Immunotherapy in Estrogen Receptor Positive (ER+) Breast Cancer: Current Technologies and Challenges Targeting *ESR1* mutations

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Abstract

Metastatic breast cancer remains without a cure and *ESR1* mutations have emerged as potential contributors in metastatic ER+ breast cancer. Immunotherapy harnesses the immune system to fight cancer and is utilized for various cancer types. However, the effectiveness and use of immunotherapy are limited when it comes to ER+ breast cancer. This subtype has been considered immunologically “cold,” meaning that the tumor is able to suppress the immune response and prevent T cells from attacking and killing tumor cells. Recent studies have shown a subset of ER+ metastatic breast cancer patients have an increased likelihood of response to immunotherapy often associated with the presence of common *ESR1* mutations D538G, Y537S, and E380Q. Emerging data shows that these mutations confer a basal-like phenotype that is more similar to that of triple-negative breast cancer, a subtype more sensitive to immunotherapy, which indicates that the mutant ER cells may be responsive. *ESR1* mutants have been shown to produce neoepitopes that are being explored as potential drug targets. This intersection of traits could suggest that *ESR1* mutations may confer some sensitivity to traditional immunotherapy in some ER+ metastatic breast cancer. This review will consolidate the recent findings in the field of immunotherapeutic targeting, where the generation of *ESR1* mutant neoepitopes for novel targeting and the phenotypic shift toward more basal-like traits combined with new understandings and technologies shine light on possibilities for the future.

Introduction

Breast cancer is the most common cancer in females with 80% of the women diagnosed with the ER+ subtype. This subtype includes breast cancer cells that express the estrogen receptor in >1% of the tumor. ER+ subtype is considered estrogen-dependent for proliferation. Currently, the standard of care for ER+ breast cancer is surgery followed by 5-10 years of adjuvant endocrine therapy to reduce estrogen signaling (Cleveland Clinic, 2024). ER+ breast cancer tends to have more favorable outcomes than estrogen receptor-negative breast cancer, as well as being less aggressive. However, in spite of better prognosis, the risk of recurrence remains a concern.

Recurrence is influenced by factors such as cancer stage and size, and it often necessitates more aggressive treatment due to developed resistance (Cleveland Clinic, 2023). Metastatic breast cancer is the most advanced stage (stage four) that has grown and spread to different body organs beyond the breast. Due to the intense spread of metastatic cancer, there is no known cure for it. Instead the goal is on a way to manage it with a focus on shrinking the tumors to improve the quality of life for them (Pennmedicine.org, 2023).

In ER+ breast cancer, *ESR1* mutations are common and generally tend to add more resistance to treatments (Brett et al, 2021). When an *ESR1* mutation is present, the estrogen receptors, proteins that receive signals from estrogen to tell breast cancer to grow, change form. This shape change activates the receptors, which causes the cancer to grow even when estrogen is

not present. The most common types of *ESR1* mutations in ER+ breast cancer are D538G, Y537, and L536R. These mutations are said to be the main instigators of metastatic ER+ breast cancer (Goldberg et al, 2024). For that reason, targeting the *ESR1* mutations and focusing on improving treatment for those can provide better outcomes with the decreasing amount of metastatic ER+ breast cancer cases.

An important feature of ER+ breast cancer is the fact that it is immunologically cold. Immunologically cold cancers tend to not have strong immune responses since “cold” tumors are surrounded by cells that suppress the immune response and prevent T cells from attacking and killing the tumor cells (Bates et al, 2020). This means that ER+ breast cancer does not usually have a good response to immunotherapy. However, now due to new clinical trials and drugs that have been found, the use of immunotherapy has become a possibility to improve the outcome of breast cancer patients (Shatsky, 2024). In order for that to be possible, breast cancer would have to be made more immunologically hot to get a response to immunotherapy.

Immunotherapy is a treatment that uses a person’s own immune system to fight cancer by helping the immune system identify and attack cancer cells. Immunotherapy is used as a standard for many cancers today and is still being trialed for potential uses in the future for others. Currently, immunotherapy is actively used on triple-negative breast cancer by healthcare providers. Due to the fact that ER+ breast cancer is immunologically cold, immunotherapy has not been effective because of the cancer’s resistance to it. However, new studies have recently shown otherwise and shown a possibility for immunotherapy to improve ER+ breast cancer patient outcomes by using *ESR1* mutations as a potential source for neoepitopes for immunotherapy to target (Goldberg et al, 2024).

