Endocrine Therapy Resistance in Breast Cancer: Mechanisms and Treatments

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i. Abstract

Cancer, a disease in which abnormal cells divide to form tumors, is considered one of the most lethal diseases, ranked number two in the United States and resulting in almost 600,000 deaths annually. Breast cancer is the number one diagnosed form of cancer in women, with 1 in 8 women diagnosed. About 80% of breast cancers are dependent on estrogen—a hormone prevalent in females—which binds to the estrogen receptor. Estrogen drives excess proliferation of cells in which estrogen receptors are overexpressed, causing breast cancer. Estrogen receptor overexpression can be targeted by endocrine treatments, however, therapy resistance eventually develops in most cases. The four major methods of endocrine therapy are Aromatase Inhibitors (AIs), Luteinizing Hormone-Releasing Hormone Agents (LHRHs), Selective Estrogen Receptor Degraders (SERDs), and Selective Estrogen Receptor Modulators (SERMs). Treatments can be administered either adjuvantly—after primary surgery to further suppress the remaining breast cancer cells—or neoadjuvantly—prior to surgery to try and shrink the tumor. Still, resistance to these therapies occurs over 30% of the time after 2-3 years. There are multiple mechanisms that can cause resistance to endocrine therapy. These mechanisms include the upregulation of HER2 and the associated PI3K/AKT and MAPK/ERK pathways, action of the AIB1 coactivator, dysregulation of Cytochrome P450, overexpression of FOXA1 and IL-8, overexpression of NFκB, and the cyclin D/CDK4/6 complex. Two major clinical trials have been studied over the past decade for the treatment of endocrine therapy resistance, which resulted in FDA-approved interventions. These are the BOLERO-2, PALOMA-1, and PALOMA-3 trials, which have indicated that mTOR and CDK4/6 inhibitors, respectively, can reduce the resistance to endocrine therapy in ER+ breast cancer patients.

1. Introduction

Cancer, a disease which affects millions of people around the world, stems from the excessive proliferation of cells and lack of apoptosis, causing mutations in cells and leading to the formation of tumors almost anywhere in the human body. Research has identified six major characteristics that contribute to the development of cancerous tumors, which are: "sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis", (Figure 1) (Hanahan and Weinberg, 2011). Each hallmark shows part of the progression of tumor formation, ultimately leading to malignancy, and gives a more complete understanding of the development of cancer. The first characteristic is sustaining proliferative signaling, which could be considered the major difference between cancerous cells and normal cells. Normal cells in the body grow, develop, and divide normally through the cell cycle, and are specialized, so they have specific roles and functions. Cancer cells ignore the regulating signals and continuously divide with the help of growth factors that bind cell-surface receptors. Following excessive proliferation is the hallmark of evading growth suppressors. Cancer cells not only grow and divide with growth factor signals, but they ignore growth suppressors whose job is to regulate proliferation and limit cell growth. Two important tumor suppressors encode the retinoblastoma-associated (RB) and TP53 proteins. The RB protein receives outside

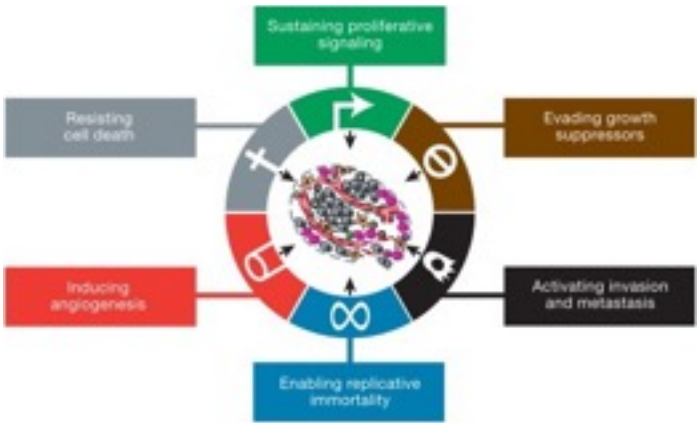

Figure 1: The six hallmarks of cancer (Adapted from Hanahan and Weinberg, 2011)

