

## Research Article

# Simultaneous effect of physical activity and anti-PDL1 injection on tumor weight and life span of mice with breast cancer

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## Abstract

Cancer is now the leading global cause of death, with breast cancer being particularly deadly for women. This study investigates how physical activity combined with anti-PD-L1 antibody administration affects tumor weight and lifespan in mice with breast cancer. In this study, 30 male and female BALB/c mice, averaging 17.76 grams in weight, were selected and divided into the following groups: PCG (n=6), EIC (n=6), EIE (n=6), EIA (n=6), and EIE+A (n=6). Following an acclimation period on the treadmill, mice underwent two initial 4-week training protocols, followed by a 2-week protocol after cancer induction. A one-way analysis of variance (ANOVA) was performed to analyze the research variables. Statistical analysis showed no significant changes in survival time or tumor weight in the PCG, EIC, and EIE groups of breast cancer mice. However, the EIA and EIE+A groups demonstrated significant improvements in both survival and tumor weight reduction ( $p < 0.05$ ). The findings of this study suggest that the combination of physical activity and anti-PDL-1 antibody administration may lead to tumor apoptosis by enhancing positive immunological effects, which could be effective for better cancer management. However, it is recommended that future studies investigate the immunological and physiological effects of the tumor microenvironment with the combination of physical activity and anti-PDL-1 antibody administration.


**Key Words:** Anti-PDL-1 antibody, Exercise oncology, Physical activity

## Introduction

Today, cancer is recognized as the leading cause of death in the world, a disease caused by cell mutations (Vincze et al., 2022). While many mutations are harmless, others can enable cells to evade cell cycle control, leading to uncontrolled growth and multiplication, which can result in cancer (Novikov et al., 2021). It is crucial to prevent cancer development, regardless of its causes (Novikov et al., 2021). However, it has been established that breast cancer (Obeagu & Obeagu, 2024) is one of the most dangerous cancers known, and so far, different treatment methods such as chemotherapy, surgery, and immunotherapy have been proposed for breast cancer (Obeagu & Obeagu, 2024). However, immunotherapy has had significant therapeutic effects compared to other treatment methods (Dvir et al., 2024). One of the characteristics of tumors is their ability to evade the immune system, which leads to tumor growth. Therefore, immunotherapy has been able to significantly prevent the tumor from evading the body's immune system (Ahmadi et al., 2024). Immunotherapy focuses on inhibiting two immune system regulators: PD-1 and PD-L1. Within the tumor microenvironment, PD-1 and its ligand PD-L1 play critical roles in tumor progression and survival by evading immune surveillance. PD-1 is expressed on various immune cells, including monocytes, T cells, B cells, dendritic cells, and tumor-infiltrating lymphocytes. In the cancer immune cycle, the immune checkpoint PD-1 and its ligand PD-L1 collaborate to help tumors resist immune-induced apoptosis and promote tumor progression. However, PD-L1 is expressed on tumor cells and antigen-presenting cells (APCs), and the binding of PD-L1 to T-cell PD-1 disrupts the function of these cells (don't eat me) and other T cells cannot enter the tumor (Dammeijer et al., 2020). Therefore, the function of a tumor that overexpresses PD-L1 is to protect itself from cytotoxic T cell (CD+8)-mediated cell killing (Dammeijer et al., 2020). Due to the reduced function of CD+8 cells, tumor cells become highly aggressive and secrete several proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-2 (IL-2), and interferon gamma (IFN- $\gamma$ ) (Dammeijer et al., 2020). It also see-

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-ms that the injection of Anti PD-L1 antibody can lead to apoptotic changes in the tumor as well as increase the survival time of mice with breast cancer (Kitagawa et al., 2020; Lei et al., 2020).