ESR1 Mutations

An *ESR1* mutation is a change in the estrogen receptor gene that can occur in breast cancer. The most common *ESR1* mutations in ER+ breast cancer include L536R, Y537S, and D538G. *ESR1* mutations are not as prevalent in primary ER+ breast cancer for they only exist in about 1% of primary tumors. However, they are relatively a lot more common in metastatic ER+ breast cancer since they exist in the range of 10-50% of metastatic breast cancer. This indicates that *ESR1* mutations are a lot more common in endocrine therapy-resistant cancers with lower survival rates (Zundelevich et al, 2020). Even though Y53S7 and D538G are both *ESR1* mutations, they do have differences between them that differentiate their effects. In a study done, the data suggested that the Y537S mutant possesses a higher affinity for SRC3, a protein that regulates transcriptional activity, as compared to D538G (Fanning et al, 2016). This was specific to the aspects of the mutations that helped the cells be endocrine resistant.

ESR1 mutations are associated with poorer overall and relapse-free survival in ER+ breast cancer patients than those without mutations. *ESR1* mutations can also cause some hormone therapies to stop working, allowing the cancer to progress. *ESR1* mutations have been found to have lower survival rates and were supported by one study that found that patients with *ESR1* mutations had a 1-year overall survival of 62% on exemestane and 80% on fulvestrant, compared to 79% and 81% for patients without mutations (Turner et al, 2020). *ESR1* mutations

can also cause endocrine resistance in patients treated with endocrine therapy. In one study, 75% of patients with *ESR1* mutations and endocrine therapy had primary endocrine resistance, compared to 24.4% of patients without mutations (Ahn et al, 2022). *ESR1* mutations also exhibit many enriched immune pathways in ER+ breast cancer. *ESR1* mutant tumors have basal features that are associated with increased immune activation, suggesting more research should be done on immune therapeutic vulnerabilities (Li et al, 2022).

Current Immunotherapy in ER+ breast cancer

Currently, the most common treatments of immunotherapy used in different types of cancers are monoclonal antibodies, chimeric antigen receptor (CAR)T cells, immune checkpoint inhibitors (ICI), and immune system modulators. Immunotherapy has been deemed effective and is cleared to treat many other types of cancers (Cleveland Clinic, 2020).

Monoclonal Antibodies

Monoclonal antibodies (mAbs) are lab-made proteins that can act as a type of immunotherapy. They work to trigger the immune system to attack the cancer cells by identifying and then latching on to specific proteins of the cancer cells (Cancer Research UK, 2021). Monoclonal antibodies can work in different ways, including marking cancer cells, bringing T cells closer to cancer cells, and blocking signals. Marking the cancer cells by binding to them makes the cancer cells easier to identify to be targeted and destroyed by the immune system. Another type of monoclonal antibody helps T cells get closer to cancer cells, which helps the immune cells to kill them. Other monoclonal antibodies can block the signals that cause the cancer cells to divide, decreasing the spread of the cancer. Studies focusing on binding monoclonal antibodies to ER mutants suggest that future research may need to explore mutant-specific mAbs if those against ER wild-type are ineffective (Ross et al, 2019).

CART cells

Chimeric antigen receptor (CAR)T cells are a type of immunotherapy that uses genetically altered T cells to find and treat cancer. This is done by isolating T cells from a patient's blood and genetically modifying them in a lab to produce the receptors that bind to proteins on cancer cells, CARs. These cells are infused back into the patient after being grown in the lab. The CART cells then find the cancer cells and destroy them. The CART cells process is long and takes time due to the lab work necessary and also has serious side effects, including cytokine release syndrome, nervous system problems, and more (American Cancer Society, 2022).

Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs) are immunotherapy drugs that block checkpoint proteins to treat cancer. These surface receptor checkpoints help regulate the immune system and can sometimes lead to prohibiting T cells from killing the cancer cells. ICIs work by blocking checkpoint proteins from binding with their partner proteins. This allows T cells to stay active and continue to kill cancer cells.