signals and determines whether each cell should continue on in the cycle or move on to apoptosis, but cancer cells have mutations in the RB pathway and allow every cell, even mutated ones, onto the next phase. The TP53 proteins function with signals inside the cell and can stop the cell cycle and induce apoptosis when necessary. A mutation in TP53, however leads to proliferation as well as a lack of apoptosis. The third hallmark is resisting cell death, or the natural occurrence of apoptosis in the body. Normal cells have signals that can lead to apoptosis when it is clear there is an excess of cells or one has a mutation, but high levels of anti-apoptotic proteins—BCL2—and suppressing pro-apoptotic proteins—BAK, BIK, and caspase 9—lead to excess proliferation and tumor formation. Hallmark number four, enabling replicative immortality, describes the lack of one of two important processes involved in normal cells: senescence—the loss of a cell's power of growth and the irreversible halt of proliferation—and crisis—ultimate cell death. While most cells undergo either of these processes after a certain amount of growth and division, cancer cells immortalize, and can grow and divide without senescence or crisis. The fifth hallmark of cancer is inducing angiogenesis, the formation and development of new blood vessels. In normal tissues, angiogenesis is activated under specific circumstances, for example, in wound healing and female reproductive cycling (Hanahan and Weinberg, 2011). But angiogenesis is abnormally promoted by cancer cells, forming new blood vessels at an excessive rate. In recent studies, angiogenesis has been found to contribute to the development and progression of tumors in both animals and humans. The final hallmark of cancer is activating invasion and metastasis, which usually occurs in a sequence of steps, or a cascade. Once all five of the previous hallmarks have occurred within a human body, the final step is invasion of cancer cells into the blood and lymphatic vessels, through the lymphatic system, and tumor formation in sur-

rounding tissue and organs in the body. This process is called metastasis, whereby cancer can spread from the primary site to almost any part of the body (Hanahan and Weinberg, 2011). In general, the treatment for metastatic cancer includes controlling the growth of the cancer and to relieve the symptoms caused by the cancerous mass, but the damage done by metastatic tumors is often irreversible, and most cancer-related deaths are due to metastatic cancer. To determine how far the cancer has progressed, doctors diagnose each patient's disease in stages. Stage 1 or 2 refers to cancer that has not spread beyond its primary site, whereas stage 3 or 4, with 4 being the highest, indicate that the cancer has spread to many organs or parts of the body (cancer.gov).

2. Background of Breast Cancer

2.1 Statistics

While heart disease ranks as the leading cause of death in the United States, cancer is rapidly approaching, and on the verge of taking over that number one spot. Although each patient's experience with the disease is unique, and may not directly reflect each statistic, it is important to gain a general idea of diagnoses, prognoses, and more, in order to help future patients, as well as healthcare professionals, governments, and researchers. In 2016, an estimated 1,685,201 new cases of cancer were diagnosed in the United States and 595,690 people died from the disease. Based on 2008-2012 cases, 454.8 per 100,000 men and women were diagnosed with cancer, and 171.2 per 100,000 men and women died. Cancer mortality is higher among men than women (207.9 per 100,000 men and 145.4 per 100,000 women) and it is highest in African American men (261.5 per 100,000) and lowest in Asian/Pacific Islander women (91.2 per 100,000). Approximately 39.6% of men and women will be diagnosed with cancer at some point during their lifetimes, and in 2014, around 15,780 children ages 0-19 were diagnosed with

cancer and 1,960 died. Although these numbers are astounding, and it is clear that many people in the United States are affected by this disease, the overall cancer death rate has declined since the 1990's. Since September 2016, studies have shown that cancer death rates decreased by 1.4% per year in women and children, and 1.8% per year in men. Still, while many individual cancer types have decreased in number of new cases, specific kinds of cancer, including melanoma in men and women, prostate, kidney, liver, and bladder cancers in men, and lung breast, uterine, and thyroid cancers in women, are likely to increase in number of cases in the United States. (cancer.gov)

As previously mentioned, over 1.5 million people were diagnosed with cancer in 2016, and around the same number of diagnoses are estimated to have occurred in 2017. Out of those 1.5 million people, it is estimated that about 252,710 women will be diagnosed with invasive breast cancer, along with 63,410 diagnosed with non-invasive breast cancer. In other words, about 1 in 8 U.S. women, or 12%, will be diagnosed with invasive breast cancer in their lifetime, and about 40,610 women in the U.S. are expected to die in 2017. Approximately 1% of breast cancer diagnoses are in men. Breast cancer death rates are higher in U.S. women than those for any other cancer besides lung, and breast cancer is the most commonly diagnosed cancer in U.S. women. (breastcancer.org)