Examining the history of exercise immunology reveals that PE can be a pivotal and safe means of enhancing the body's immune system function (Nieman, 2024). The enhancement of immune system function through PE has been particularly noted in the immunological changes of the adaptive immune system, specifically the improvement of T cell function (Nieman, 2024; Simpson et al., 2024). These positive effects could include improved CD+4 and CD+8 function and anti-inflammatory cytokines (Nieman, 2024; Simpson et al., 2024). These immunological effects caused by physical activity have been shown in several studies that can lead to positive apoptotic changes in tumor tissue (Fortner et al., 2023; Friedenreich et al., 2021). Also, on the other hand, several studies showed that physical activity can increase the duration of mice with breast cancer (Cannioto et al., 2021; Friedenreich et al., 2021; Wang & Zhou, 2021).

Several studies have investigated the separate effect of anti-PDL-1 antibody injection and physical activity on tumor changes and the quality of life of mice. However, so far, no study has investigated the simultaneous effect of anti-PDL-1 antibody and physical activity on tumor changes. Since anti-PDL-1 antibody injection is very useful in immunotherapy to prevent immune system evasion, and on the other hand, physical activity plays a significant role as a cost-free drug to improve immune system function, combining physical activity with anti-PDL-1 antibody to examine tumor changes can be effective in filling the research gap and starting a new scientific research. This study investigates how physical activity combined with anti-PD-L1 antibody administration affects tumor weight and lifespan in mice with breast cancer.

## Materials and Methods

The Ethics Committee in Biomedical Research of Tarbiat Modares University of Tehran (IR.MODARES.AEC.1403.023) approved this study. All animal procedures were conducted in compliance with the guidelines for the care and use of laboratory animals and ethical principles in animal research as endorsed by the Iranian Council for the Control of Animal Experiments. This study adheres to the ARRIVE guidelines.

## Animals

Thirty female mice, weighing between 18-20 grams and approximately at 4 weeks of age, were purchased from the mouse-breeding center of Rafsanjan University of Medical Sciences. They were housed in special Plexiglas cages under controlled environmental conditions, with an average temperatu-

-re of  $22 \pm 1.4$  °C, humidity of  $50 \pm 4\%$ , and a 12-hour light-dark cycle. The mice had free access to laboratory animal food and water. At the beginning of the training program, the mice were familiarized with the treadmill for adaptation. Five cancer-afflicted mice and five healthy mice warmed up for 10 minutes and began training at a speed of 6 m/min. The mice were habituated to the treadmill for four consecutive days before treadmill performance testing. The adaptation period involved 5 minutes of running at a speed of 6 m/min, followed by 5 minutes at speeds ranging from 6 to 12 m/min. For the exercise test, the mice warmed up for 3 minutes at a speed of 6 m/min. Then, they trained at a speed of 3 m/min for 3 minutes with no incline, and every 3 minutes, the treadmill speed was increased by 3 m/min until the mice reached fatigue (Conner et al., 2014; Ferreira et al., 2007; Shamsi et al., 2017; Tobias et al., 2023). Finally, the exercise-training program was formulated based on the maximum running speed achieved by the mice.

## Cell line

The 4T1 mouse breast cancer cell line was cultured in RPMI1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin (PenStrep) under conditions of 80% humidity, 5% CO<sub>2</sub>, and a temperature of 37°C, all maintained under sterile conditions. After incubation, the culture medium was renewed every 48 hours, continuing this process three times until approximately 70% of the cell culture plate was populated with cancer cells. Then, using 5% trypsin enzyme and rapid pipetting, the cells were detached. The harvested cells were washed with RPMI1640 medium, creating a suspension with a density of one million cells per milliliter. After trypsinization and washing with PBS buffer, the cells were counted, and dilutions of  $510^5$  to  $110^6$  cells were prepared. Finally, to induce cancer, an injection was administered near the breast area.

## Protocol 1 (exercise before cancer induction)

All groups underwent training for 60 minutes per session, 5 sessions per week, during the first 6 weeks without the induction of cancer or administration of antibodies. The mice were trained on a 5-lane treadmill.

