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Effect and mechanism of local radiotherapy combined with immune checkpoint inhibitor in the treatment of metastatic triple negative breast cancer

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Qiong Lu[*]*, Fei Wang, Lei Yu and Ping Lv*

Shanghai Concord Medical Cancer Center,Shanghai, China

***For Correspondence:** Email: *dangwenxie85981@163.com*

Abstract

Triple negative breast cancer is highly sensitive to immunotherapy due to its high PD-L1 expression; however, many patients exhibit poor response or develop resistance. This study evaluates the efficacy and mechanism of local radiotherapy combined with PD-1/PD-L1 inhibitors in metastatic triple negative breast cancer treatment. A retrospective analysis of 40 patients divided into a combination group (local radiotherapy + PD-1/PD-L1 inhibitor) and a single-agent group (PD-1/PD-L1 inhibitor) was conducted. Clinical efficacy, progression-free survival, immune cytokine levels (Tumor necrosis factor-α, Interleukin-1, and Interleukin-8), and adverse effects were compared. Results showed that the combination therapy significantly improved clinical outcomes and prolonged progression-free survival without increasing toxicity. Patients receiving local radiotherapy and PD-1/PD-L1 inhibitors exhibited reduced immune cytokines, with no significant post-treatment increase in Tumor necrosis factor-α, Interleukin-1, and Interleukin-8 levels. These findings suggest that local radiotherapy enhances the effectiveness of PD-1/PD-L1 inhibitors in TNBC treatment without raising the risk of adverse effects. *(Afr J Reprod Health 2025; 29 [7]: 27-35).*

Keywords: Triple negative breast cancer; Radiotherapy; Programmed cell death protein 1; Programmed cell death protein ligand 1; Immune checkpoints

Résumé

Le cancer du sein triple négatif (CSTN) est particulièrement sensible à l'immunothérapie en raison de sa forte expression de PD-L1 ; cependant, de nombreux patients présentent une réponse médiocre ou développent une résistance. Cette étude évalue l'efficacité et le mécanisme de la radiothérapie locale associée aux inhibiteurs de PD-1/PD-L1 dans le traitement du CSTN métastatique. Une analyse rétrospective de 40 patientes a été réalisée, réparties en deux groupes : un groupe combiné (radiothérapie locale + inhibiteur de PD-1/PD-L1) et un groupe monothérapie (inhibiteur de PD-1/PD-L1 seul). L'efficacité clinique, la survie sans progression, les niveaux de cytokines immunitaires (facteur de nécrose tumorale α, interleukine-1 et interleukine-8) et les effets indésirables ont été comparés. Les résultats montrent que le traitement combiné améliore significativement les résultats cliniques et prolonge la survie sans progression, sans augmenter la toxicité. Les patientes ayant reçu la radiothérapie locale en association avec les inhibiteurs de PD-1/PD-L1 présentaient une réduction des cytokines immunitaires, sans élévation significative post-traitement des niveaux de TNF-α, d'IL-1 et d'IL-8. Ces résultats suggèrent que la radiothérapie locale renforce l'efficacité des inhibiteurs de PD-1/PD-L1 dans le traitement du CSTN sans accroître le risque d'effets indésirables. *(Afr J Reprod Health 2025; 29 [7]: 27-35).*

Mots-clés: : Cancer du sein triple négatif ; Radiothérapie ; Protéine de mort cellulaire programmée 1 ; Ligand de la protéine de mort cellulaire programmée 1 ; Points de contrôle immunitaires

Introduction

Breast cancer is a malignancy that arises from genetic mutations of breast epithelial cells under the action of multiple oncogenic factors. The 2021 global cancer survey report indicated that breast cancer is the most frequent malignancy in women[1]. Among the different subtypes of breast cancer, the aggressiveness, recurrence rate, and case fatality rate of Triple negative breast cancer (TNBC) are higher than those of the other types[2,3].