2.2 Anatomy of the Breast

It is clear that breast cancer has affected so many people, both men and women, and although it is treatable if detected early, there is still no cure. Prior to discussing treatment methods and whether or not we are any closer to finding a cure, it is important to understand exactly what breast cancer is and why it occurs. In order to understand this, we must first familiarize

ourselves with the anatomy of a normal breast. The female breast is made up of fat cells called adipose tissue, which extends from the collarbone down to the underarm and across to the middle of the ribcage. Within the breast there are 15-20 sections called lobes, which are made up of many smaller, milk-producing glands, called lobules—highlighted in pink in Figure 2—that are connected to the nipple by milk ducts. Nerves, blood vessels, and lymphatic channels to the lymph nodes make up the rest of the breast. Breast cancer usually develops in the lobes, lobules (lobular carcinoma), or ducts (ductal carcinoma) within the breast, and the type of cancer is determined by the origin of the growth of cancerous cells. Breast cancer usually begins when cancer cells invade healthy breast tissue, forming a tumor in the breast. Once the cancer cells are prominent in the breast, they can spread to the lymph system, which is a network of lymph vessels throughout the body that connects lymph nodes, small organs that filter our foreign substances in the body. The detection of cancerous cells in your lymph nodes is usually a clear hint that the cancer has metastasized and the cancer has spread to other organs. (webmd.com)

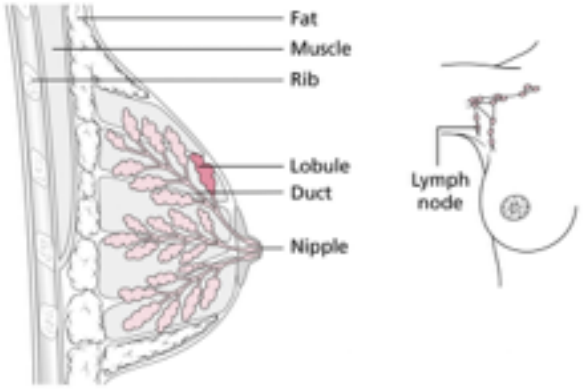

Figure 2: Basic anatomy of the breast, including lymph node (Image © Patient Pictures)

2.3 Risk Factors

Although there is currently no clear cause of breast cancer, there are many known risk factors; some of which can be changed, some which cannot. For example, just being a woman is the largest risk factor for developing breast cancer. As previously mentioned, over 250,000 women are diagnosed with breast cancer every year, and while men do develop breast cancer,

there are only around 2,000 cases in men diagnosed every year, or under 1%. The reason for increased rates of breast cancer in women versus men is due to the fact that women's breast development is usually complete by age 14, whereas male breasts do not usually fully form. This means that male breasts are made up of fat, not formed glands—a common location within the breast where cancerous cells form a tumor. Unlike women, the male breasts are not exposed to common growth hormones, such as estrogen and progesterone. Another risk factor that is static is age; the risk of developing breast cancer increases as you get older. While breast cancer in women age 45 and younger occurred about 1 in 8 times, it was found in women aged 55 or older 2 out of 3 times. This is because the older we get, the more opportunities for genetic damage or mutations occur. Yet another risk factor that you cannot change is one's family history. There is a higher risk of developing the disease if a woman's close female relatives have been diagnosed. A woman with a first-degree female relative (sister, mother, daughter) who has been diagnosed with breast cancer has double the risk of developing the disease, and if two first-degree female relatives have been diagnosed, the risk is 5 times greater. One final risk factor of breast cancer that you cannot change is genetics. While most cases of breast cancer occur due to mutations in the body, about 5% to 10% of breast cancers are hereditary, or passed from parent to child. The most common genes affected in inherited cases of breast cancer are mutations in the Breast Cancer Susceptibility Genes 1 and 2, or *BRCA1* and *BRCA2* genes. These genes' function is to repair DNA damage; but when mutant alleles of the gene are passed down from parent to child, they don't act properly in DNA repair, and thus may cause up to 10% of all breast cancers. Women with an abnormal *BRCA1* or *BRCA2* gene can have up to an 80% risk of diagnosis. (mcancer.org); (breastcancer.org)

