RESEARCH ARTICLE

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Radiotherapy Improves Antitumor Immunity by Inducing Immunogenic Cell Death in Breast Cancer Patients

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Abstract

Background: Radiotherapy, a significant cancer treatment, stimulates localized tumor cell death and causes immune modulation. Increasing data has indicated that radiation strengthens tumor-related immunity. **Aim of the Study:** The aim of this work was to investigate the effect of radiotherapy on soluble forms of the primary immune checkpoints *CTLA-4*, *PD-1* and *PD-L1* in patients with breast cancer. **Patients and Methods:** The study was conducted on70 subjects were separated into 2 groups: Group I included 40 patients with breast cancer who received fractionated radiation. Group II: Includes 30 healthy females free from any disease as control group, age and gender matched the patients' group. ELISA was used to assay *CTLA-4*, *PD-1* and *PD-L1*. **Results:** The current study revealed that soluble *PD-1* and *PD-L1* are significantly elevated in sera of patients with breast cancer pre radiotherapy treatment and significantly higher than in control group. Exposure to radiotherapy significantly drops *PD-1* levels and brings its levels in patients closer to those of healthy individuals but not affects *PD-L1* levels. Soluble *CTLA-4* is insignificantly elevated in sera of breast cancer patients pre radiotherapy treatment and significantly decreased post radiotherapy. **Conclusion:** Radiotherapy can enhance immune recognition of the tumor cells by dropping the primary immune checkpoints *PD-1* and *CTLA-4* in breast cancer patients and improve response to immune checkpoints inhibition.

Keywords: Radiotherapy- Antitumor- Immunogenic- Breast- Cancer

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Introduction

Breast cancer (BC) is the most diagnosed malignant tumor and the main reason of cancer related death among women globally [1]. It has become the cancer with the greatest incidence [2]. Despite its high prevalence, the rate of mortality from BC has fallen dramatically, by 43% between 1989 and 2020, with the decline being more pronounced in larger locations [3]. Postoperative radiation following breast-conserving surgery has become an optimum option for treat patients with BC [4].

Radiation treatment causes immunogenic cell death (ICD) is becoming recognized as a significant advancement in breast cancer treatment. Historically, radiation has been used to eliminate tumor cells by causing DNA damage. However, recent research has shown that radiotherapy can boost the immune system by converting tumor cells into an endogenous cancer vaccine. This process, known as immunogenic cell death (ICD), enhances radiotherapy's therapeutic efficacy by inducing a strong immune response against the tumor. ICD is defined by a series of biological processes, including calreticulin surface exposure, ATP secretion, and HMGB1 protein release. These signals are critical in attracting and activating dendritic cells, which process and deliver antigens of tumor to T cells, resulting in a strong immune response against the tumor [5].

Recent study indicates the potential benefits of combining radiation with immunotherapy to boost the immune response. This combined technique appears to be effective in minimizing metastasis and recurrence, perhaps giving long-term protection against BC. Ongoing clinical trials attempt to improve therapy regimens and get a better knowledge of the mechanisms behind ICD [6]. According to a previous study, radiotherapy induced ICD increases the release of tumor-derived CXCL16, which attracts effector T cells and strengthens the anti-tumor immune response [7]. Furthermore, a study in Cancer Immunology Research found that combining radiotherapy with immune checkpoint inhibitors could be especially effective in treating severe BC subtypes, including triple-negative BC [8].

Cancer immunotherapy is a relatively new field that

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seeks to find effective treatments that boost the power and specificity of the anti-tumor immune response [9]. Nobel Prize in 2018 was awarded by Tasuku Honjo and James P. Allison for developing a treatment of cancer that prevents negative immune modulation. Their study of the immune checkpoints programmed cell death protein 1 (*PD-1*) and cytotoxic T-lymphocyte-associated protein 4 *CTLA-4*revealed that they played a "brake" role in immune function and showed that inhibiting immune checkpoints could reactivate T cells and reduce cancer cells more effectively [10]. *CTLA-4* was initially recognized as an inhibitory signal involved in the termination of immunological responses [11].

Growing evidence reports that prohibiting *PD-1* is critical for activating a strong immunological reaction to cancer cells [12]. Suppressing the *PD-1* signaling pathway has demonstrated clinical success in patients with diverse hematological cancers and solid tumors are mostly determined by T lymphocytes' ability to efficiently infiltrate the tumor [13]. Furthermore, targeting programmed cell death ligand 1 (*PD-L1*) has been linked to strong therapeutic responses in a diverse group of cancer patients [14].