## Protocol 2 (exercise after cancer induction) + Anti-PDL-1 injection

After 6 weeks, the mice were injected subcutaneously with the 4T1 cell line in the upper right thigh area. Two weeks after the cell line injection, the tumor was palpable at the injection site. Upon tumor appearance in the first week, the mice were randomly assigned into the following groups: PCG (N=6), EIC (N=6), EIE (N=6), EIA (N=6), and EIE+A (N=6). The mice in the exercise groups trained 3 times a week, performing 3 sets of 10 minutes each, with 2 minutes and 30 seconds of active rest between sets (Jafari et al., 2021; Shamsi et al., 2017; Tobias et al., 2023) The exercise intensity of the rats was 60% of the maxi-

-mum intensity they achieved (Jafari et al., 2021; Shamsi et al., 2017; Tobias et al., 2023).

In the entire length of the research, the incline of the treadmill was zero. In the second protocol, the PCG group did not participate in physical activity during the initial 6 weeks and subsequently refrained from both physical activity and antibody administration following cancer induction. The EIC group engaged in physical activity during the first 6 weeks but ceased physical activity and did not receive antibody treatment after cancer induction. The EIE group participated in physical activity during the initial 6 weeks and resumed physical activity exclusively following cancer induction. The EIA group engaged in physical activity during the first 6 weeks and received antibody treatment only after cancer induction. Lastly, the EIE+A group participated in physical activity during the initial 6 weeks and continued with both physical activity and antibody administration following cancer induction. Anti-PDL-1 antibody was obtained in EIA and EIE+A groups according to Table 1. Antibody injection was performed in the tail.

### Tumor weight measurement

To measure the tumor weight, after the surgery of the mice, the tumor weight was used with a Scale MH digital scale made in Germany.

### The lifespan of mice

To measure the survival time of the mice, starting with protocol 2, the survival of the mice in each group was monitored daily. The investigation involved recording the number of days each mouse survived from the start of the protocol until surgery (Magaki et al., 2019).

### Data analysis

To analyze the data, descriptive statistics were used to calculate descriptive indices, including mean and standard deviation. All statistical procedures were performed using IBM SPSS Statistics version 24 software at a significance level of  $P < 0.05$ . The Kolmogorov-Smirnov test was used to check the normality of data

distribution. Additionally, a one-way analysis of variance (ANOVA) was used to measure the research variables.

## Results

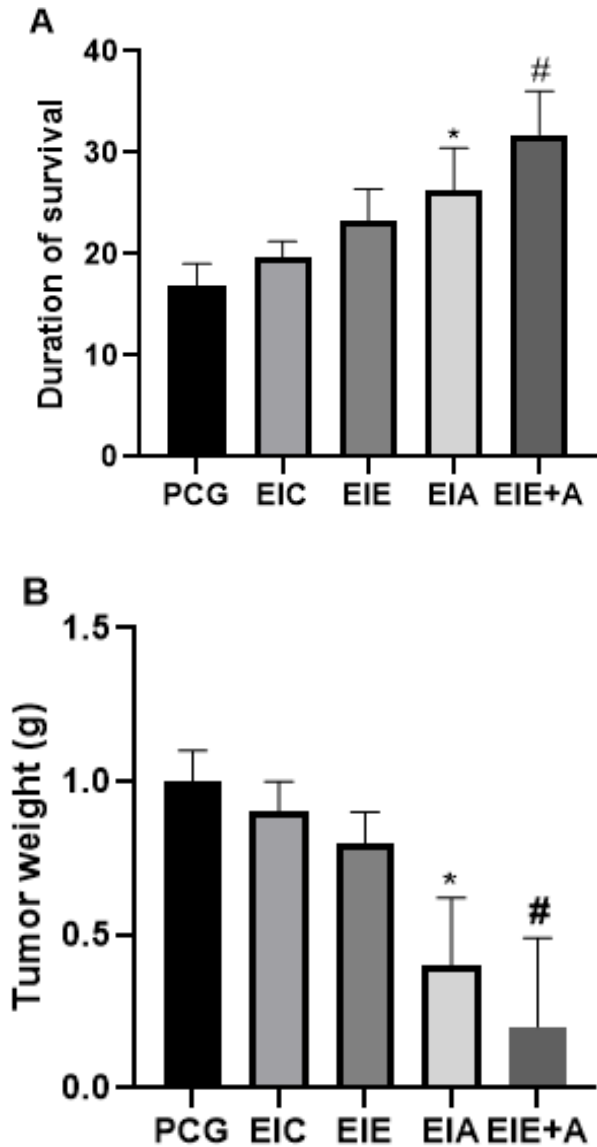
The results of statistical analysis showed that in PCG, EIC and EIE groups, no significant changes were observed in relation to the survival time of mice with breast cancer, but significant changes were observed in EIA and EIE+A ( $P < 0.05$ ) (Figure 1-A). On the other hand, the results of statistical analysis regarding the tumor weight of mice with breast cancer showed that no significant changes were observed in PCG, EIC and EIE groups, but significant changes were observed in EIA and EIE+A groups. ( $P < 0.05$ ) (Figure 1-B).