TNBC is characterized by its unique biological behavior and clinical manifestations: it usually has a higher proliferation rate and more

aggressive behavior, and is prone to distant metastasis at an early stage, especially in important organs such as lung, brain and liver. In addition, tumor cells in TNBC often exhibit a high gene mutation burden, which makes them potentially sensitive to certain immunotherapy strategies, but at the same time increases the complexity of treatment, as tumor cells may evade immune surveillance through multiple mechanisms[4,5]. The treatment of TNBC is mainly chemotherapy, but its clinical efficacy is not ideal in view of current data. Especially for metastatic TNBC patients, the survival time after chemotherapy is generally only around 13 months[6,7]. Therefore, the treatment of TNBC has become a hot and difficult problem in the field of breast cancer research. With a deeper understanding of the biological characteristics and molecular mechanisms of TNBC in clinical practice, progress has been made in the treatment of TNBC[8]. Firstly, based on the understanding of TNBC molecular subtypes, treatment strategies are shifting towards precision medicine. Research has shown that TNBC is not a single disease, but encompasses multiple biological subtypes such as immunomodulatory, basal like, and mesenchymal types [9]. These subtypes have different biological characteristics and clinical manifestations, and their response to treatment also varies. Therefore, selecting individualized treatment plans based on molecular typing has become a focus of research. In addition, PARP inhibitors have also shown hope in the treatment of TNBC[10]. PARP is an enzyme involved in DNA repair, and its inhibitors can block the DNA repair mechanism of cancer cells, thereby increasing their sensitivity to chemotherapy[11]. In TNBC patients with BRCA1/2 gene mutations, PARP inhibitors such as Olaparib and Talazoparib have shown good therapeutic effects[12].TNBC is characterized by high mutational load, immune cell infiltration as well as high expression of programmed cell death protein 1 (PD-L1) and high sensitivity to immunotherapy[13]. PD-1/PD-L1 is a pair of immune co stimulatory molecules[14]. In the tumor microenvironment, the binding of PD-1 to PD-L1 can attenuate the T cell-mediated immunosurveillance effect through multiple mechanisms, thereby providing cancer cells with a route to evade the immune response[15]. In clinical experiments, promising results have been observed with anti-PD-1/PD-L1 agents when used as monotherapy or in combination with conventional therapies[16]. In the research and application of TNBC treatment, blocking the PD-1/PD-L1 signaling pathway has been a breakthrough in the systemic treatment of TNBC. However, only 10% - 30% of TNBC patients can produce long-term, sustained efficacy after receiving PD-1/PD-L1 inhibitor, and many patients do not respond significantly to this treatment regimen or will still develop resistance[17]. Therefore, how to further improve the efficacy of PD-1/PD-L1 inhibitor has become a hotspot of research at home and abroad. Radiotherapy is currently the first method of clinical tumor treatment, by inducing malignant tumor cells to DNA double strand break damage through radiotherapy, is able to promote tumor cell apoptosis, thereby achieving the purpose of treating tumors[18]. The main purpose of radiotherapy is to treat metastatic cancer cells, thereby reducing recurrence in patients. But the therapeutic effect of this regimen on metastatic TNBC patients at present remains to be explored. In this study, we intended to administer local radiotherapy to multiple or even all metastatic foci of metastatic TNBC patients, and we treated them with PD-1/PD-L1 inhibitor before radiotherapy to observe the synergistic effects between radiotherapy induction and PD-1/PD-L1 inhibitor on TNBC.

Methods

General information

Forty patients with TNBC were enrolled in Shanghai Concord Medical Cancer Center from January 2019 to January 2021 were collected as study subjects. Inclusion criteria were: (1) patients as TNBC by pathology; (2) clear metastasis of tumor cells shown to distant sites on imaging; (3) presence of measurable lesions; (4) no anti-tumor therapy had been administered for two months prior to enrollment; (5) tolerance to the treatment methods used in this study; and (6) survival expected to be no less than 3 months.

The exclusion criteria were: (1) those with primary tumors in other sites; (2) co=morbid heart, liver, kidney, thyroid and other serious primary diseases; (3) pregnant or lactating women; (4) those with mental disorders and abnormal coagulation; and (5) those with incomplete clinical data.