Other Immune System Modulators

Immune system modulators are substances that stimulate or suppress the immune system, which include cytokines, BCG, and immunomodulatory drugs. Cytokines are proteins that help the immune system manage the response to foreign substances, including cancer cells. There are two different types of cytokines: interferons and interleukins. Interferons help the immune system decrease cancer growth while interleukins start an immune response and assist in immune system cells communication. Immunomodulatory drugs are medications that enhance your immune system. For example, thalidomide, lenalidomide, and pomalidomide are immunomodulatory drugs that make cells release cytokine to help the body fight the cancer by making more white blood cells (Cleveland Clinic, 2020).

Vaccines

Vaccines are used as a form of immunotherapy against cancer by helping the immune system identify antigens in the cancer cells. An example of cancer vaccines is the Bacillus Calmette-Guérin (BCG) which is a tuberculosis vaccine that acts as an immune stimulant. It was the first immunotherapy approved by the FDA and is currently still used for bladder cancer. There are currently four vaccines that are approved by the FDA to help prevent cancer along with BCG and Sipuleucel-T (Provenge), which are therapeutic cancer vaccines: Cervarix, Gardasil, Gardasil-9, and Hepatitis B (HBV) vaccine (Dunn, 2024).

Table 1: Immunotherapy Trials Done in ER+ Breast Cancer

	In vitro	*In vivo*	Clinical
mAb	(Ross et al, 2019)		
carT cells	(Schettini et al, 2021)		
ICI	(Williams et al 2021)		
Vaccines	(Dailey et al, 2024), (Goldberg et al, 2024)	(Dailey et al, 2024)	

The table highlights papers that have tested the corresponding immunotherapy treatment either *in vitro*, *in vivo*, or clinical for ER+ breast cancer.

ER+ breast cancer has not been responsive to immunotherapy in the past due to it being considered immunologically cold. ER+ breast cancer's low levels of immune cell infiltration and PD-L1 expression limit the effectiveness of immunotherapy. ER+ breast cancer also has fewer

somatic mutations and tumor-infiltrating lymphocytes than other types of breast cancer (O'Leary et al, 2023). The tumor microenvironment has potent interactions with tumor cells and there is a focus in the field to alter the tumor microenvironment making it more immunologically hot to maximize the efficacy of immunotherapy (Gatti-Mays, 2019).

Vaccine-based Immunotherapy for *ESR1* Mutations

There is a recent study done on the effectiveness and possibilities of vaccines targeting *ESR1* mutations to get an immune response on the cancer cells. The study tested different *ESR1* mutant forms by developing many recombinant vaccines that encoded those forms and confirmed the ability to get T cell responses. Then, the anti-tumor potential of the vaccines were tested and were found to suppress tumor growth. The results were able to support the presence of human T cells reactive to the *ESR1* mutant epitopes in an ER+ breast cancer patient which led to the start of the development of the vaccines. However, this immunotherapy technique is still under clinical trial, meaning it is not cleared as a type of treatment yet and has only been tested on mice (Dailey et al, 2024).

Conclusions / Findings

Even though immunotherapy has not been shown to be effective on primary ER+ breast cancer, there is ongoing research that suggests *ESR1* mutations are novel targets for immunotherapy in metastatic ER+ breast cancer (Goldberg et al, 2024). These mutations have been shown to play a role in resistance against endocrine therapy. However, the mutations also show the possibility of using them as neoepitopes that the immunotherapy can target. Recent studies have looked at the immunogenicity of mutant ER peptides using machine learning algorithms as well as classic molecular biology assays. The data supported the potential of the peptides to be targeted by novel immunotherapy strategies by confirming the immunogenicity of the *ESR1* epitopes (Goldberg et al, 2024).

The most common types of immunotherapy used in cancer are currently not used clinically in ER+ breast cancer with *ESR1* mutations. In order for this to change, more research and technology would need to be found to work on the tumor to make the cancer more immunologically "hot" so that immunotherapy would work better on the tumor. Currently, it is shown that most immunotherapy treatments for ER+ breast cancer are not used clinically or even *in vivo*. However, there is potential shown with vaccine-based immunotherapy that is currently being tried *in vivo* that is found to get T cell responses and suppress tumor growth showing the future for immunotherapy in ER+ breast cancer.

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