The aim of this work was to investigate the effect of radiotherapy on soluble forms of the primary immune checkpoints*CTLA-4*, *PD-1* and *PD-L1* in BC patients.

Materials and Methods

Subjects

This study included 70 subjects divided into the following groups:

Group I: 40 BC patients treated with fractionated radiotherapy.

Group II: Includes 30 healthy females free from any disease as control group, age and sex matched with patients group.

All participants are asked to volunteer for the study freely as the approval of the Ethics committee of Medical Research Institute obtained (IRB00010526), informed written consent are gathered before their inclusion in the study protocol.

Patients selected from those admitted to Bahia foundation, Cairo, Egypt.

Inclusion Criteria

• Patients aged 18 years or older, confirmed diagnosis of BC, candidates for radiotherapy as part of their treatment plan and consent to participate in the study and provide biological samples for analysis.

Exclusion Criteria

• Patients with a history of other malignancies within the past five years and patients who have received prior immunotherapy for BC.

Methods

Blood Samples Collection

- Two venous blood samples (5ml each) are collected from all BC patients: one blood sample gathered before radiotherapy and the other after completing radiotherapy. One venous blood sample (5ml) withdrawn from healthy controls. Blood samples are processed to isolate serum for analysis.

- Enzyme-linked immunosorbent assay (ELISA) is used to measure the soluble forms of *CTLA-4*, *PD-L1*, and *PD-1* in the serum according to the manufacture instructions (Cloud Clone, USA)

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp).The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level. Student t-test used for normally distributed quantitative variables, to compare between two studied groups. Paired t-test used for normally distributed quantitative variables, to compare between two periods. Mann Whitney test used for abnormally distributed quantitative variables, to compare between two studied groups and finally Wilcoxon signed ranks test used for abnormally distributed quantitative variables, to compare between two periods.

Results

Serum levels of PD-1 in patients with BC pre and post radiotherapy

Before radiotherapy, patients with BC had a higher *PD-1* levels with a mean of 9.10 ± 1.16 (ng/ml). After radiotherapy, there was a significant decrease in *PD-1* levels, with the mean dropping to 6.56 ± 1.91 (ng/ml) (p2< 0.001). This decrease suggests that radiotherapy may help lower *PD-1* levels in BC patients, possibly influencing immune checkpoint activity. The control group, consisting of healthy individuals, had lower *PD-1*levels, with a mean of 5.50 ± 3.01 (ng/ml).When comparing patients' *PD-1* levels before treatment to those of the control group, there was a significant difference (p1< 0.001), indicating that BC patients have elevated *PD-1* levels prior to treatment. However, after radiotherapy, there was insignificant difference in *PD-1* levels between the treated patients and the control group (p1= 0.387), suggesting that radiotherapy brings levels of *PD-1* in patients closer to those of healthy individuals (Table 1).

Table 1. Statistical Analysis of Serum PD-1 Levels in BC Patients.

	BC Patients (n = 40)		Controls
	Pre	Post	(n = 30)
PD - 1 (ng/ml)			
Min. – Max.	7.50 – 11.50	2.50 – 9.50	0.50 – 8.50
Mean ± SD.	9.10 ± 1.16	6.56 ± 1.91	5.50 ± 3.01
p_1	<0.001*	0.387	
p_2	<0.001*		

SD, Standard deviation; p_1, p value for comparing between pre and post radiotherapy with Controls; p_2, p value for comparing between pre and post radiotherapy; *, Statistically significant at p ≤ 0.05

Table 2. Statistical Analysis of Serum *PD-L1* Levels in BC Patients

	BC Patients (n = 40)		Controls
	Pre	Post	(n = 30)
PD-L1 (pg/ml)			
Min. – Max.	170.0 – 340.0	160.0 – 315.0	90.0 – 290.0
Mean ± SD.	257.5 ± 46.84	257.8 ± 47.11	203.3 ± 71.48
p_1	0.001*	0.001*	
p_2	0.892		

SD, Standard deviation; p_1, p value for comparing between pre and post radiotherapy with Controls; p_2, p value for comparing between pre and post radiotherapy; *, Statistically significant at $p \leq 0.05$

Table 3. Statistical Analysis of Serum *CTLA-4* Levels in BC Patients

	BC Patients (n = 40)		Controls
	Pre	Post	(n = 30)
CTLA- 4 (ng/ml)			
Min. – Max.	32.50 – 77.50	20.0 – 62.50	45.0 – 67.50
Mean ± SD.	59.19 ± 10.82	46.19 ± 10.62	56.67 ± 8.13
p_1	0.289	<0.001*	
p_2	<0.001*		

