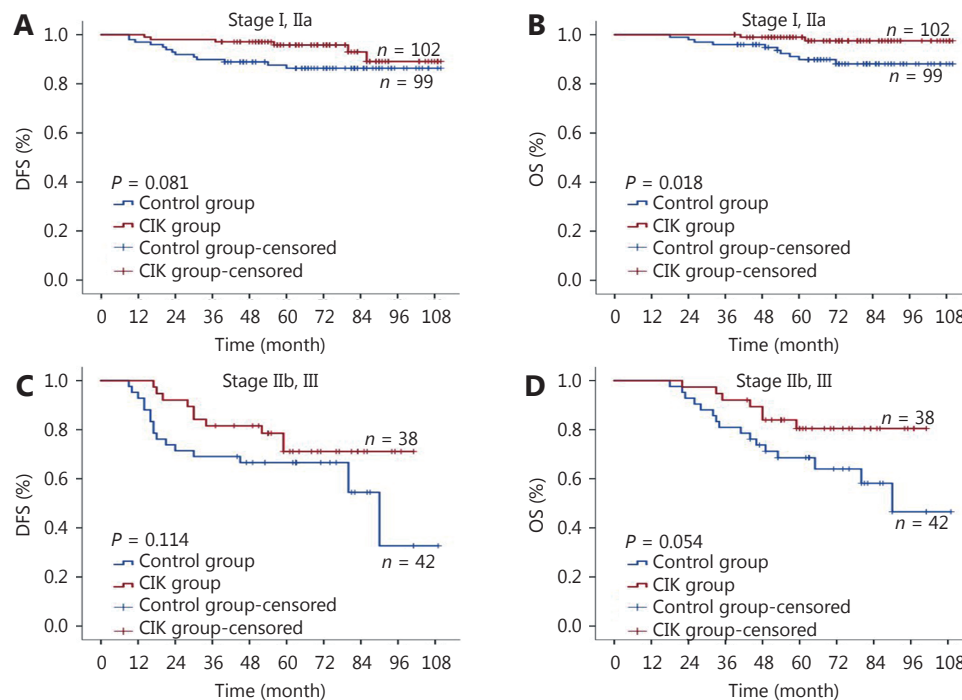


Figure 3 (A) Subgroup analysis to estimate the benefits of CIK treatment according to TNM stages. A, disease-free survival (DFS) curves of TNBC patients with stage I, IIa. (B) Subgroup analysis to estimate the benefits of CIK treatment according to TNM stages. B, overall survival (OS) curves of TNBC patients with stage I, IIa. (C) Subgroup analysis to estimate the benefits of CIK treatment according to TNM stages. C, disease-free survival (DFS) curves of TNBC patients with stage IIb, III. (D) Subgroup analysis to estimate the benefits of CIK treatment according to TNM stages. D, overall survival (OS) curves of TNBC patients with stage IIb, III.

been supported by some preclinical studies. These studies have shown that there is a synergy and complementation relationship between immunotherapy and chemotherapy³¹.

The left-out tumor cells after chemotherapy and some chemotherapy-insensitive tumor cells can be removed by CIK cells³². Furthermore, it was demonstrated in previous

Table 3 Multivariate analysis of DFS and OS in patients with TNBC

Parameter	DFS		OS	
	HR (95%CI)	P	HR (95%CI)	P
TNM stage	1.449 (1.118-1.877)	0.005*	1.581 (1.204-2.077)	0.001*
Tumor size	1.943 (0.847-4.459)	0.117	2.018 (0.761-5.352)	0.158
Lymph node metastasis	1.001 (0.426-2.352)	0.998	1.258 (0.465-3.403)	0.652
Histological grade	1.510 (0.821-2.778)	0.185	1.576 (0.796-3.119)	0.191
Radiotherapy	0.578 (0.198-1.692)	0.317	0.738 (0.209-2.605)	0.637
CIK treatment	0.520 (0.271-0.998)	0.049*	0.414 (0.190-0.903)	0.027*