Table 1: Comparison of baseline data among patients treated with different regimens

Factors	Combination (n=20)	Single (n=20)	t/χ^2	P
Years (years)	49.25±4.61	48.72±4.73	0.359	0.722
Disease duration (months)	9.76±2.15	9.07±2.41	0.956	0.345
Tumor types			0.170	0.919
Invasive ductal carcinoma	9 (45.00)	8 (40.00)		
Invasive lobular carcinoma	7 (35.00)	7 (35.00)		
Other	4 (20.00)	5 (25.00)		
Clinical stages			0.114	0.736
Stages III	13 (65.00)	14 (70.00)		
Stages IV	7 (35.00)	6 (30.00)		
Metastatic sites			0.700	0.705
Bone metastasis	11 (55.00)	9 (45.00)		
Lymph node metastasis	6 (30.00)	6 (30.00)		
Other	3 (15.00)	5 (25.00)		

The 40 study participants were divided into a combination group and a single group. Specifically, patients who received combination therapy (local radiotherapy plus PD-1/PD-L1 inhibitors) were assigned to the combination group, while those who received only PD-1/PD-L1 inhibitors were assigned to the single group. As shown in Table 1, there were no differences in baseline data such as age, disease duration, tumor type, clinical stage, and metastatic sites between the two groups.

Treatment

The single group used anti PD-1/PD-L1 immunotherapy. They received intravenous injection of 200 mg of Carrelian bead (produced by Suzhou Shengdiya Biological Medicine Co., Ltd., National Drug Approval No.: S20190027). The drug was injected once a week for 60 minutes, for a treatment cycle of 4 weeks. The efficacy evaluation was conducted once every two treatment cycles until the patient's condition improved or the treatment was intolerable.

The combination group followed up with local radiotherapy after treatment with a single group of immunosuppressants. The patients were placed in the prone position or supine position. Medical staff used hot plastic molds to fix the patients on the special integrated plate for radiotherapy. The medical staff took the body surface projection of the patient with obvious pain and image concentration as the center of the radiotherapy target area, and marked the projection on the thermoplastic mold. The doctor conducted the X-ray CT scanning in SPECT/CT, imported the CT image into the radiotherapy planning system (TPS), outlined the lesion and formulated the radiotherapy plan. Radiotherapy plan includedtarget area 30 gY/3 gY/10F, once a day, Monday to Friday radiotherapy, with weekends suspension over two consecutive weeks. Before radiotherapy, doctors calibrated the patient's radiotherapy target area under the analog positioning machine to ensure the accuracy of the radiotherapy target area. Radiotherapy was carried out after confirming that the radiotherapy target area was correct.

Observed indicators

(1) Clinical efficacy: Imaging examination was carried out before and after the treatment of the patients. The short-term efficacy of patients was evaluated according to the evaluation of short-term efficacy of solid tumors (RECIST) 1.1[19]. Complete response (CR) : complete resolution of all visible tumor lesions lasting more than 4 weeks. Partial response (PR) was defined as a reduction of 50% or more in the product of the maximum diameter of the tumor lesion and its maximum vertical transverse diameter, with no enlargement of other lesions and no new lesions appearing, and lasting

for more than 4 weeks. Stable (SD) : the tumor lesion was reduced by less than 50% in the product of the maximum diameter and its maximum vertical transverse diameter, or increased by no more than 25%, and no new lesions appeared, and lasted for more than 4 weeks. Progression (PD) : the product of the maximum diameter of the tumor and its maximum vertical transverse diameter increased by more than 25%, or new lesions appeared.

(2) Progressive free survival (PFS). (3) Immune indexes: the immune indexes of the two groups of patients were detected by automatic flow cytometry before and after treatment. The detected immune indexes include PD-1 CD4+T cells, PD-1 CD8+T cells and PD-L1 CD4+ monocytes. (4) Detection of inflammatory cytokines: TNF-α, IL-1, and IL-8 were detected by enzyme-linked immunosorbent assay before and after treatment. Detailed operations were performed according to the manufacturer's instructions.

(5) The toxic and side effects of the two groups of patients were recorded, mainly including bone marrow depression, nausea and vomiting, rash, fever, liver and kidney function damage, diarrhea, leukopenia, and thrombocytopenia.

These adverse effects were assessed and documented based on the patients' medical records.

Statistical analysis

SPSS 25.0 statistical software (International Business Machines Corporation, USA) was used to analyze the research data. Enumeration data were expressed as frequency (%) and compared between groups χ 2-test, the rank sum test was used for count data with hierarchical nature.

The measurement data that conformed to normal distribution were expressed in the form of ($\bar{\mathbf{x}}\pm$ SD), t-tests are used for comparison between two groups, and ANOVA analysis is used for comparison between multiple groups. All data comparisons were deemed to significant at $P < 0.05$.

Ethical considerations

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the Ethics Committee of Shanghai Concord Medical Cancer Center.