SD, Standard deviation; p_1, p value for comparing between pre and post radiotherapy with Controls; p_2, p value for comparing between pre and post radiotherapy; *, Statistically significant at $p \leq 0.05$

Serum levels of PD-L1 in patients with BC pre and post radiotherapy

Before radiotherapy, the mean serum levels of *PD-L1* in patients with BC were 257.5 ± 46.84 (pg/ml).After radiotherapy, the levels of *PD-L1* in patients remained almost unchanged, with a mean of 257.8 ± 47.11 (pg/ml). The lack of significant change between pre- and post-treatment PD-L levels (p2= 0.892) suggests that radiotherapy did not have a substantial effect on *PD-L1* levels in these patients. The control group had significantly lower mean *PD-L1* levels of 203.3 ± 71.48 (pg/ml).There was a significant difference between both pre-treatment and post-treatment *PD-L1* levels in patients compared to the healthy controls (p1= 0.001)for both comparisons. This indicates that BC patients, regardless of radiotherapy, exhibit persistently higher *PD-L1* levels than control group, suggesting an inherent difference in *PD-L1* expression associated with BC (Table 2).

Serum levels of CTLA-4 in patients with BC pre and post radiotherapy

Before radiotherapy, BC patients had mean *CTLA-4* levels of 59.19 ± 10.82(ng/ml).After radiotherapy, there was a significant decrease in *CTLA-4* levels, with the mean dropping to 46.19 ± 10.62 (ng/ml). The change was statistically significant (p2< 0.001), suggesting that radiotherapy reduces *CTLA-4* levels in BC patients. This reduction may indicate an alteration in immune checkpoint pathways following treatment. The control group had mean *CTLA-4* levels of 56.67 ± 8.13 (ng/ml). When comparing pre-treatment levels in patients to those of the healthy controls, there was insignificant difference (p = 0.289). However, after radiotherapy, the levels of *CTLA-4* in patients with BC were significantly lower than in the control group (p1< 0.001). This indicates that radiotherapy reduced *CTLA-4* levels in BC patients to below those seen in healthy individuals, potentially indicating a shift in immune modulation post-treatment (Table 3).

Discussion

BC is the most frequent malignant tumor in women and the primary cause of cancer-related mortality in females, with an increasing prevalence [15]. BC is highly mixed, with significant variations in biological behavior, cell types, and genetic profiles, which contribute to disparities in patient responses to conventional treatments such as hormone therapy, chemotherapy, radiotherapy and targeted therapy [16]. Ionizing radiation treatment is an important method for local tumor management, as well as in the therapy of BC and other malignancies. Previous research has suggested that, local radiation and immunotherapy may work together to treat cancer [17]. According to emerging evidence, ionizing radiation causes immunized tumor cell killing and modifies the microenvironment of tumor, encouraging the recruitment of anti-tumor T lymphocytes. This lends support to the concept that radiation can improve both the priming and effectors phases of the anti-tumor immune response [18,19]. Cancer cells avoid immune surveillance by expressing immunological checkpoints, which block the capacity of immune system to attack and eradicate malignancies [20, 21]. Immune checkpoint inhibitors (ICIs) target these inhibitory substances, thus releasing the immune system's "brakes" and increasing anti-tumor immunity to eliminate cancer cells. Currently, clinically licensed ICIs target the *PD-1*, *PD-L1*, and *CTLA-4* pathways [22]. This study will look at how radiation affects the soluble versions of ICIs such as *PD-L1*, *PD-1*, and *CTLA-4*in patients with BC.

The current study revealed that soluble *PD-1* and *PD-L1* are significantly elevated in sera of BC patients pre radiotherapy and significantly higher than in healthy controls. This indicates that breast cancer patients, regardless of radiotherapy, exhibit persistently higher *PD-1* and *PD-L1* levels than control group, suggesting an inherent difference in their expression associated with BC. Exposure to radiotherapy significantly drops *PD-1* levels and brings its levels in patients closer to those of healthy individuals but not affects *PD-L1* levels. This decrease in *PD-1* suggests that radiotherapy may help lower *PD-1* levels in BC patients, possibly influencing immune checkpoint activity. Our findings agree with previous reports [23, 24].