*P < 0.05

studies that CIK cells could function as anti-cancer stem cells³³. In this way, CIK cell therapy can reduce tumor recurrence and metastasis. Additionally, CIK cells secrete cytokines, such as IFN- γ , IL-2, and TNF- α , which can activate the anti-tumor properties of macrophages, reduce the immunological damage resulting from chemotherapeutic drugs, and facilitate the immune surveillance function of the body, to inhibit the growth of tumor cells³⁴. Some chemotherapeutics, such as anthracycline can not only kill tumor cells directly, but also increase the sensitivity of tumor cells to immune effector cells³⁵, thereby promoting their eradication by immune cells. With weakly immunogenic and immunosuppressive properties, immune escape is a typical biological feature of tumor cells³⁶. Regulatory T (Treg) cells could limit the anti-tumor effect of immune cells by hindering CD3⁺CD4⁺ and CD3⁺CD8⁺ T lymphocytes from activation and proliferation, preventing NK cells proliferation, producing inhibitory cytokines, and eliminating effector cells, thereby promoting the immune escape of tumor cells, thus stimulating tumor progression³⁷. CIK cells are capable of decreasing Treg cells ratio in peripheral blood of tumor patients, thereby increasing the proportion of CD3⁺CD4⁺T cells and the ratio of CD4⁺/CD8⁺T cells, thus the immunosuppressive status of tumor patients could be reduced or eliminated³⁸. Therefore, chemotherapy can significantly lower the tumor burden, and then immune suppression can be alleviated or restored, hence sequential immunotherapy could achieve better therapeutic efficacy.

Additionally, besides the synergic effect with chemotherapy, immunotherapy also shows synergy with radiotherapy. Preclinical and clinical evidence suggests that RT may be a motivating factor to enhance the therapeutic benefits of immunotherapy for cancers^{39, 40}. The potential effects of radiotherapy combined with immunotherapy are complex and multifactorial. Briefly, a combination of RT and

immunotherapy induces the release of antigens during cancer cell death in association with proinflammatory signals that trigger the innate immune system to activate the tumor-specific T cells; thus, tumor targeted radiation therapy can be converted into an *in-situ* tumor vaccine⁴¹. To summarize, RT could improve the efficacy of immunotherapy and the immune system also functions in the action of radiotherapy.

No significant difference was found in the adverse reactions to chemotherapy plus CIK immunotherapy or chemotherapy alone, which indicates that the adverse effects of CIK immunotherapy are minor. The number of cycles for CIK treatment in this study depended on the patient's disease progression, willingness to treat, and family economic status, ranging from 1 to 26 cycles, with the median of 6 treatment cycles. Survival analysis showed that the patients treated for more than 6 cycles with CIK cells had greater DFS and OS intervals than those treated with less than 6 cycles, demonstrating that the prognosis of patients was related to the frequency of CIK administration. However, the specific connection between the number of CIK treatment cycles and survival remains to be explored. Furthermore, the equilibrium of treatment efficacy and costs, and the exploration of the number of cycles to the greatest benefit of patients remains to be studied.

The study was novel for a number of reasons. First, the objects of the study were TNBC patients without distant metastasis, which forms a complementation with existing clinical studies of the patients at an advanced stage of breast cancer or metastatic breast cancer using CIK cells for treatment¹⁴⁻¹⁷. Therefore, this study enhanced our understanding of the potential of CIK cells in breast cancer treatment. Additionally, previous studies mostly concentrated on treatment using a single chemotherapy regimen⁴²; in this study, the chemotherapy regimens were classified into anthracycline-based, anthracycline- and taxane-based, and taxane-based regimens, which provided a

Table 4 Adverse events of the two groups

Adverse events	CIK group (n = 147)	Control group (n = 147)	p
Bone marrow suppression			0.222
0	98	84	
I	20	35	
II	18	16	
III	8	10	
IV	3	2	
Fever			0.491
0	118	112	
I	28	32	
II	1	3	
III	0	0	
IV	0	0	
Nausea and vomiting			0.545
0	98	94	
I	18	13	
II	23	31	
III	8	9	
IV	0	0	
Liver dysfunction			0.641
0	120	117	
I	17	21	
II	10	8	
III	0	1	
IV	0	0	
Kidney dysfunction			0.415
0	137	134	
I	9	13	
II	1	0	
III	0	0	
IV	0	0	
Peripheral nerve toxicity			0.542
0	132	126	
I	10	13	
II	5	8	
III	0	0	
IV	0	0	

more comprehensive description of the efficacy of CIK cell therapy. However, this study also has some limitations. First, the precise assessment of CIK cell-induced treatment might be limited by the patient selection bias in the retrospective study. Second, the data collected in this study spanned from January 1, 2009 to January 1, 2015 and the follow-up period was up to May 1, 2018. The short follow-up time did not reflect the effect of CIK cell therapy on the long-term survival of patients with TNBC after surgery. A previous study revealed that CIK cells have a long half-life *in vivo*⁴³, which could explain the long-lasting effects of CIK cells. Even if the disease progresses, the remaining active CIK cells can eliminate tumor cells and slow down the disease progression. Therefore, a prospective, multi-center, long-lasting follow-up assessment of CIK cell therapy for TNBC is required.