Results

Treatment effects

The CR rate and PR rate of patients in the combination were higher than single group. As shown inTable 2, there was a significant a great difference in clinical outcomes between the two groups, as determined by the rank sum test ($z = 127.000$, $P = 0.036$).

Progression free survival in the two groups

Based on patient follow-up data, the median follow-up time was 11 months (range, 4-18 months). As shown in Figure 1, there was a significant difference in median progression free survival (PFS) between the 2 groups, with a median PFS of 14 months in the combination group and 10 months in the single group. However, survival analysis curves showed no great difference in PFS time between the two groups (log rank χ 2 = 3.612, $P = 0.057$), as shown in Fig 1. Table 3

Immune cell expression landscape

Upon comparing the immune cell expression levels before and after treatment in both groups, we found that in the group treated with local radiotherapy combined with immunosuppressive agents, the levels of PD-1 CD4+ T cells, PD-1 CD8+ T cells, and PD-L1 CD4+ monocytes were significantly reduced after treatment compared to before ($P < 0.05$). In contrast, in the group treated with only immunosuppressive agents, there were no significant changes in these immune cell expressions before and after treatment ($P > 0.05$). Furthermore, the levels of PD-1 CD4+ T cells, PD-1 CD8+ T cells, and PD-L1 CD4+ monocytes were significantly lower in the group treated with local radiotherapy combined with immunosuppressive agents compared to the group treated with only immunosuppressive agents ($P < 0.05$). These results are detailed in Table 4.

Table 2: Comparison of treatment effects among patients under different treatment regimens

Grouping	CR	PR	SD	PD
Combination (n=20)	2 (10.00)	11 (55.00)	5 (25.00)	2 (10.00)
Single (n=20)	1 (5.00)	5 (25.00)	9 (45.00)	5 (25.00)
Z	-2.096			
P	0.036			

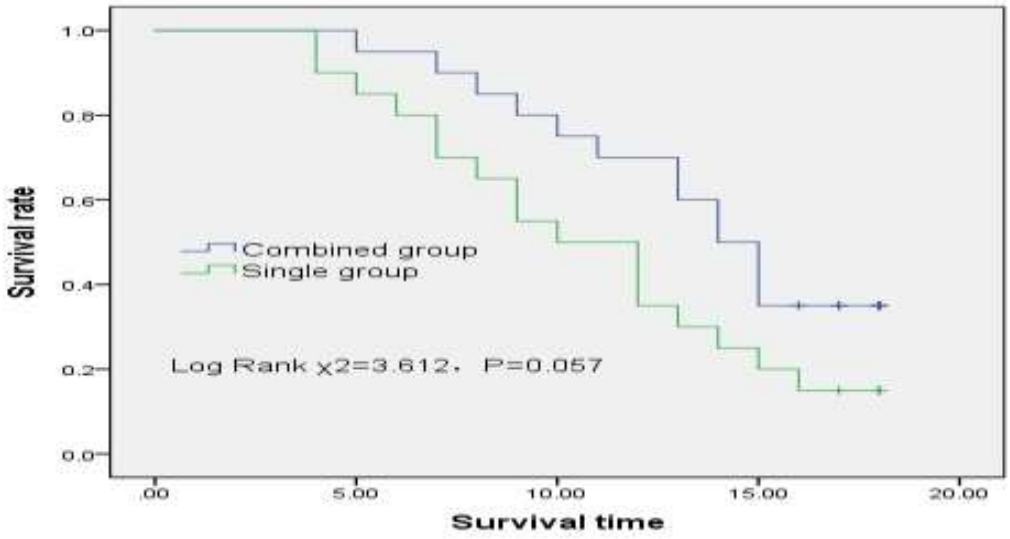

Figure 1: Curves for the analysis of progression free survival in the two groups

Table 3: Mean and median of survival analysis times

Grouping	Average value		Median	
	Estimate	95%CI	Estimate	95%CI
Combination (n=20)	13.750	11.965-15.535	14.000	12.247-15.753
Single (n=20)	10.850	8.859-12.841	10.000	6.713-13.287
Total	12.300	10.890-13.710	13.000	10.944-15.056

Table 4: Immune cell expression

Grouping	Time of treatment	PD-1 CD4+ T cells (%)	PD-1 CD8+ T cells (%)	PD-L1 CD4+ monocytes (%)
Combination (n=20)	Before	48.51±6.94	42.33±7.15	67.29±5.94
	After	33.26±5.18[ab]	31.45±6.21[ab]	49.62±6.85[ab]
Single (n=20)	Before	47.29±7.05	41.18±7.29	68.07±6.27
	After	45.33±6.83	39.84±7.05	66.52±7.04