The type 1 trans membrane protein *PD-1* is present on bone marrow cells, T cells, B cells, and NK cells [25]. *PD-L1*, which is present in various malignancies, including melanoma, colon adeno carcinoma, and BC, demonstrated to be is over expresses in triple-negative BC in contrast to luminal and HER2+ subtypes [26]. *PD-L1*, which interacts with the *PD-1* receptor on T cells is found on a wide range of immune cells, including T cells, B cells, antigen-presenting cells [27, 28]. The interaction between *PD-L1* and *PD-1* causes programmed

T cell death, allowing malignant cells to avoid immune identification and eradication [29]. Immune checkpoint drugs that target *PD-1* or *PD-L1* have showed promise in treating metastatic BC. Pembrolizumab, an anti-*PD-1* antibody, was recently shown to increase overall survival (OS) and progression-free survival when coupled with chemotherapy as first-line therapy for *PD-L1*-expressing metastatic triple-negative BC [30].

A 2018 study by Ruyi H demonstrated that *PD-1* expression was substantially related with recurrence of BC. Analysis of Kaplan-Meier curve demonstrated that, the *PD-1* low-expression group had a considerably longer mean recurrence-free survival (68 months) than the *PD-1* high-expression group (56 months). Over expression of *PD-1* may reduce the host immune response and facilitate immune evasion by malignant cells, resulting in a greater recurrence rate. Thus, *PD-1* could be used as a therapeutic target to prevent recurrence [31].

In preclinical bladder cancer models, anti-*PD-1* or anti-*PD-L1* antibodies were shown to improve radiotherapy-induced anti-tumor immunity [32]. Another study conducted on mice model found that, in a non-small cell lung cancer a synergistic anti-tumor immunological response when radiation was paired with anti-*PD-L1* antibody treatment [33]. Recent research has demonstrated that, radiation not only reduces immunity but also increases anti-tumor immunity and generates bystander effects. *PD-L1* is frequently expressed on tumor cell membranes and binds to *PD-1* on immune cells (CD8+ T cells), playing vital role in tumor immune evasion. Furthermore, several studies have shown that, Radiotherapy increases *PD-L1* levels. Du et al. found the upregulation of *PD-L1* caused by the activation of the cGAS-STING pathway after treatment with radiation [34]. A number of studies have evaluated combined Radiotherapy, *PD-1*/*PD-L1*, and *CTLA-4* inhibition, while other studies have investigated Radiotherapy and immune checkpoint therapy in relation to other combinations of vaccine therapies, chemotherapy, or targeted therapies for variety of malignant tumors [35, 36].

CTLA-4, at times referred to as CD152, is a trans membrane protein that is mostly expressed on regulatory T cells (Tregs), CD4+ T cells, and CD8+ T cells. It is an alternate target for immunological checkpoint suppression [37]. *CTLA-4* is present in T cells, non-lymphoid cells, B cells, dendritic cells, stromal cells, and malignancies [38, 39]. It has been shown that *CTLA-4* is expressed on the surface and in the cytoplasm of BC cells in addition to being present on Tregs and T cells [40]. Our study showed that soluble *CTLA-4* is insignificantly elevated in sera of BC patients pre radiotherapy treatment and significantly decreased post radiotherapy. Suggesting that radiotherapy reduces *CTLA-4* levels in patients with BC and this reduction may indicate an alteration in immune checkpoint pathways following treatment.

Previous reports have demonstrated that, *CTLA-4* is primarily expressed on T cells, where it plays a critical role in maintaining immune tolerance by inhibiting proliferation and activation of T cells, thereby protecting the body from autoimmune reactions [41]. In one study involving 1,087patients with BC, the relationship between activation of Tcellsand*CTLA-4* expression was evaluated, and the results demonstrated that, elevated *CTLA-4* expression was related with lower activation of T cell, which inversely correlated with survival rates [42]. However, the clinical importance of *CTLA-4* in predicting the prognosis of different malignancies remains uncertain. *CTLA-4* over expression in tumors has been linked to reduced OS and may be used as an independent prognostic indicator in esophageal carcinoma, as well as pharyngeal and laryngeal squamous cell carcinoma [43, 44].Conversely, a study of *CTLA-4* expression found that, *CTLA-4* over expression was related to improve OS in patients with radically resected stage I-III non-small cell lung cancer [45]. According to previous research, patients with gastric cancer with low or high expression of *CTLA-4* had an insignificant difference in OS [46].