Conclusions

In summary, the strategy of CIK cell therapy after adjuvant chemotherapy could reduce recurrence and metastasis in postoperative TNBC patients, thereby prolong the overall survival time with minimum side effects. Therefore, CIK cell immunotherapy could be a potential new strategy for systemic adjuvant therapy after surgery for TNBC patients in the near future. Recently, the development of a gene expression profile facilitated re-classification of TNBC into six new subtypes, which showed varied sensitivities to different therapies. As precision medicine develops, precision therapy may be directed at various, potentially actionable molecular mutations in different subtypes of TNBC.

Conflict of interest statement

No potential conflicts of interest are disclosed.

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Supplementary materials

Table 1 Clinical characteristics of patients in the two groups

Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
1	CIK group	77	IIb	Positive	III	No	Yes	Alive
2	CIK group	52	IIa	Positive	II	Yes	No	Alive
3	CIK group	38	Unable to value	Positive	III	No	No	Alive
4	CIK group	60	I	Negative	II	No	No	Alive
5	CIK group	58	I	Positive	III	Yes	No	Alive
6	CIK group	60	Unable to value	Negative	III	No	No	Alive
7	CIK group	37	IIb	Negative	II	No	No	Alive
8	CIK group	49	I	Negative	II	No	No	Alive
9	CIK group	51	I	Negative	II	No	No	Alive
10	CIK group	50	IIIa	Positive	II	Yes	No	Alive
11	CIK group	60	I	Negative	II	No	No	Alive
12	CIK group	61	IIa	Negative	II	No	No	Alive
13	CIK group	76	IIa	Negative	II	No	No	Alive
14	CIK group	46	I	Negative	II	No	No	Alive
15	CIK group	54	IIa	Positive	II	Yes	No	Alive
16	CIK group	64	IIb	Positive	II	No	No	Alive
17	CIK group	58	IIb	Positive	II	No	No	Alive
18	CIK group	44	IIb	Positive	III	Yes	No	Alive
19	CIK group	50	I	Negative	III	No	No	Alive
20	CIK group	33	IIb	Negative	II	No	No	Alive
21	CIK group	57	IIIc	Positive	III	Yes	Yes	Death
22	CIK group	52	Unable to value	Negative	III	No	No	Alive
23	CIK group	56	IIa	Positive	II	Yes	No	Alive
24	CIK group	38	I	Negative	II	No	No	Alive
25	CIK group	33	IIa	Negative	III	No	No	Alive
26	CIK group	51	IIb	Positive	III	No	No	Alive
27	CIK group	58	IIIc	Positive	III	No	No	Alive
28	CIK group	53	IIIc	Positive	II	No	Yes	Alive
29	CIK group	40	IIa	Negative	II	Yes	No	Alive
30	CIK group	54	IIa	Negative	II	No	No	Alive
31	CIK group	56	IIa	Positive	III	Yes	No	Alive
32	CIK group	51	I	Negative	II	No	No	Alive
33	CIK group	53	IIa	Positive	III	No	No	Alive
34	CIK group	56	IIa	Negative	II	No	No	Alive