Note: [a]$P < 0.05$, contrast to the combined before treatment, [b]$P < 0.05$, contrast to the single after treatment

Table 5: The expression of inflammatory factors

Grouping	Time of treatment	TNF-α (pg/mL)	IL-1 (pg/mL)	IL-8 (pg/mL)
Combination (n=20)	Before	30.16±4.91	15.16±2.37	23.08±3.15
	After	31.84±4.25[b]	16.09±1.58[b]	24.85±2.91[b]
Single (n=20)	Before	29.44±4.86	15.94±2.49	23.49±3.27
	After	27.46±5.03	14.16±1.97	22.16±3.04

Table 6: Incidence of toxicities in the two groups

Adverse effects	Combination (n=20)	Single (n=20)	t/χ^2	P
Overall incidence	8 (40.00)	13 (65.00)	2.506	0.113
Bone marrow depression	2 (10.00)	2 (10.00)		
Nausea and vomiting	5 (25.00)	7 (35.00)		
Rash	1 (5.00)	2 (10.00)		
Fever	2 (10.00)	3 (15.00)		
Liver and kidney Function damage	2 (10.00)	2 (10.00)		
Diarrhea	6 (30.00)	7 (35.00)		
Leukopenia	2 (10.00)	3 (15.00)		
Thrombocytopenia	1 (5.00)	2 (10.00)		

Inflammatory factor expression levels

When comparing the levels of inflammatory cytokines (TNF-α, IL-1, and IL-8) within each treatment group before and after therapy, we found that in the group treated with immunosuppressive agents alone, these cytokine levels decreased after treatment, but the changes were not statistically significant ($P > 0.05$). In contrast, in the group treated with local radiotherapy combined with immunosuppressive agents, the cytokine levels increased after treatment, yet again without significant differences ($P > 0.05$). However, when comparing the levels of these inflammatory cytokines between the two groups after treatment, we observed that the combined treatment group (local radiotherapy + immunosuppressive agents) had significantly higher levels of TNF-α, IL-1, and IL-8 compared to the group treated with immunosuppressive agents alone ($P < 0.05$). These results are detailed in Table 5.

Occurrence of adverse reactions

To explore the safety of local radiotherapy combined with immunosuppressive therapy, we compared the occurrence of toxic side effects after treatment between the two groups. As shown in Table 6, after comparison, we found that the occurrence of toxic side reactions in patients treated with local radiotherapy and immunosuppressive agents was not statistically different from those treated with immunosuppressive agents only ($P > 0.05$). Table 6.

Discussion

PD-1 is an inhibitory receptor expressed on the surface of T cells, which plays a crucial role in modulating the immune response by downregulating T cell activation and proliferation, thereby preventing excessive immune reactions and maintaining immune homeostasis[20]. PD-L1 and PD-L2 are ligands of PD-1, of which PD-L1 is highly expressed within a variety of tumor cells[21]. In vitro T cell responses and enhanced antitumor activity by inhibiting the interaction of PD-1 with PD-L1 have been indicate to be important in combating cancer, anti infection, anti autoimmune disease, and organ transplant survival[22]. Radiotherapy induces both anti-tumor immune responses and pro tumor immune responses in the tumor microenvironment. In terms of radiotherapy induced pro tumour immune modulation, radiotherapy can mediate radiotherapy resistance by activating the nuclear transcription factor kB pathway in a radiation associated antigen protein manner such as PD-1/PD-L1 and CD47[23,24].

The results of this study demonstrate that the combination of local radiotherapy and PD-1/PD-L1 inhibitors significantly enhances the clinical efficacy in patients with metastatic TNBC. This improvement can be attributed to several key mechanisms. Firstly, local radiotherapy induces DNA damage in tumor cells, leading to the release of tumor antigens. These antigens can be recognized by the immune system, thereby enhancing the activation of T cells.