There are also significant risk factors that one can change to reduce the risk of developing breast cancer. These include being overweight, drinking alcohol and smoking. All three of these can increase a women's risk of breast cancer. Being overweight can attribute to the development of breast cancer because fat cells make estrogen, and estrogen binding to a estrogen receptor-overexpressing breast cancer cells can help in their transformation to the cancerous phenotype. Alcohol can also increase estrogen levels, which increases the risk of hormone-receptor-positive breast cancers. Smoking is a known cause of many cancers, and it can also increase complications from breast cancer treatment. (mcancer.org); (breastcancer.org)

2.4 Subtypes of Breast Cancer

Breast cancer can be separated into molecular subtypes to better specify the disease and determine the best next step in treatment. These four major subtypes are luminal A, luminal B, basal-like, and HER2 type. Luminal A tumors occur in 30-70% of breast cancers, and they are usually estrogen receptor (ER) positive and human epidermal growth factor receptor 2 (HER2) negative, which will be discussed later in this paper. Luminal A tumors tend to be the most receptive to cancer drugs and hormone therapy, and therefore have the best prognosis and higher survival rates. Luminal B tumors are usually ER positive and HER2 positive or negative, and they occur in 10-20% of breast cancer cases. Luminal B tumors have a worse prognosis than luminal A tumors because they tend to be larger tumors and lymph node positive, but they still have fairly high survival rates. Basal-like tumors are found mostly, but not always, in triple-negative cancer, and about 15-20% of all breast cancers are basal-like. Triple-negative and basal-like tumors have a poor prognosis compared to the ER positive breast cancers. These tumors are also usually ER negative, and often lymph node positive. About 5-15% of breast cancers are

HER2 type. Prior to the discovery and FDA approval of HER2-targeting treatments, HER2 type had a very poor prognosis, but with the use of these drugs, the prognosis has improved. (komen.org)

3. Estrogen and Estrogen Receptor

Once a patient is diagnosed with breast cancer, the next step is to determine whether the cancer is estrogen-receptor positive (ER+), HER2+ or progesterone-receptor-positive (PR+). Testing is important to determine what treatment plan will work best for the patient. Specific plans, for example hormone therapy, have the possibility of slowing down or even arresting the growth of breast cancer cells expressing hormone receptors, but hormone-receptor-negative cells will not be affected by this type of therapy. Prior to discussing treatment methods, or even how hormones—specifically estrogen—affects breast cancer cells, it is important to learn what estrogen is and its functions within the body. Estrogen is a hormone that promotes the development and maintenance of female characteristics in the body, including breasts, pubic and armpit hair, and the regulation of the menstrual cycle and reproductive system. Estrogen dictates specific tissues in the body to behave in a certain way, starting in females around puberty, when the ovaries release estrogen during their monthly cycle. Once the hormone is released, it will travel through the bloodstream, and relay messages throughout the body when needed. Although estrogen is mainly found in women's bodies, it is also found in men, albeit in much lower levels. Estrogen primarily benefits organs found in the female body, including the ovaries, vagina, and uterus. It helps develop women's sex organs, increases fat storage around the thighs and hips of women to determine a more curved body shape, and even stimulates the muscles in the uterus that cause contractions during labor and delivery. Estrogen isn't only important when found nat-

urally in the body; synthetic estrogen has been created and used for many different medical objectives. The main uses for estrogen are the birth control pill—which prevents ovulation, regulates the menstrual cycle, and may even reduce hormone-related acne—and hormone replacement therapy, which relieves symptoms of menopause by elevating the hormone levels in females (hormone.org, 2014).

Now that we've described the functions and importance of estrogen, it is vital to understand exactly how estrogen relays these important messages to the different organs of the body. Estrogen hormones act through hormone receptors, which are special proteins found within cells throughout the body. Estrogen receptors are commonly found in normal breast cells. When bound to estrogen receptor, estrogen will turn on the transcriptional activity of the estrogen receptor in control of growth, function, and maintenance of breast cells. Increased proliferation of breast cells, which eventually increases cell growth and division, can cause accumulation of mutations that could lead to breast cancer. Excess estrogen can also produce genotoxic waste, and both processes will then lead to abnormal cell growth, disruption of apoptosis, and lack of DNA repair, which can cause tumor formation. ER+ breast cancer is the most common breast cancer diagnosed today, and according to the American Cancer Society, about 2 of every 3 cases of breast cancer are hormone receptor positive, and about 80% of breast cancers are specifically ER +. There are two types of estrogen receptors, and they are clearly expressed differently in different types of tissues throughout the body. The human ERα gene is located on chromosome 6, and the ERβ gene is located on chromosome 14; the two proteins indeed have very distinct functions. While ERα is expressed in much greater quantities in women, men still have low levels, and a balanced expression of that coupled with ERβ expressions is required for normal development of