Continued

Continued

Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
35	CIK group	59	I	Negative	II	No	No	Alive
36	CIK group	66	IIa	Negative	II	No	No	Alive
37	CIK group	56	I	Negative	II	No	No	Alive
38	CIK group	42	IIb	Positive	III	Yes	Yes	Death
39	CIK group	50	IIa	Negative	III	No	No	Alive
40	CIK group	49	I	Negative	II	No	No	Alive
41	CIK group	69	I	Negative	III	No	No	Alive
42	CIK group	47	IIa	Negative	II	No	No	Alive
43	CIK group	55	I	Positive	II	No	No	Alive
44	CIK group	50	IIa	Negative	II	No	No	Alive
45	CIK group	59	I	Negative	III	No	No	Alive
46	CIK group	59	IIa	Positive	II	No	No	Alive
47	CIK group	50	IIIc	Positive	III	No	No	Alive
48	CIK group	56	Unable to value	Positive	III	Yes	No	Alive
49	CIK group	57	I	Negative	II	No	No	Alive
50	CIK group	60	IIa	Negative	II	No	No	Alive
51	CIK group	63	I	Negative	II	No	No	Alive
52	CIK group	62	IIa	Positive	II	Yes	No	Alive
53	CIK group	60	I	Negative	III	No	No	Alive
54	CIK group	54	IIa	Negative	III	No	No	Alive
55	CIK group	48	IIb	Positive	II	No	No	Alive
56	CIK group	58	IIa	Negative	II	No	No	Alive
57	CIK group	31	I	Negative	III	Yes	No	Alive
58	CIK group	56	IIa	Positive	II	Yes	No	Alive
59	CIK group	60	IIa	Positive	II	No	No	Alive
60	CIK group	52	I	Negative	II	No	No	Alive
61	CIK group	42	IIIc	Positive	III	Yes	Yes	Death
62	CIK group	52	IIa	Negative	III	No	No	Alive
63	CIK group	53	I	Negative	II	No	No	Alive
64	CIK group	61	IIIa	Positive	II	Yes	No	Alive
65	CIK group	48	IIa	Positive	II	No	No	Alive

Continued

Continued

Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
66	CIK group	34	I	Negative	III	No	No	Alive
67	CIK group	71	IIb	Negative	II	No	No	Alive
68	CIK group	48	Unable to value	Negative	II	No	Yes	Alive
69	CIK group	46	I	Negative	II	No	No	Alive
70	CIK group	37	IIb	Negative	II	Yes	No	Alive
71	CIK group	45	IIIa	Positive	II	No	No	Alive
72	CIK group	61	IIa	Negative	II	No	No	Alive
73	CIK group	45	IIIc	Positive	III	Yes	Yes	Death
74	CIK group	56	I	Negative	III	No	No	Alive
75	CIK group	59	IIa	Negative	II	No	No	Alive
76	CIK group	46	IIa	Negative	II	No	No	Alive
77	CIK group	41	I	Negative	II	No	No	Alive
78	CIK group	46	IIb	Positive	III	No	No	Alive
79	CIK group	49	IIa	Negative	III	No	No	Alive
80	CIK group	54	I	Negative	III	No	No	Alive
81	CIK group	61	IIb	Positive	III	Yes	No	Alive
82	CIK group	49	IIa	Negative	II	No	No	Alive
83	CIK group	55	I	Negative	II	No	No	Alive
84	CIK group	50	IIa	Negative	II	Yes	Yes	Death
85	CIK group	46	I	Negative	II	No	No	Alive
86	CIK group	57	IIIb	Positive	II	Yes	No	Alive
87	CIK group	33	IIb	Positive	III	No	No	Alive
88	CIK group	61	IIa	Negative	III	No	No	Alive
89	CIK group	52	I	Negative	III	No	No	Alive
90	CIK group	47	IIa	Negative	II	Yes	No	Alive
91	CIK group	58	IIIa	Positive	II	Yes	No	Alive
92	CIK group	45	IIa	Negative	II	No	No	Alive
93	CIK group	53	I	Negative	II	No	No	Alive
94	CIK group	32	IIa	Negative	III	Yes	Yes	Alive
95	CIK group	38	IIa	Positive	III	Yes	No	Alive
96	CIK group	31	Unable to value	Negative	III	Yes	Yes	Death
97	CIK group	50	IIb	Negative	III	No	No	Alive
98	CIK group	44	IIb	Negative	II	No	No	Alive
99	CIK group	62	IIa	Positive	III	No	No	Alive
100	CIK group	54	I	Negative	II	Yes	No	Alive
101	CIK group	50	IIa	Positive	II	No	No	Alive
102	CIK group	68	I	Negative	III	No	No	Alive
103	CIK group	26	Unable to value	Negative	II	Yes	No	Alive