Previous reports revealed that, tumor cells expressed *CTLA-4*, with *CTLA-4*+ dots in the cytoplasm of tumor cell mimicking transport vesicles. BC cells have also been reported to contain *CTLA-4*, largely localized in the cytoplasm of the cancer cells [47, 48]. Lu et al found that *CTLA-4* expression could act as a predictive predictor in breast carcinomas. According to this study, a better prognosis was linked to a higher number of infiltrating *CTLA-4*+ lymphocytes, whereas a worse prognosis has been linked to a higher expression of *CTLA-4*. However, a high density of *CTLA-4*+ lymphocytes was associated with a better prognosis only when tumor *CTLA-4* expression was modest [49].

Cancer cells express both *PD-L1* and *CTLA-4*, allowing malignancies to avoid immune detection and killing. Blocking *PD-1* and *CTLA-4* using particular antibodies can trigger the anti-tumor immune response, resulting in tumor regression. The combination of radiation and *CTLA-4* inhibitors has been investigated in several clinical trials across various tumor types, with intriguing results, while overall evidence of consistent benefits is lacking [44, 50-52]. While immunotherapies have been successful in treating a variety of tumor types, they frequently fail to control cancers in the majority of patients [53]. Focal radiation therapy can boost the immune system's detection of malignancies [54] and has been demonstrated to improve responses to *CTLA-4* suppression in animal models [55, 56] and certain human patients [57, 58]. Previous research has shown that combining local radiation to the primary tumor with *CTLA-4* inhibition might result in considerable anti-tumor immunity, especially in less immunogenic metastatic mammary carcinoma models like 4T1 [59, 60].

In conclusion, radiotherapy can enhance immune recognition of the tumor cells by dropping the primary immune checkpoints *CTLA-4*and *PD-1* in BC patients and improve response to immune checkpoints inhibition.

Author Contribution Statement

Ibrahim G. Abdelrahman conducted the statistical analysis, wrote the results section, and contributed to writing the manuscript. Osama O. Almesrati Contributed to the conceptualization, writing of the manuscript, and reviewing of relevant literature, and critically reviewed

the manuscript to ensure accuracy and clarity. Sanaa A. El-Benhawy research proposal idea, doing practical part of the study and contributor in writing the manuscript. Moustafa A. Soula Contributed to Searching the literature and Manuscript drafting. Amira Atef Mahmoud: interpreting the results and writing the manuscript

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All authors have contributed significantly to this work.

Approval by Scientific Body

This study has not been reviewed or approved by any specific scientific body. It does not constitute part of a student thesis or academic dissertation.

Availability of data

The data sets are not publicly available but are available from the corresponding author on reasonable request.

Ethics Committee Approval

This study was approved by ethical and scientific committees of Medical Research Institute, Alexandria University, Alexandria, Egypt. (Approval number: IRB00010526).

