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# HR-Positive Breast Cancer and New Potentially Synergistic Approaches with CDK Inhibition of the Retinoblastoma Protein and CAR T-Cell Immunotherapy

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**Abstract**

Breast cancer is the most prevalent cancer in women and 25% of all cancers worldwide (Ghoncheh et al., 2016). It is the second most common cause of female death from cancers (Alkabban & Ferguson, 2022). A silent-forming cancer, breast cancer is highly metastatic in later stages and is often revealed through routine screening. It is caused by an accumulation of genetic mutation and damage, allowing for uncontrolled proliferation and tumorigenesis. Multiple hallmark changes of cancer are often seen in breast cancer, such as in the PI3K/AKT pathway, the RAS/MEK/ERK pathway, or the RB pathway. Additionally, it is also possible to inherit pro-cancerous genes, such as BRCA1/2, carcinogens that cause DNA crosslinks and double strand breaks (Patel et al., 1998).

The retinoblastoma tumor suppressor gene plays a key role in controlling the cell cycle. Its product, pRB, is able to prevent the G1/S transition through attachment to the E2F transcription factor in its unphosphorylated state, which in turn prevents transcription of genes involved in DNA replication and cell cycle progression (Weinberg, 1995). The cyclin-dependent kinases CDK4 and 6, activated by cyclin D, are then able to phosphorylate pRB when the cell is ready, releasing E2F and allowing for progression (Shah et al., 2018). It has been observed that breast cancer cells have various defects in the RB pathway, such as overexpressing cyclin D or lacking CDK4/6 regulation through the INK4 family, leading to a failure in cell growth control and uncontrolled proliferation (Hamilton and Infante, 2016). Thus, CDK4/6 inhibition has become a key point of study by artificially inducing cell cycle arrest. Currently, palbociclib, ribociclib, and abemaciclib are the only approved CDK4/6 inhibitors for first-line use in HR+/HER2- breast cancer, the most common subtype, possessing hormone (estrogen/progesterone) receptors and lacking the human epidermal growth factor 2 receptor (SEER, 2020). While they differ structurally, they all interchangeably serve the role of potently blocking pRB phosphorylation (Zhang et al., 2021).

Chimeric antigen receptor (CAR) T-cell therapy is a highly experimental and novel cancer treatment, currently employed for various hematological malignancies (National Cancer Institute, 2022). Developed from adoptive T cell transfer (ACT), it allows a patient to use their own T cells against cancer. The cells are extracted and artificially given the CAR receptors which bind to receptors found on the cells, activating them, and then are cultured and readded to the blood, essentially acting as manmade antigen presentation (June et al., 2018). The specialization of the T cells' receptors allows this treatment to be highly targeted and specific, and the T cells' innate habitat in the blood and lymph make it an obvious choice for treating leukemias and myelomas. Its potential in other cancers, however, is undeniable and research is ongoing to find more applications of this novel treatment.

**Keywords:** HR-Positive Breast Cancer, CAR T-Cell immunotherapy, proposal, limitations, treatment

**1. Current Limitations**

The three approved CDK4/6 inhibitors, palbociclib, ribociclib, and abemaciclib, have been extensively studied and trialed with promising results. However, pathway inhibition is ultimately still susceptible to tumor heterogeneity and changes within the cellular complex. One such issue is the loss of functional pRB, which renders any inhibition ineffective as there is no longer effective binding to E2F (Watt and Goel, 2022). Likewise, inhibition can also be overridden through overexpression of E2F (Guarducci et al., 2016). It has also been found that FAT1 receptor loss can also lead to CDK inhibitor resistance through the Hippo pathway (Li et al., 2018). Lastly, CDK inhibitors also exhibit traditional chemotherapeutic adverse effects, in the form of neutropenia, anemia, or thrombopenia, due to diminished myelogenous production (Spring et al., 2019).

While excellent for treating hematological cancers, CAR T therapy may face several issues when treating solid tumor growths, such as the tumor microenvironment, as well as inherent problems of the treatment, namely targeting and toxicity (Sterner & Sterner, 2021). As the movement of T cells is dependent on vascularization of the target area, tumors that are more isolated or possess physical barriers, such as stroma, are less likely to be targeted effectively. Furthermore, the tumor microenvironment may emit immunosuppressive signals, shutting down T cell activity and rendering the treatment inert (John et al., 2013). Additionally, the treatment may also fail as the antigen target originally designated by the CAR receptor may no longer be present within the tumor cells due to heterogeneity (Yang et al., 2021). Lastly, T cell toxicity may prove to be highly dangerous for the patient. The CAR T cells may be “on-target/off-tumor”, where the target antigen is found on non-tumor, healthy cells, thereby needlessly harming bodily tissue. Additionally, if the targeted tumor is properly targeted and damaged at a rapid rate, tumor lysis syndrome may occur fatally, where cancerous cell contents are released into the bloodstream and surrounding vascularization (Howard et al., 2012).