Continued

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Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
104	CIK group	60	IIa	Negative	II	No	No	Alive
105	CIK group	35	IIb	Positive	II	No	No	Alive
106	CIK group	36	IIa	Negative	II	No	No	Alive
107	CIK group	52	IIb	Positive	III	Yes	No	Alive
108	CIK group	60	IIa	Negative	II	No	No	Alive
109	CIK group	43	IIa	Negative	III	No	No	Alive
110	CIK group	57	IIa	Positive	III	No	No	Alive
111	CIK group	73	IIIa	Positive	III	No	No	Alive
112	CIK group	72	IIa	Negative	II	No	No	Alive
113	CIK group	47	IIa	Negative	III	No	No	Alive
114	CIK group	49	IIa	Negative	III	No	Yes	Alive
115	CIK group	40	I	Negative	II	No	No	Alive
116	CIK group	52	IIb	Positive	III	No	Yes	Alive
117	CIK group	38	IIb	Positive	III	Yes	No	Alive
118	CIK group	53	IIa	Negative	III	No	No	Alive
119	CIK group	50	IIa	Negative	II	No	No	Alive
120	CIK group	57	I	Negative	II	No	Yes	Alive
121	CIK group	54	IIa	Positive	III	No	No	Alive
122	CIK group	50	IIb	Positive	III	Yes	No	Alive
123	CIK group	64	I	Negative	II	No	No	Alive
124	CIK group	61	I	Negative	II	No	No	Alive
125	CIK group	49	I	Negative	II	Yes	No	Alive
126	CIK group	59	I	Negative	II	No	No	Alive
127	CIK group	68	IIb	Positive	II	No	No	Alive
128	CIK group	59	IIb	Positive	III	No	No	Alive
129	CIK group	59	IIa	Negative	III	No	No	Alive
130	CIK group	48	IIa	Negative	II	No	Yes	Alive
131	CIK group	53	I	Negative	II	No	No	Alive
132	CIK group	60	I	Negative	III	No	No	Alive
133	CIK group	45	IIIc	Positive	III	Yes	Yes	Death
134	CIK group	60	I	Negative	II	No	No	Alive
135	CIK group	69	IIIc	Positive	II	Yes	Yes	Death
136	CIK group	45	IIa	Negative	III	No	No	Alive
137	CIK group	39	I	Negative	II	No	No	Alive
138	CIK group	42	I	Negative	II	No	No	Alive
139	CIK group	44	I	Negative	III	Yes	No	Alive
140	CIK group	44	I	Negative	II	Yes	No	Alive
141	CIK group	32	IIa	Positive	II	Yes	No	Alive

Continued

Continued

Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
142	CIK group	55	I	Negative	III	No	No	Alive
143	CIK group	43	IIa	Negative	II	No	Yes	Death
144	CIK group	40	IIa	Negative	II	No	No	Alive
145	CIK group	64	IIa	Negative	II	No	No	Alive
146	CIK group	69	IIIc	Positive	II	Yes	Yes	Death
147	CIK group	44	IIa	Negative	II	No	No	Alive
148	Control group	78	I	Negative	II	No	No	Alive
149	Control group	76	I	Negative	II	No	No	Alive
150	Control group	73	I	Negative	II	No	No	Alive
151	Control group	72	IIb	Positive	III	No	No	Alive
152	Control group	71	IIIc	Positive	III	No	Yes	Death
153	Control group	69	IIb	Positive	III	No	Yes	Death
154	Control group	69	IIIa	Positive	II	Yes	Yes	Death
155	Control group	69	IIIb	Negative	III	No	No	Alive
156	Control group	68	I	Negative	III	No	No	Alive
157	Control group	68	I	Negative	II	Yes	No	Alive
158	Control group	66	I	Negative	II	No	No	Alive
159	Control group	64	I	Negative	II	Yes	No	Alive
160	Control group	64	IIb	Positive	II	No	No	Alive
161	Control group	64	IIa	Negative	II	No	No	Alive
162	Control group	63	Unable to value	Negative	III	No	No	Alive
163	Control group	62	IIa	Positive	II	No	No	Alive
164	Control group	62	IIa	Negative	III	Yes	No	Alive
165	Control group	61	IIa	Negative	II	No	No	Alive
166	Control group	61	IIa	Positive	III	Yes	Yes	Death
167	Control group	61	IIIa	Positive	II	Yes	Yes	Death
168	Control group	61	IIa	Negative	II	No	No	Alive
169	Control group	61	IIa	Negative	I	No	No	Alive
170	Control group	61	IIb	Positive	II	Yes	No	Alive
171	Control group	61	IIa	Negative	II	No	No	Alive
172	Control group	60	I	Negative	II	No	No	Alive
173	Control group	60	IIa	Negative	II	No	No	Alive
174	Control group	60	IIIc	Positive	II	No	No	Alive
175	Control group	60	IIa	Negative	III	No	No	Alive
176	Control group	60	IIIa	Positive	II	Yes	Yes	Death
177	Control group	60	IIIc	Positive	II	No	No	Alive
178	Control group	60	IIIc	Positive	III	Yes	Yes	Death
179	Control group	60	IIIc	Positive	II	Yes	Yes	Death