male reproductive tissues. Research has found that ERα is found more in breast cancer cells, ovarian cells, and the hypothalamus, while ERβ cells are expressed more in kidney, brain, bone, heart, lungs, and prostate. Therefore, ERα is the important hormone receptor regarding breast cancer, and for the purpose of this paper, we will be referring to ERα merely as ER (Hye-Rim, et al., 2012).

4. Diagnosis and Treatment

In order to confirm whether or not a patient has hormone-receptor positive breast cancer, a biopsy of the cancerous tissue is subjected to a special staining process, called ImmunoHisto-Chemistry (IHC). This process determines how many hormone receptors show up in the sample; the tumor will be hormone receptor positive if at least 1% of the cells have estrogen or progesterone receptors. Once the IHC staining process is completed, and if the patient is diagnosed with ER+ breast cancer, the next step is proper treatment of this type of cancer. Although there is no cure as of now for any type of cancer, ER+ breast cancer has very high odds of being successfully treated, especially when detected in the early stages. Women diagnosed with ER+ breast cancer will usually require hormone therapy, also called endocrine therapy. This usually occurs after surgery, chemotherapy, and radiation, to help reduce the risk of the cancer reoccurring, but can also occur prior to surgery at times. There are different surgical options, including a lumpectomy, which removes breast tissue but not the entire breast, and a mastectomy, which removes the entire breast. Often, removal of cancer cell positive lymph nodes from under the arm is necessary. Hormone therapy is usually taken for at least five years. While there are several types of hormone therapy, they all have the same goal: to keep estrogen from helping the cancer grow, either by lowering estrogen levels in the body or blocking estrogen from binding to the breast

cancer cells. Two significant treatments that lower estrogen levels are Aromatase inhibitors (AI's) and Luteinizing hormone-releasing hormone agents (LHRH's), and two that block estrogen are selective estrogen-receptor response modulators (SERM's) and selective estrogen-receptor degrader (SERD's) (Shah, et al, 2014).

4.1 Aromatase Inhibitors

Aromatase inhibitors, or AI's, stop estrogen production to lower total estrogen levels in the body in postmenopausal women. Before menopause, the ovaries generate most of the estrogen in a woman's body, but after menopause—when the ovaries stop producing estrogen—the hormone androgen is converted to estrogen in the fat tissue by an enzyme called aromatase. AI's work by blocking aromatase, so less estrogen is synthesized—and therefore, available—to stimulate the growth of ER+ breast cancer cells. AI's do not stop the ovaries from producing estrogen, so these medications will not be helpful in premenopausal women with ER+ breast cancer (Lonning, 2010). Specific Aromatase inhibitors include Femara (chemical name: letrozole), Arimidex (chemical name: anastrozole), and Aromasin (chemical name: exemestane). It is recommended that postmenopausal women with ER+ breast cancer take AIs, either alone or with another hormone therapy treatment, for at least five years. AI's have few side effects, most of which are not very serious. It can, however, cause muscle pain and joint stiffness or pain, and possible bone thinning due to the lack of estrogen in the body, which can lead to osteoporosis and fractures. When taking AI's, it could be beneficial to take additional medication to strengthen your bones (healthline.com, 2017).

4,2 Luteinizing Hormone-Releasing Hormone Agents

The second type of hormone therapy to lower estrogen levels is called Luteinizing hormone-releasing hormone agents, or LHRH's. LHRH's will shut down and stop the function of the ovaries in pre-menopausal women, in order to stop the production of estrogen. A lack of estrogen available means less growth of ER+ breast cancer. LHRH's are usually injections that are given once a month for several months, and once the therapy is no longer needed, the ovaries can begin functioning again. This is the "best case" method of ovarian suppression, because it can be reversed and the ovaries can function once again after the injections. Other methods of ovarian suppression include an oophorectomy, which is the permanent removal of the ovaries, or chemotherapy drugs, which can damage the ovaries and prevent it from producing estrogen. Examples of LHRH's are Zoladex (chemical name: goserelin), Lupron (chemical name: leuprolide), and Trelstar (chemical name: triptorelin). The side effects of using LHRH's as hormone therapy are similar to many symptoms of menopause, including hot flashes, night sweats, and mood swings (Goel, 2009).