**2. Proposal**

To overcome the limitations of the individual treatments, I propose a combination of CDK4/6 inhibition and CAR T cell therapy should be tested using mice and organoid modelling to evaluate their synergistic effects and change in overall effectiveness. It combines a well-researched and approved treatment with a novel treatment, yet to be used prevalently in breast cancer. This combination may result in a favorable outcome and will improve treatment options, or may yield valuable knowledge and insight if failure occurs. Additionally, as both outlined treatments suffer from loss-of-response due to random mutation and heterogeneity, a combination therapy with contrasting targets may lower probability of resistance being mutated. Resistance most likely will occur first with one treatment while the other continues unaffected, improving prognosis. Lastly, the treatments may be mutually beneficial in situations such as if CDK inhibition can limit tumor growth and decrease the load on CAR T cells, or if CAR T cells can aid in improving remission times and decrease the probability of relapse by actively eliminating tumor cells and/or tumor stem cells.

**3. Modeling Systems and Methodology**

It is important to model breast cancer tumors with high accuracy and replicate the tumor microenvironment. The primary line of testing will be carried out on humanized mouse (hu-mouse) models with functional immune systems and patient-derived xenograft (PDX) mice. Given human tumors, hu-mice are able to give an accurate representation of CAR T cell activity working alongside a grafted human immune system, as well as demonstrate any adverse effects of CDK inhibition, such as neutropenia. However, this model, as demonstrated by Jin et al., is difficult to procure and has not been used previously to replicate the environment and interactions of HR+/HER2- breast cancer (Jin et al., 2018). Thus, for greater accessibility and testing effectiveness, PDX mice are also to be used to obtain theoretical results using induced murine tumor tissue. CAR T cells will be procured from human donors, cultivated, and administered to the mice. The three different CDK4/6 inhibitors will each be given to see which one has the best potential in combination therapy. The outcome of treatment will be measured using bioluminescent imaging to view any changes in macroscopic features. Control, CAR T-only, and CDK inhibitor-only mice will also be tracked to identify any changes over the standard single therapies.

Although murine models are beneficial for viewing treatment/cancer interactions, they are also limited as they do not possess the cellular environment of a human. Hu-mouse immune systems lack the depth of development required to simulate accurate interactions. Additionally, xenografting does not ensure that the tumor will behave in the same manner in the host, leading to potential differences in the tumor microenvironment and surrounding vascularization, affecting CAR T effectiveness.

Additionally, organoids will be used to further provide insight into the direct tumor suppression efficacy of the two drugs. They can give near-in-vivo reconstructions of the breast microenvironment and are under a higher degree of control than true in-vivo models (Mohan et al., 2021). Not only does it replicate tumor growth, but also the surrounding human breast environment, making for more authenticity in surrounding signaling and vascularization, key for evaluating the effectiveness of CAR T therapy. Similar to with mice models, the

mammary organoids will be administered cultivated CAR T cells from a donor and then the three CDK4/6 inhibitors, as well as a trial for only T cells, only inhibitors, and control. Breast cancer will be induced through genetic modification to remain constant through the assays, and again bioluminescence will be used to determine overall tumor size and treatment efficacy.

**4. Limitations of Proposed Treatment**

A combination therapy still poses risks and the potential of treatment failure. Although resistance to both treatments have greater improbability than each individual treatment, chance mutations and tumor heterogeneity may still lead to loss-of-response. It is unlikely that a single mutation is able to cause total treatment failure, but multiple mutations in a short timespan could cause a cascading resistance, first to one treatment then the other, thus reducing the time available to detect reductions in efficacy. Furthermore, the adverse effects associated with CDK inhibitor drugs and toxicity from CAR T-cells may be cumulative in risk. Cytopenia or neutropenia may develop due to both treatments negatively affecting myeloid-line proliferation, commonly seen in CDK inhibition. CAR T therapy, if off-target, could worsen the problem by attacking developing myeloid progenitors or other marrow cells. If allowed to progress, this will severely immunocompromise the patient, requiring further intensive care aside from cancer treatment.

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