Continued

Continued

Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
180	Control group	60	Unable to value	Positive	III	No	No	Alive
181	Control group	59	IIIc	Positive	II	No	No	Alive
182	Control group	59	IIb	Positive	III	No	No	Alive
183	Control group	59	IIa	Positive	II	No	No	Alive
184	Control group	59	I	Negative	III	No	No	Alive
185	Control group	59	IIa	Positive	II	No	Yes	Death
186	Control group	59	IIa	Positive	II	No	No	Alive
187	Control group	59	IIa	Negative	II	Yes	No	Alive
188	Control group	58	IIb	Negative	II	Yes	No	Alive
189	Control group	58	IIa	Positive	II	No	No	Alive
190	Control group	58	IIa	Negative	II	No	No	Alive
191	Control group	58	IIIa	Positive	II	Yes	No	Alive
192	Control group	58	IIa	Negative	II	Yes	No	Alive
193	Control group	56	I	Negative	II	No	No	Alive
194	Control group	57	I	Negative	II	No	Yes	Alive
195	Control group	57	IIIa	Positive	III	No	No	Alive
196	Control group	57	I	Negative	II	Yes	No	Alive
197	Control group	57	IIa	Negative	III	No	No	Alive
198	Control group	56	IIIc	Positive	II	No	No	Alive
199	Control group	56	IIIa	Positive	III	No	No	Alive
200	Control group	56	IIIb	Negative	III	No	Yes	Death
201	Control group	56	I	Negative	III	No	Yes	Death
202	Control group	56	IIa	Negative	II	Yes	No	Alive
203	Control group	56	I	Negative	III	No	No	Alive
204	Control group	56	IIa	Negative	III	No	No	Alive
205	Control group	55	IIa	Positive	III	No	No	Alive
206	Control group	55	IIa	Positive	III	Yes	No	Alive
207	Control group	55	I	Negative	III	No	Yes	Death
208	Control group	54	I	Negative	II	No	No	Alive
209	Control group	54	I	Negative	III	No	No	Alive
210	Control group	54	IIa	Positive	II	No	No	Alive
211	Control group	54	IIIc	Positive	II	No	Yes	Death
212	Control group	54	IIa	Negative	II	No	No	Alive
213	Control group	54	IIIa	Positive	II	Yes	No	Alive
214	Control group	53	IIIc	Positive	II	No	Yes	Death
215	Control group	53	IIa	Negative	II	No	Yes	Death
216	Control group	53	I	Negative	II	No	No	Alive
217	Control group	53	IIa	Positive	II	No	Yes	Death