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145-64: https://doi.org/10.3322/caac.21601.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021;71(1):7-33. https://doi.org/10.3322/caac.21654.
3. Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, et al. Breast cancer statistics, 2022. CA Cancer J Clin. 2022;72(6):524–41. https://doi.org/10.3322/caac.21754.
4. Speers C, Pierce LJ. Postoperative radiotherapy after breast-conserving surgery for early-stage breast cancer: a review. JAMA Oncol. 2016;2(8):1075–82. https://doi.org/10.1001/jamaoncol.2015.5805.
5. Zhu M, Yang M, Zhang J, Yin Y, Fan X, Zhang Y, et al. Immunogenic cell death induction by ionizing radiation. Front Immunol. 2021:12;705361. https://doi.org/10.3389/fimmu.2021.705361.
6. Guo W, Jia L, Xie L, Kiang JG, Wang Y, Sun F, et al. Turning anecdotal irradiation-induced anticancer immune responses into reproducible in situ cancer vaccines via disulfiram/copper-mediated enhanced immunogenic cell death of breast cancer cells. Cell Death Dis. 2024;15(4):298 https://doi.org/10.1038/s41419-024-06644-3.
7. Zhu S, Wang Y, Tang J, Cao M. Radiotherapy induced immunogenic cell death by remodeling tumor immune microenvironment. Front Immunol. 2022;13:1074477. https://doi.org/10.3389/fimmu.2022.1074477.
8. David S, Tan J, Siva S, Karroum L, Savas P, Loi S. Combining radiotherapy and immunotherapy in metastatic breast cancer: current status and future directions. Biomedicines. 2022;10(4):821. https://doi.org/10.3390/biomedicines10040821.
9. Salmaninejad A, Valilou SF, Shabgah AG, Aslani S, Alimardani M, Pasdar A, et al. *PD-1*/*PD-L1* pathway: basic biology and role in cancer immunotherapy. J Cell Physiol. 2019;234(10):16824-37. https://doi.org/10.1002/jcp.28358.
10. Ljunggren HG, Jonsson R, Höglund P. Seminal immunologic discoveries with direct clinical implications: the 2018 Nobel Prize in Physiology or Medicine honours discoveries in cancer immunotherapy. Scand J Immunol. 2018;88(6):e12731. https://doi.org/10.1111/sji.12731.
11. Hosseini A, Gharibi T, Marofi F, Babaloo Z, Baradaran B. *CTLA-4*: From mechanism to autoimmune therapy. Int Immunopharmacol. 2020;80:106221. https://doi.org/10.1016/j.intimp.2020.106221.
12. Messenheimer DJ, Jensen SM, Afentoulis ME, Wegmann KW, Feng Z, Friedman DJ, et al. Timing of *PD-1* blockade is critical to effective combination immunotherapy with anti-OX40. Clin Cancer Res. 2017;23(20):6165-77. https://doi.org/10.1158/1078-0432.CCR-16-2677.
13. Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the *PD-1* signaling pathway. J Biomed Sci. 2017;24:26. https://doi.org/10.1186/s12929-017-0329-9.
14. Sacher AG, Gandhi L. Biomarkers for the clinical use of *PD-1*/*PD-L1* inhibitors in non–small-cell lung cancer: a review. JAMA Oncol. 2016;2(9):1217-22. https://doi.org/10.1001/jamaoncol.2016.0639.
15. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health. 2019;9(4):217-22. https://doi.org/10.2991/jegh.k.191008.001.
16. Rani A, Stebbing J, Giamas G, Murphy J. Endocrine resistance in hormone receptor positive breast cancer–from mechanism to therapy. Front Endocrinol. 2019;10:245. https://doi.org/10.3389/fendo.2019.00245.
17. Demaria S, Bhardwaj N, McBride WH, Formenti SC. Combining radiotherapy and immunotherapy: a revived partnership. Int J Radiat Oncol Biol Phys. 2005;63(3):655-66 https://doi.org/10.1016/j.ijrobp.2005.06.032.
18. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4–dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007;13(9):1050–9 . https://doi.org/10.1038/nm1622.
19. Lugade AA, Moran JP, Gerber SA, Rose RC, Frelinger JG, Lord EM. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. J Immunol. 2005;174(12):7516-23. https://doi.org/10.4049/jimmunol.174.12.7516.
20. Demaria S, Formenti SC. Sensors of ionizing radiation effects on the immunological microenvironment of cancer. Int J Radiat Biol. 2007;83(11-12):819-25. https://doi.org/10.1080/09553000701481816.
21. Matsumura S, Wang B, Kawashima N, Braunstein S, Badura M, Cameron TO, et al. Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol. 2008;181(5):3099-107. https://doi.org/10.4049/jimmunol.181.5.3099.
22. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nat Commun. 2020;11(1):3801. https://doi.org/10.1038/s41467-020-17670-y.
23. Al-Muskakeh MK, Yaseen AN, Aldabagh MA. Assessment of Soluble *PD-1* and *PD-L1* in Iraqi women patients with breast cancer with toxoplasmosis. Indian J Forensic Med Toxicol. 2022;16(1). https://doi.org/10.37506/ijfmt.v16i1.17692.
24. Krutzek F, Kopka K, Stadlbauer S. Development of