The combination with PD-1/PD-L1 inhibitors further supports this process by blocking the inhibitory signals that tumor cells use to evade immune detection. This dual approach effectively enhances the immune system's ability to target and eliminate cancer cells[25]. Secondly, the significant reduction in PD-1 CD4+ T cells, PD-1 CD8+ T cells, and PD-L1 CD4+ monocytes in the combination group suggests that local radiotherapy may downregulate the expression of these immune checkpoints. This downregulation is crucial because it reduces the ability of tumor cells to suppress the immune response, thereby enhancing the effectiveness of the PD-1/PD-L1 inhibitors[26]. Thirdly, the absence of a significant increase in inflammatory cytokines (TNF-α, IL-1, and IL-8) in the combination group indicates that the treatment did not induce excessive inflammation. This is important because uncontrolled inflammation can lead to tissue damage and other adverse effects. The controlled inflammatory environment suggests that the combination therapy is not only effective but also safe, as it does not exacerbate the inflammatory burden on the patient[27].

Furthermore, the combination therapy's ability to improve clinical outcomes without increasing adverse effects highlights a potential synergistic effect between local radiotherapy and PD-1/PD-L1 inhibitors. This synergy suggests that the two treatments work together to enhance antitumor activity while maintaining a favorable safety profile. This finding is supported by previous studies that have shown similar synergistic effects in other cancer types, such as non-small cell lung cancer and melanoma[28]. Lastly, these results suggest that the combination of local radiotherapy and PD-1/PD-L1 inhibitors could be a promising treatment strategy for metastatic TNBC. Given the high aggressiveness and poor prognosis of TNBC, this combined approach offers a potential avenue for improving patient outcomes. Future studies should explore the optimal timing and dosing of radiotherapy in combination with immunotherapy to maximize therapeutic benefits while minimizing adverse effects.

Study strengths and limitations

In summary, the findings of this study provide valuable insights into the mechanisms underlying the enhanced efficacy of the combination therapy. The results highlight the potential for local radiotherapy to augment the effects of PD-1/PD-L1 inhibitors, offering a new direction for the treatment of metastatic TNBC.

However, there are still two limitations to this study. Firstly, this study is a retrospective analysis and may not be able to fully control for all potential confounding factors and time series biases, which may lead to biases in the evaluation of treatment efficacy and prognostic factors. Meanwhile, the sample size of this study is relatively small, and small sample studies may be more susceptible to the influence of random errors, thereby affecting the reliability of the research results

Conclusion

Radiotherapy, as an important treatment for TNBC, is able to improve the short - and long-term outcomes of patients. The advent of immunotherapy has changed the treatment patterns and strategies for patients with metastatic TNBC. Immunosuppressive agents prolong PFS in patients with TNBC. The results of this study demonstrate that PD-1/PD-L1 inhibitor have synergistic effects with local radiotherapy in the treatment of patients with metastatic TNBC. This brings new hope for patients with metastatic TNBC. However, the current clinical treatment of PD-1/PD-L1 inhibitor combined with local radiotherapy has not completed its maturity, and the choice of timing for the combination remains controversial.

Competing interests

The authors declare no competing interests

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Author contributions

Qiong Lu were responsible for conception and design. Fei Wang and Qiong Lu was responsible for manuscript writing. Lei Yu, Ping Lv, were responsible for collection and assembly of data. Fei