## Discussion

This study aimed to explore the impact of physical activity and the administration of an anti-PDL-1 antibody on both tumor weight and overall lifespan in mice with breast cancer. Recognizing the potential of immunotherapy in cancer treatment, particularly through immune checkpoint inhibitors like anti-PDL-1 antibodies, the study investigated the synergistic effects when combined with physical activity, an intervention known to influence immune system responses. The findings revealed that in the EIA group (anti-PDL-1 antibody administration) and the EIE+A group (combined anti-PDL-1 administration and physical activity), both tumor weight was reduced, and lifespan was extended in comparison to control and other experimental groups. The results underscore the significant impact of physical activity and anti-PDL-1 administration on enhancing survival rates in breast cancer-bearing mice, marking a promising combination approach for improved outcomes. Aligning with prior research, which has shown that mice with advanced-stage breast cancer typically experience a rapid decline in survival due to aggressive metastasis to organs like the lungs and ovaries within days following tumor cell injection (Alagheband et al., 2016; Poff et al., 2014; Schonberg et al., 2012; Schrag et al., 2000; Thomas et al., 2017; Zheng et al., 2007), this study suggests that introducing physical activity can delay this progression. Notably, other studi-

**Table 1.** Training protocol and injection dose of anti-PDL-1 antibody

Protocol	First week	second week	third week	fourth week	Five Week	Six Week		First week	second week	third week	fourth week
treadmill speed	14	16	18	18	20	20		14	14	16	16
duration	60 minutes	60 minutes	60 minutes	60 minutes	60 minutes	60 minutes	Cancer induction	3 sets of 10 minutes	3 sets of 10 minutes	3 sets of 10 minutes	3 sets of 10 minutes
Number of sessions per week	5	5	5	5	5	5		3	3	3	3
The amount of antibody injection								10 mg per session	10 mg per session	10 mg per session	10 mg per session



**Figure 1.** Survival time of mice and changes in tumor weight in mice with breast cancer.

studies have shown that physical activity alone can positively impact survival by inducing beneficial immune system changes and reducing tumor volume in breast cancer models (Bronikowski et al., 2003; Garcia-Valles et al., 2013; Nilsson et al., 2019). These improvements likely result from activated immune pathways that enhance tumor response and reduce metastasis risk. Furthermore, this study confirms that both physical activity and anti-PDL-1 administration independently contribute to a significant reduction in tumor weight. This result complements previous findings that moderate-intensity physical activity stimulates beneficial immunological changes within the tumor microenvironment, potentially enhancing the effects of anti-PDL-1 therapy. Immunological changes observed in other research include increased infiltration of T-cells, reduced immunosuppres-