Radiotracers for Imaging of the *PD-1*/*PD-L1* Axis. Pharmaceuticals. 2022;15(6):747. https://doi.org/10.3390/ph15060747.
25. Kumar H, Bot A. In this issue: Role of immune cells, immune modulating factors and immunotoxins in cancer immunotherapy. Int Rev Immunol. 2017;36(4):205-6. https://doi.org/10.1080/08830185.2017.1326784.
26. Qin G, Wang X, Ye S, Li Y, Chen M, Wang S, et al. NPM1 upregulates the transcription of *PD-L1* and suppresses T cell activity in triple-negative breast cancer. Nat Commun. 2020;11(1):1669. https://doi.org/:10.1038/s41467-020-15364-z.
27. Zou W, Wolchok JD, Chen L. *PD-L1* (B7-H1) and *PD-1* pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. Sci Transl Med. 2016;8(328):328rv4. https://doi.org/10.1126/scitranslmed.aad7118.
28. Lin H, Wei S, Hurt EM, Green MD, Zhao L, Vatan L, et al. Host expression of *PD-L1* determines efficacy of *PD-L1* pathway blockade–mediated tumor regression. J Clin Invest. 2018;128(2):805–15. https://doi.org/10.1172/JCI96113.
29. Nathan MR, Schmid P. The emerging world of breast cancer immunotherapy. Breast. 2018;37:200-6. https://doi.org/10.1016/j.breast.2017.05.013.
30. Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im SA, Yusof MM, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. Lancet. 2020;396(10265):1817-28. https://doi.org/10.1016/S0140-6736(20)32531-9.
31. Huang R, Cui Y, Guo Y. Programmed cell death protein-1 predicts the recurrence of breast cancer in patients subjected to radiotherapy after breast-preserving surgery. Technol Cancer Res Treat. 2018;17:1533033818793425. https://doi.org/10.1177/1533033818793425.
32. Walshaw RC, Honeychurch J, Illidge TM, Choudhury A. The anti-*PD-1* era an opportunity to enhance radiotherapy for patients with bladder cancer. Nat Rev Urol. 2018;15(4):251-9. https://doi.org/10.1038/nrurol.2017.172.
33. Gong X, Li X, Jiang T, Xie H, Zhu Z, Zhou F, et al. Combined radiotherapy and anti–*PD-L1* antibody synergistically enhances antitumor effect in non–small cell lung cancer. J Thorac Oncol. 2017;12(7):1085-97. https://doi.org/10.1016/j.jtho.2017.04.014.
34. Park SS, Dong H, Liu X, Harrington SM, Krco CJ, Grams MP, et al. *PD-1* restrains radiotherapy-induced abscopal effect. Cancer Immunol Res. 2015;3(6):610-9. https://doi.org/10.1158/2326-6066.CIR-14-0138.
35. McLaughlin M, Patin EC, Pedersen M, Wilkins A, Dillon MT, Melcher AA, et al. Inflammatory microenvironment remodelling by tumour cells after radiotherapy. Nat Rev Cancer. 2020;20(4):203-17. https://doi.org/10.1038/s41568-020-0250-y.
36. Du SS, Chen GW, Yang P, Chen YX, Hu Y, Zhao QQ, et al. Radiation therapy promotes hepatocellular carcinoma immune cloaking via *PD-L1* upregulation induced by cGAS-STING activation. Int J Radiat Oncol Biol Phys. 2022;112(5):1243-55. https://doi.org/10.1016/j.ijrobp.2021.12.162.
37. Narits J, Tamm H, Jaal J. *PD-L1* induction in tumor tissue after hypofractionated thoracic radiotherapy for non-small cell lung cancer. Clin Transl Radiat Oncol. 2020;22:83-7. https://doi.org/10.1016/j.ctro.2020.04.003.
38. Mori Y, Sato H, Kumazawa T, Permata TB, Yoshimoto Y, Murata K, et al. Analysis of radiotherapy-induced alteration of CD8+ T cells and *PD-L1* expression in patients with uterine cervical squamous cell carcinoma. Oncol Lett. 2021;21(6):446. https://doi.org/10.3892/ol.2021.12707.
39. Wu CJ, Tsai YT, Chang CC, Lee IJ, Wu PY, Tao MH. Efficacy of combining radiotherapy and immunotherapy for metastatic colorectal cancer and the modulation of tumor microenvironment. J Immunol. 2017;198(Suppl 1):79-15. https://doi.org/10.4049/jimmunol.198.Supp.79.15.
40. Wang S, Kuczma M, Pi W, Kong V, Campbell J, Jin JY, et al. Combined stereotactic body radiation therapy and immunotherapy on 4T1 triple-negative breast cancer murine model. Int J Radiat Oncol Biol Phys. 2016;96(2):E583. https://doi.org/10.1016/j.ijrobp.2016.06.2088.
41. Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunol Rev. 2009;229(1):12-26. https://doi.org/10.1111/j.1600-065X.2009.00770.x.
42. Lüönd F, Tiede S, Christofori G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. Br J Cancer. 2021;125(2):164-75. https://doi.org/10.1038/s41416-021-01351-8.
43. Navarrete-Bernal MG, Cervantes-Badillo MG, Martínez-Herrera JF, Lara-Torres CO, Gerson-Cwilich R, Zentella-Dehesa A, et al. Biological landscape of triple negative breast cancers expressing *CTLA-4*. Front Oncol. 2020;10:1206. https://doi.org/10.3389/fonc.2020.01206.
44. Contardi E, Palmisano GL, Tazzari PL, Martelli AM, Fala F, Fabbi M, et al. *CTLA-4* is constitutively expressed on tumor cells and can trigger apoptosis upon ligand interaction. Int J Cancer. 2005;117(4):538-50. https://doi.org/10.1002/ijc.21155.
45. Schaer DA, Murphy JT, Wolchok JD. Modulation of GITR for cancer immunotherapy. Curr Opin Immunol. 2012;24(2):217-24. https://doi.org/10.1016/j. coi. 2011. 12.011.
46. Lu L, Bai Y, Wang Z. Elevated T cell activation score is associated with improved survival of breast cancer. Breast Cancer Res Treat. 2017;164(3):689-96. https://doi.org/10.1007/s10549-017-4281-x.
47. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. Cancer Cell. 2018;33(5):843-52. https://doi.org/10.1016/j.ccell.2018.03.018.
48. Karpathiou G, Casteillo F, Giroult JB, Forest F, Fournel P, Monaya A, et al. Prognostic impact of immune microenvironment in laryngeal and pharyngeal squamous cell carcinoma: Immune cell subtypes, immunosuppressive pathways and clinicopathologic characteristics. Oncotarget. 2016;8(12):19310. https://doi.org/10.18632/oncotarget.14242
49. Lu H, Yang J, Jiao S, Li Y, Zhang W, Wang J. Cytotoxic T lymphocyte antigen 4 expression in human breast cancer: implications for prognosis. Cancer Immunol Immunother. 2015;64(7):853-60. https://doi.org/10.1007/s00262-015-1683-8.
50. Chang H, Jung WY, Kang Y, Lee H, Kim A, Kim HK, et al. Programmed death-ligand 1 expression in gastric adenocarcinoma is a poor prognostic factor in a high CD8+ tumor infiltrating lymphocytes group. Oncotarget. 2016;7(49):80426. https://doi.org/10.18632/oncotarget.12603
51. Mao H, Zhang L, Yang Y, Zuo W, Bi Y, Gao W, et al. New insights of *CTLA-4* into its biological function in breast cancer. Curr Cancer Drug Targets. 2010;10(7):728-36. https://doi.org/10.2174/156800910793605811.
52. Zhang XF, Pan K, Weng DS, Chen CL, Wang QJ, Zhao