Wang, Lei Yu and Ping Lv were responsible for data analysis and interpretation. All authors were responsible for manuscript writing. All authors were responsible for the final approval of the manuscript.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, and Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, 2021;71(3):209-249.
2. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, and Chen Y. Recent advances in therapeutic strategies for triple-negative breast cancer. *J Hematol Oncol*, 2022;15(1):121.
3. Sun X, Tang H, Chen Y, Chen Z, Hu Z, Cui Z, Tao Y, Yuan J, Fu Y, Zhuang Z, He Q, Li Q, Xu X, Wan X, Jiang Y, and Mao Z. Loss of the receptors ER, PR and HER2 promotes USP15-dependent stabilization of PARP1 in triple-negative breast cancer. *Nat Cancer*, 2023;4(5):716-733.
4. Zhu H, Yang K, Yao H, Chen X, Yan S, He Y, Cao Y, Luo J, and Wang D. Multifunctional Nanoplatform-Mediated Chemo-Photothermal Therapy Combines Immunogenic Cell Death with Checkpoint Blockade to Combat Triple-Negative Breast Cancer and Distant Metastasis. *Int J Nanomedicine*, 2023;18:3109-3124.
5. Gao C, Li H, Liu C, Xu X, Zhuang J, Zhou C, Liu L, Feng F, and Sun C. Tumor Mutation Burden and Immune Invasion Characteristics in Triple Negative Breast Cancer: Genome High-Throughput Data Analysis. *Front Immunol*, 2021;12:650491.
6. Chow R, Hasan S, Choi JI, Fox J, Chhabra AM, Marshall DC, Bakst RL, and Simone CB 2nd. Effect of treatment interruptions on overall survival in patients with triple-negative breast cancer. *J Natl Cancer Inst*, 2023;115(9):1029-1035.
7. Marra A, and Curigliano G. Adjuvant and Neoadjuvant Treatment of Triple-Negative Breast Cancer With Chemotherapy. *Cancer J*, 2021;27(1):41-49.
8. Derakhshan F, and Reis-Filho JS. Pathogenesis of Triple-Negative Breast Cancer. *Annu Rev Pathol*, 2022;17:181-204.
9. Karim AM, Eun Kwon J, Ali T, Jang J, Ullah I, Lee YG, Park DW, Park J, Jeang JW, and Kang SC. Triple-negative breast cancer: epidemiology, molecular mechanisms, and modern vaccine-based treatment strategies. *Biochem Pharmacol*, 2023;212:115545.
10. Bustos MA, Yokoe T, Shoji Y, Kobayashi Y, Mizuno S, Murakami T, Zhang X, Sekhar SC, Kim S, Ryu S, Knarr M, Vasilev SA, DiFeo A, Drapkin R, and Hoon DSB. MiR-181a targets STING to drive PARP inhibitor resistance in BRCA-mutated triple-negative breast cancer and ovarian cancer. *Cell Biosci*, 2023;13(1):200.
11. Zhu Y, Liu Z, Gui L, Yun W, Mao C, Deng R, Yao Y, Yu Q, Feng J, Ma H, and Bao W. Inhibition of CXorf56 promotes PARP inhibitor-induced cytotoxicity in triple-negative breast cancer. *NPJ Breast Cancer*, 2023;9(1):34.
12. Teo ZL, O'Connor MJ, Versaci S, Clarke KA, Brown ER, Percy LW, Kuykhoven K, Mintoff CP, Savas P, Virassamy B, Luen SJ, Byrne A, Sant S, Lindeman GJ, Darcy PK, and Loi S. Combined PARP and WEE1 inhibition triggers anti-tumor immune response in BRCA1/2 wildtype triple-negative breast cancer. *NPJ Breast Cancer*, 2023;9(1):68.
13. Luo K, Yang L, Yan C, Zhao Y, Li Q, Liu X, Xie L, Sun Q, and Li X. A Dual-Targeting Liposome Enhances Triple-Negative Breast Cancer Chemoimmunotherapy through Inducing Immunogenic Cell Death and Inhibiting STAT3 Activation. *Small*, 2023;19(40):e2302834.
14. Kumar S, Chatterjee M, Ghosh P, Ganguly KK, Basu M, and Ghosh MK. Targeting PD-1/PD-L1 in cancer immunotherapy: An effective strategy for treatment of triple-negative breast cancer (TNBC) patients. *Genes Dis*, 2022;10(4):1318-1350.
15. Yu Y, Jin X, Zhu X, Xu Y, Si W, and Zhao J. PD-1/PD-L1 immune checkpoint inhibitors in metastatic triple-negative breast cancer: a systematic review and meta-analysis. *Front Immunol*, 2023;14:1206689.
16. Liu C, Qian X, Yu C, Xia X, Li J, Li Y, Xie Y, Gao G, Song Y, Zhang M, Xue H, Wang X, Sun H, Liu J, Deng W, and Guo X. Inhibition of ATM promotes PD-L1 expression by activating JNK/c-Jun/TNF-α signaling axis in triple-negative breast cancer. *Cancer Lett*, 2024;586:216642.
17. Wang L, Sfakianos JP, Beaumont KG, Akturk G, Horowitz A, Sebra RP, Farkas AM, Gnjatic S, Hake A, Izadmehr S, Wiklund P, Oh WK, Szabo PM, Wind-Rotolo M, Unsal-Kacmaz K, Yao X, Schadt E, Sharma P, Bhardwaj N, and Zhu J. Myeloid Cell-associated Resistance to PD-1/PD-L1 Blockade in Urothelial Cancer Revealed Through Bulk and Single-cell RNA Sequencing. *Clin Cancer Res*, 2021;27(15):4287-4300.
18. Zeng L, Ding S, Cao Y, Li C, Zhao B, Ma Z, Zhou J, Hu Y, Zhang X, Yang Y, Duan G, Bian X, and Tian G. A MOF-Based Potent Ferroptosis Inducer for Enhanced Radiotherapy of Triple Negative Breast Cancer. *ACS Nano*, 2023;17(14):13195-13210.
19. Banerji U, van Herpen CML, Saura C, Thistlethwaite F, Lord S, Moreno V, Macpherson IR, Boni V, Rolfo C, de Vries EGE, Rottey S, Geenen J, Eskens F, Gil-Martin M, Mommers EC, Koper NP, and Aftimos P. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncol*, 2019;20(8):1124-1135.
20. Huang R, Wang Z, Hong J, Wu J, Huang O, He J, Chen W, Li Y, Chen X, and Shen K. Targeting cancer-associated adipocyte-derived CXCL8 inhibits triple-negative breast cancer progression and enhances the