4.3 Selective Estrogen Receptor Degraders

The other important type of hormone therapy for ER+ breast cancer is reducing the levels of estrogen receptors in breast cancer cells. One way to block the receptors is with selective estrogen receptor degrader, or SERD's. SERD's bind directly to the receptors, causing them to fold abnormally, blocking their activity and targeting them for degradation. One type of SERD is Faslodex (chemical name: fulvestrant), which can either be used as the first-line endocrine therapy treatment in ER+, advanced stage breast cancer, or used to treat metastatic breast cancer in postmenopausal women, after other hormone therapy drugs have stopped working (Boer, 2017). SERDs are administered by injections, every two weeks for the first month, then monthly there-

after. Common side effects can be similar to menopausal symptoms, along with bone pain (due to the blocking of estrogen action) (cancer.org).

4.4 Selective Estrogen Receptor Modulators

The second method of hormone therapy that inhibits the receptors is called selective estrogen receptor modulators, or SERM's. SERM's bind directly to the estrogen receptor to compete with estrogen, thereby blocking the estrogen effects. SERM's are used for women in all stages of breast cancer. For women with ER+ breast cancer that have already undergone surgery, it can help lower the chances of the cancer recurrence, and help lower the risk of developing cancer in the other breast. For women with hormone receptor-positive ductal carcinoma in situ undergoing treatment, SERM's will lower the chance of the cancer recurring. For women whose ER+ breast cancer has metastasized, SERM's can help slow or stop the growth of the cancer, with the potential to shrink some tumors. And finally, for women at high risk of breast cancer, SERM's can lower the risk of development. The most commonly used SERM to treat ER+ breast cancer is tamoxifen. In a study published in the *Journal of the National Cancer* Institute, it was shown that tamoxifen reduced the occurrence of ER+ tumors in patients by 69% (Fisher, 1998). Evista (chemical name: raloxifene) can also be used as a SERM, but is less common. The most common side effects of these SERM's include hot flashes, vaginal dryness, and mood swings, and the rare, but more serious side effects include blood clots or deep vein thrombosis, strokes in postmenopausal women, and an increased risk of developing uterine cancer in postmenopausal women (cancer.org).

4.5 HER2 Breast Cancer

Although eighty percent of all breast cancers are ER+, 65% of which are also PR positive, there are other types of breast cancer, each with its own treatment plan. Twenty percent of all breast cancers are human epidermal growth factor receptor 2—or HER2—positive. The HER2 gene makes HER2 proteins, which are receptors on breast cells that help control how a healthy breast cell grows, divides, and repairs itself. If a patient has HER2 positive breast cancer, HER2 gene amplification has occurred, where too many copies of the gene are present. This will lead to too many HER2 receptors being made, which is called HER2 protein overexpression (Kumar, 2017). HER2 amplified breast cancer can be found and diagnosed in a number of ways. The IHC test, as mentioned above, determines the amount of HER2 protein in the cancer cells, while the Fluorescence In Situ Hybridization (FISH), the Subtraction Probe Technology Chromogenic In Situ Hybridization (SPot-Light HER2 CISH), and the Inform Dual In Situ Hybridization (Inform HER2 Dual ISH) tests all determine the number of copies of the HER2 gene in the cancer cells. Once it is confirmed that the type of breast cancer is HER2 positive, there are many specific treatments available. Herceptin (chemical name: trastuzumab), and Perjeta (chemical name: pertuzumab), are monoclonal antibodies that bind to HER2 and inhibit its activity. Kadcyla (chemical name: ado-trastuzumab emtansive) is a combination of herceptin and emtansine, a chemotherapy agent, and work together to reach the HER2 positive cancer cells. Nerlynx (chemical name: neratinib) and Tykerb (chemical name: lapatinib) are small molecule inhibitors of HER2. Herceptin is the most commonly used drug for HER2 positive breast cancer, and is usually given along with chemotherapy after surgery (breastcancer.org).