JJ, et al. Cytotoxic t lymphocyte antigen-4 expression in esophageal carcinoma: Implications for prognosis. Oncotarget. 2016;7(18):26670-9. https://doi.org/10.18632/oncotarget.8476.
53. Hiniker SM, Chen DS, Reddy S, Chang DT, Jones JC, Mollick JA, et al. A prospective clinical trial combining radiation therapy with systemic immunotherapy in metastatic melanoma. Int J Radiat Oncol Biol Phys. 2016;96:578–88. https://doi.org/10.1016/j.ijrobp.2016.07.005.
54. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature. 2015;520(7547):373–7. https://doi.org/10.1038/nature14292.
55. Boutros C, Chaput-Gras N, Lanoy E, Larive A, Mateus C, Routier E, et al. Dose escalation phase 1 study of radiotherapy in combination with anti-cytotoxic-T-lymphocyte-associated antigen 4 monoclonal antibody ipilimumab in patients with metastatic melanoma. J Immunother Cancer. 2020;8(2):e000627. https://doi.org/10.1136/jitc-2020-000627.
56. Tang C, Welsh JW, De Groot P, Massarelli E, Chang JY, Hess KR, et al. Ipilimumab with stereotactic ablative radiation therapy: phase I results and immunologic correlates from peripheral T cells. Clin Cancer Res. 2017;23(6):1388–96. https://doi.org/10.1158/1078-0432.CCR-16-1432.
57. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168(4):707–23. https://doi.org/10.1016/j.cell.2017.01.017.
58. Demaria S, Golden EB, Formenti SC. Role of local radiation therapy in cancer immunotherapy. JAMA Oncol. 2015;1(9):1325–32. https://doi.org/10.1001/jamaoncol.2015.2756.
59. Demaria S, Kawashima N, Yang AM, Devitt ML, Babb JS, Allison JP, et al. Immune-mediated inhibition of metastases after treatment with local radiation and *CTLA-4* blockade in a mouse model of breast cancer. Clin Cancer Res. 2005;11(2):728–34. https://doi.org/10.1158/1078-0432.728.11.2.
60. Dewan MZ, Galloway AE, Kawashima N, Dewyngaert JK, Babb JS, Formenti SC, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti–*CTLA-4* antibody. Clin Cancer Res. 2009;15(17):5379–88. https://doi.org/10.1158/1078-0432.CCR-09-0265.