efficacy of anti-PD-1 immunotherapy. *Cell Death Dis*, 2023;14(10):703.
21. Chen M, Bie L, and Ying J. Cancer cell-intrinsic PD-1: Its role in malignant progression and immunotherapy. *Biomed Pharmacother*, 2023;167:115514.
22. Ali LR, Lenehan PJ, Cardot-Ruffino V, Dias Costa A, Katz MHG, Bauer TW, Nowak JA, Wolpin BM, Abrams TA, Patel A, Clancy TE, Wang J, Mancias JD, Reilley MJ, Stucky CH, Bekaii-Saab TS, Elias R, Merchant N, Slingluff CL Jr, Rahma OE, and Dougan SK. PD-1 Blockade Induces Reactivation of Nonproductive T-Cell Responses Characterized by NF-κB Signaling in Patients with Pancreatic Cancer. *Clin Cancer Res*, 2024;30(3):542-553.
23. Dai D, Zhou H, Yin L, Ye F, Yuan X, You T, Zhao X, Long W, Wang D, He X, Feng J, and Chen D. PELI1 promotes radiotherapy sensitivity by inhibiting noncanonical NF-κB in esophageal squamous cancer. *Mol Oncol*, 2022;16(6):1384-1401.
24. Liu Z, Zhao L, Liu H, Dong N, Zhou N, Zhang Y, Yin T, He H, Gou J, Tang X, Yang L, and Gao S. Norcantharidin liposome emulsion hybrid delivery system enhances PD-1/PD-L1 immunotherapy by agonizing the non-canonical NF-κB pathway. *Int J Pharm*, 2022;628:122361.
25. Stenmark Tullberg A, Sjöström M, Tran L, Niméus E Killander F, Kovács A, Lundstedt D, Holmberg E and Karlsson P. Combining histological grade, TILs, and the PD-1/PD-L1 pathway to identify immunoenic tumors and de-escalate radiotherapy in early breast cancer: a secondary analysis of a randomized clinical trial[J]. *J Immunother Cancer*, 2023,11(5):e006618.
26. Yang X, en H, Li Z, Peng X and Fu J. Combinations of radotherapy with immunotherapy in nasopharyngeal carcinoma[J]. *Int Immunopharmacol*, 2023,125(Pt A):111094.
27. Zhu Y, en J, Li Q, Chen B, Zhao L, Liu S, Yang Y, Wang S Lv Y,Li J, Zhang L, Hu Y, Liu M and Xi M. Toripalimab combined with definitive chemoradiotherapy in locally advanced oesophageal squamous cell carcinoma (EC-CRT-001): a single-arm, phase 2 trial[J]. *Lancet Oncol*, 2023,24(4):371-382.
28. Oyoshi , Du J, Sakai SA, Yamashita R, Okumura M, Motegi A,Hojo H, Nakamura M, Hirata H, Sunakawa H, Kotani D, Yano T, Kojima T, Nakamura Y, Kojima M, Suzuki A, Zenkoh J, Tsuchihara K, Akimoto T, Shibata A, Suzuki Y and Kageyama SI. Comprehensive single-cell analysis demonstrates radiotherapy-induced infiltration of macrophages expressing immunosuppressive genes into tumor in esophageal squamous cell carcinoma[J]. *Sci Adv*, 2023,9(50):eadh9069