-sive cells in the tumor microenvironment, and enhanced cytokine activity, which collectively aid in decreasing tumor size and weight (Ahmadi Hekmatikar et al., 2023; Rami et al., 2023; Slattery et al., 2010; Suzuki et al., 2022; Van Blarigan et al., 2015; Zhang et al., 2015). The combination of anti-PDL-1 administration with physical activity seems to offer a particularly potent approach, leveraging both immune stimulation and checkpoint blockade to suppress tumor growth (Ahmadi Hekmatikar, 2024; Tayebi et al., 2020). One of the primary strengths of this study is its novel approach in examining the combined effects of physical activity and anti-PDL-1 antibody on tumor reduction and lifespan extension, offering valuable insight into potential non-invasive strategies for cancer management. By assessing these interventions together, this study highlights a promising area for developing integrative cancer therapies that may improve patient outcomes without the toxicity often associated with traditional cancer treatments. However, this study is limited by its lack of in-depth immunological analyses, such as assessments of specific immune cell activity, cytokine levels, and tumor size measurements over time. These factors are essential for fully understanding the mechanisms driving the observed benefits. Future research should therefore include comprehensive immunological profiling and monitor tumor progression at various stages to further elucidate the processes involved. Additionally, investigating how different intensities and durations of physical activity influence tumor progression and immune response may provide a more nuanced understanding of the optimal conditions for combining physical activity with immunotherapy. Such studies would help establish guidelines for integrating physical activity into cancer treatment plans, potentially enhancing the effectiveness of immune-based therapies like anti-PDL-1 antibody administration.

## Conclusion

The present study broadly demonstrated that the combination of physical activity and anti-PD-L1 antibody injection exerts a synergistic effect, leading to reduced tumor weight and extended survival time in mice. However, due to the study's limitations, the detailed mechanisms underlying these effects remain insufficiently understood. Future research should prioritize exploring the immunological and physiological changes within the tumor microenvironment. Additionally, examining the effects of varying exercise intensities in combination with anti-PD-L1 antibody administration may yield novel insights.

## What is already known on this subject?

**Anti-PDL-1 Antibodies in Cancer Treatment:** Anti-PDL-1 antibodies are a class of immune checkpoint inhibitors that block the interaction between PD-L1 (programmed death-ligand 1) on tumor cells and PD-1 receptors on T-cells, allowing the immune system to better recognize and attack cancer cells. Studies have

shown that anti-PDL-1 therapy can be effective in reducing tumor growth and improving survival rates in various cancers, including breast cancer, particularly in advanced stages.

**Physical Activity and Cancer Progression:** Regular physical activity has been shown to slow down cancer progression and improve survival rates across multiple types of cancers, including breast cancer. Physical activity may exert its anti-cancer effects through immune system enhancement, improved metabolic function, and reduction of inflammation. In animal models, exercise has been associated with decreased tumor growth, potentially due to increased immune cell infiltration in the tumor microenvironment and other favorable immunological changes.

**Combined Effects of Physical Activity and Immunotherapy:** Emerging evidence suggests that physical activity may enhance the effectiveness of immunotherapies like anti-PDL-1 antibodies. Exercise is thought to stimulate the immune system, increase T-cell activation, and improve circulation, which may help immunotherapeutic agents reach tumors more effectively. Studies in other cancers indicate that a combination of exercise and immunotherapy may yield synergistic effects, leading to reduced tumor size and improved survival outcomes.

## What this study adds?

Physical activity along with immunotherapy can have a significant effect on tumor changes in mice with breast cancer.

### Organ Cross-Talk Tips:

- Exercise is thought to stimulate the immune system, increase T-cell activation, and improve circulation, which may help immunotherapeutic with crosstalk between cancer and immune cells.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The Ethics Committee in Biomedical Research of Tarbiat Modares University of Tehran (IR.MODARES.AEC.1403.023) approved this study.

**Informed consent** Not applicable

## Author contributions

Conceptualization: H.A.A.N., AAH.; Methodology: A.Y.A., M.M.SH.; Software: H.A.A.N., AAH.; Validation: A.Y.A., M.M.SH.; Formal analysis; A.Y.A., M.M.SH; Investigation: H.A.A.N., AAH.; Resources: A.Y.A., M.M.SH.; Data curation: H.A.A.N., AAH.; Writing - original draft: H.A.A.N., AAH.; Writing – review & editing: H.A.A.N., AAH.; Visualization: A.Y.A., M.M.SH.; Supervision: AAH. Project administration: AAH.; Funding acquisition: AAH.

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