4.6 Triple Negative Breast Cancer

If a patient has been tested for ER, PR, and HER2, and all test results are negative, the patient is diagnosed with triple-negative cancer. This means that the cancer is not caused by mutations in the hormones estrogen or progesterone, or the amplification of HER2 genes or overexpression proteins. About 10-20% of all breast cancers are triple negative, and this type of breast cancer will not respond to hormone therapy or HER2 specific treatments. Because of the lack of specificity in treatment when targeting the cancer cells, triple-negative cancer is, unfortunately, usually more aggressive than other types of breast cancers, and five-year survival rates also tend to be lower. A study from the Lehigh Valley Heath Network determined that in 224 triple-negative breast cancer patients, the overall five year survival rate was 78.1%, compared to about 93% in women with other types of breast cancer. Still, there are treatment options for triple-negative breast cancer, including chemotherapy and radiation, and more research is conducted to find more successful treatments (Skandan, 2017).

4.7 Adjuvant vs. Neoadjuvant Therapy

Different types and subtypes of cancer require different medication and treatment plans, which have been previously mentioned. But along with different treatments, it is necessary to determine whether to use adjuvant or neoadjuvant therapy. Because the more commonly used method is adjuvant, it is important to discuss it prior to neoadjuvant methods. Adjuvant treatment is administered after primary surgery to kill any remaining breast cancer cells, as well as to prevent the cancer from returning. Adjuvant hormonal therapy is more specifically targeted for patients with ER+ breast cancer, to either lower the levels of hormones in the body (AI's and LHRH's) or block the receptors in breast cancer cells (SERD's and SERM's). A study of tamox-

ifen therapy, for example, showed that tamoxifen resulted in a 47% reduction of recurrence and a 22% reduction in mortality if taken for 5 years. Tamoxifen is often prescribed after radiation therapy and/or chemotherapy to remove existing cancer cells post-surgery and prevent relapse. Adjuvant radiation is only recommended for patients with a high risk of relapse, and while it should reduce the risk of recurrence, other risks prevent studies from finding an increase in overall survival. Adjuvant chemotherapy has been associated with a 10 year reduction in recurrence of 35% and a 10 year reduction in mortality of 27% in women younger than 50 years old, and although the percentages are lower in women ages 50 to 69, there is still significant reduction in recurrence and mortality. If the oncologist decides that chemotherapy is necessary along with hormonal therapy, it means that the doctor and patient agreed that the breast cancer is severe enough to accept that higher rate of side effects. Adjuvant therapy is frequently used because in surgery, doctors can quickly determine the stage, location, and size of the tumor or tumors that make up the cancer. Adjuvant therapy also allows for surgery soon after diagnosis, based on the premise that the quicker cancer cells are removed from the body, the better. With these specific circumstances for each patient, the treatment team can properly create a plan to best help each individual (Chew, et al., 2001).

Compared to adjuvant therapy, which targets remaining cells post-surgery and prevents the cancer from reoccurring, neoadjuvant therapy is administered prior to surgery to try and shrink the tumor and treat the cancer. At times, if the neoadjuvant therapy is successful, a patient's tumor may shrink, allowing major surgeries to be reduced to minor procedures and giving doctors more surgical options. For example, a woman who might have needed a mastectomy—removal of the entire breast—due to her tumor size, may be able to shrink her tumor with neoad-

juvant therapy and reduce it to a lumpectomy—removal of breast tissue. Neoadjuvant therapy is also indicative of the response to adjuvant therapy. Neoadjuvants are also important to target smaller lumps of breast cancer cells, called micrometastases, that wouldn't normally be seen by a doctor. Treating micrometastases with neoadjuvant therapy will prevent them from returning and growing into tumors, causing a patient to relapse. As specified in Figure 3**,** an indicator of good response to neoadjuvant endocrine therapy is tumor shrinkage and absence of cancer cells in the lymph nodes, whereas a poor response shows no tumor shrinkage, and cancer cells remain in the lymph nodes, leading to possible metastasis. Examples of neoadjuvant therapy are radiation therapy, chemotherapy, and hormonal therapy (West, 2015). Pertuzumab, as mentioned earlier, blocks cancer cells from receiving signals they need to grow and divide, and was the first medication approved by the FDA for neoadjuvant therapy for breast cancer in September 2013. This drug is specifically used for women with HER2 positive breast cancer. (Newton, 2017) Although neoadjuvant therapy seems to be extremely beneficial, it has not been used much to treat breast cancer because of its very recent introduction into treatment plans for breast cancer, as well as the lack of experience in the United States. Many clinical trials are showing that preoperative therapy can shrink the size of the tumor, allowing for easier access in surgery, but most doctors still recommend the classic adjuvant therapy that has proven to give the most successful results.