Continued

Continued

Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
218	Control group	53	I	Negative	III	No	Yes	Death
219	Control group	53	IIa	Positive	II	No	No	Alive
220	Control group	52	IIa	Negative	II	No	No	Alive
221	Control group	52	I	Negative	II	No	No	Alive
222	Control group	52	IIa	Negative	II	No	No	Alive
223	Control group	52	I	Negative	II	Yes	Yes	Alive
224	Control group	52	IIa	Positive	II	No	No	Alive
225	Control group	52	I	Negative	III	Yes	No	Alive
226	Control group	52	I	Negative	II	No	No	Alive
227	Control group	51	I	Negative	II	No	No	Alive
228	Control group	51	IIa	Positive	III	No	No	Alive
229	Control group	51	I	Negative	II	Yes	No	Alive
230	Control group	50	IIa	Negative	III	No	No	Alive
231	Control group	50	IIb	Positive	II	No	No	Alive
232	Control group	50	IIa	Negative	II	No	Yes	Alive
233	Control group	50	IIIc	Positive	III	No	No	Alive
234	Control group	50	IIa	Positive	III	No	No	Alive
235	Control group	50	I	Negative	III	No	No	Alive
236	Control group	50	IIIc	Positive	II	Yes	Yes	Death
237	Control group	50	Unable to value	Negative	I	No	No	Alive
238	Control group	50	I	Negative	II	No	No	Alive
239	Control group	50	I	Negative	III	No	No	Alive
240	Control group	49	IIa	Negative	III	No	No	Alive
241	Control group	49	IIIc	Positive	III	Yes	Yes	Death
242	Control group	49	I	Negative	III	No	No	Alive
243	Control group	49	I	Negative	II	No	No	Alive
244	Control group	49	IIa	Negative	II	No	No	Alive
245	Control group	49	I	Negative	II	No	No	Alive
246	Control group	48	IIIa	Positive	II	Yes	Yes	Alive
247	Control group	48	IIa	Negative	II	No	No	Alive
248	Control group	48	IIa	Negative	II	No	No	Alive
249	Control group	48	IIb	Positive	III	No	Yes	Death
250	Control group	47	I	Negative	II	No	No	Alive
251	Control group	47	IIa	Positive	II	Yes	No	Alive
252	Control group	47	IIa	Negative	III	No	No	Alive
253	Control group	46	I	Negative	II	No	No	Alive
254	Control group	46	I	Negative	II	No	No	Alive
255	Control group	46	IIIc	Positive	II	No	No	Alive

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Patient	Group	Age, years	TNM stage	Lymph node	Pathological grades	Radiotherapy	Recurrence	Survival
256	Control group	46	I	Negative	III	No	No	Alive
257	Control group	46	IIa	Positive	II	No	No	Alive
258	Control group	45	IIa	Negative	III	No	No	Alive
259	Control group	45	I	Negative	III	No	No	Alive
260	Control group	45	IIIa	Positive	II	No	No	Alive
261	Control group	45	I	Negative	II	No	No	Alive
262	Control group	45	IIIa	Positive	III	Yes	No	Alive
263	Control group	44	IIa	Positive	II	No	No	Alive
264	Control group	44	IIa	Negative	II	No	Yes	Death
265	Control group	44	IIa	Negative	II	No	No	Alive
266	Control group	44	I	Negative	III	No	No	Alive
267	Control group	44	IIa	Negative	III	No	No	Alive
268	Control group	43	I	Negative	III	No	No	Alive
269	Control group	43	IIa	Positive	II	No	No	Alive
270	Control group	42	IIIa	Positive	III	No	No	Alive
271	Control group	42	IIa	Negative	III	No	No	Alive
272	Control group	42	IIa	Positive	II	No	No	Alive
273	Control group	41	IIa	Negative	II	No	No	Alive
274	Control group	40	IIa	Negative	III	No	Yes	Death
275	Control group	40	I	Negative	II	No	No	Alive
276	Control group	40	Unable to value	Negative	III	No	No	Alive
277	Control group	39	IIa	Negative	II	No	No	Alive
278	Control group	38	IIb	Positive	III	No	No	Alive
279	Control group	38	Unable to value	Negative	II	No	No	Alive
280	Control group	38	IIIa	Positive	II	No	No	Alive
281	Control group	38	IIa	Positive	III	No	Yes	Death
282	Control group	37	IIIa	Positive	II	No	Yes	Death
283	Control group	37	IIIc	Positive	III	No	No	Alive
284	Control group	36	IIb	Positive	III	Yes	Yes	Death
285	Control group	35	IIIa	Positive	II	No	Yes	Alive
286	Control group	34	IIb	Positive	II	No	No	Alive
287	Control group	33	I	Negative	III	No	No	Alive
288	Control group	33	IIa	Positive	II	No	No	Alive
289	Control group	33	IIIc	Positive	III	Yes	No	Alive
290	Control group	32	I	Negative	II	No	No	Alive
291	Control group	32	Unable to value	Negative	II	No	No	Alive
292	Control group	31	I	Negative	III	No	No	Alive
293	Control group	31	IIa	Negative	III	No	No	Alive
294	Control group	26	IIa	Negative	III	No	Yes	Death