Abstract:

Immunotherapy is one of the cancer treatment methods that helps the body's immune system fight against cancer. However, despite its numerous advantages, it still faces multiple challenges. Nanoparticles are drug delivery systems that may be able to overcome these challenges in immunotherapy. On the other hand, the use of combination immunotherapy can be effective in strengthening immunotherapy as it deals with multiple suppressive elements of the immune system in the tumor microenvironment and activates multiple stages of the immune-cancer cycle. The aim of this study is to produce PLGA nanoparticles containing breast tumor cell extract as a rich source of tumor antigens and Poly (I:C) as an immune system stimulant, decorated with CD40 agonistic monoclonal antibodies as an activator of immune cells, and also to produce PLGA nanoparticles containing Linrodostat mesylate as an IDO1 enzyme inhibitor decorated with anti-PDL-1 monoclonal antibodies, and to evaluate their therapeutic effects on a breast cancer mouse model. In this study, nanoparticles were prepared using the double emulsion solvent evaporation method, and particle size, morphology, dispersibility, zeta potential, as well as encapsulation and release efficiency were examined before and after attachment to monoclonal antibodies. The 4T1 mouse breast cancer cell line was used to induce tumors. The animals were randomly divided into 5 groups of 8, including a healthy control group without tumor induction, a tumor control group without treatment, a group treated with PLGA nanoparticles containing cell extract and Poly (I:C) decorated with CD40 agonistic monoclonal antibodies, a group treated with nanoparticles containing Linrodostat mesylate decorated with anti-PDL-1 monoclonal antibodies, and a group treated with both nanoparticles simultaneously.

The treatment regimen consisted of two injections with a two-week interval starting from the 14th day after tumor induction, followed by sacrifice on the 35th day. After the mice were euthanized on the 35th day, the effectiveness of the vaccine was evaluated by measuring the proliferation of splenocytes, delayed hypersensitivity response, changes in cytokine production, specific CTL cell response, CD107 expression, and histopathological changes in breast tumor tissue. The results of this

study showed that the nanoparticles produced had uniform dispersibility, spherical shape, smooth surface, appropriate encapsulation efficiency, and stable release.

Both vaccines based on PLGA nanoparticles significantly reduced tumor growth, increased survival time, increased proliferation activity of splenic lymphocytes indicating anti-tumor cellular immune response, increased delayed hypersensitivity response indicating anti-tumor Th1 cell response, increased specific CTL responses. Anti-tumor, the shift of cytokine response towards Th1 cells, the increase in the production of cytokine IFN γ and the decrease in the production of IL-4 in mice with breast tumors, which changes in the group treated with nanoparticles containing the drug Linrodostat mesylate decorated with anti-PDL monoclonal antibody. 1-Compared to the group treated with nanoparticles containing antigen (tumor cell lysate) and adjuvant Poly (I:C) in PLGA nanoparticles attached to CD40 agonistic monoclonal antibody, all these changes in the group with both nanoparticles were used together, it was more severe. Also, pathological evaluations showed increased necrosis, increased infiltration of immune cells, and decreased density of tumor cells.

According to the results of the present study, it seems that immunotherapy using a combination of antigen (tumor cell lysate) and adjuvant Poly (I:C) inside PLGA nanoparticles attached to CD40 agonistic monoclonal antibody and nanoparticles containing Linrodostat drug mesylate decorated with anti-PDL-1 monoclonal antibody and especially using these two nanoparticles at the same time, as a therapeutic strategy for breast tumor treatment, strengthens anti-tumor immune responses and can be an effective therapeutic strategy in treatment Breast cancer is *raised.*

Keywords: Nanoparticle, Immunotherapy, PLGA ،Tumor Cell Lyset ، Poly (I:C) ،Linrodostat mesylate ،CD40 ،PDL-1