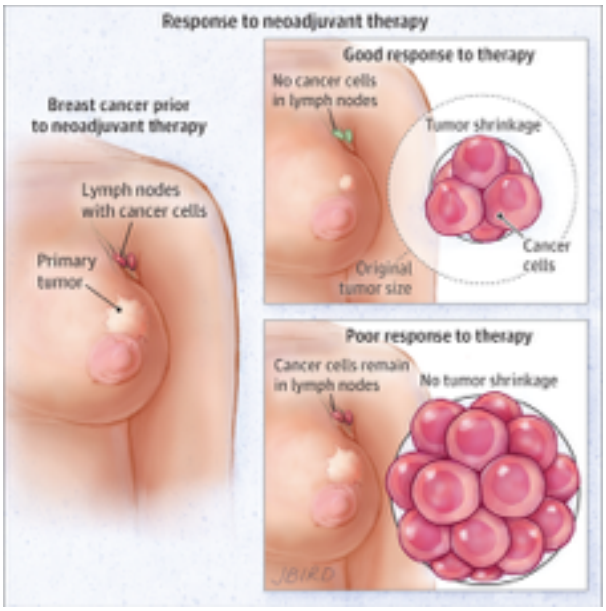

Figure 3: Visual representation of positive and negative responses to neoadjuvant therapy, including cancer cells in the lymph nodes

5. Mechanisms of Anti-Estrogen and Endocrine Therapy Resistance

Despite the progress that has been made in finding and diagnosing the correct form of breast cancer, and understanding which type of therapy to prescribe to a patient, about 30% of all patients have recurrent disease, meaning the cancer will come back, indicating some form of resistance to the endocrine therapy. Resistance to hormone therapy is, unfortunately, an extremely common occurrence in breast cancer patients, and can lead to disease progression and death. Many patients with advanced or metastatic ER+ breast cancer end up developing resistance to endocrine therapy within 2-3 years of starting. Resistance can be classified in two different ways: primary—or de novo—resistance, and acquired resistance. De novo resistance indicates that the cancer never responded to the drug administered, and there was never any shrinkage of the tumors. Acquired resistance means that the patient initially responded to the medication, and shows significant shrinking of tumors, before the disease ultimately progressed. Studies have determined that different mechanisms of endocrine therapy resistance exist. Because tamoxifen and aromatase inhibitors are the most commonly used therapies to target ER+ breast cancer, the most research has been done to learn about resistance to these types of therapies, as well as methods to best combat said resistance. While there is no one confirmed reason for endocrine therapy resistance, there are many mechanisms that could contribute to the resistance, most of which have been discovered in the past decade.

5.1 HER2 in PI3K/AKT and MAPK/ERK Pathways

One of the most commonly discussed mechanisms of resistance specifically focuses on tamoxifen. Tamoxifen resistance is majorly contributed by growth factors, including the human epidermal growth factor receptor 2—HER2—which affects two major pathways. The crosstalk

between estrogen receptors and the HER2 and other growth factors is crucial to the development of endocrine therapy resistance. Before discussing its significance in hormone resistance in breast cancer, it is important to understand both the Phosphatidylinositol-3-Kinase/Protein Kinase B (PI3K/AKT) and the Mitogen-Activated Protein Kinases/Extracellular Signal-Regulated Kinases (MAPK/ERK) pathways that have been shown to cause tamoxifen resistance, as shown in Figure 4. The PI3K/AKT pathway is important in regulation of the cell cycle, and is directly related to proliferation and, therefore, cancer. PI3K activates AKT, which is a regulator of growth and survival pathways in the cell that may mediate therapy resistance. One important factor which will be discussed later is mammalian target of rapamycin complex 1 (mTORC1), which is a protein complex that controls protein synthesis. The PI3K/AKT pathway plays an important role in cancers because an upregulation of the pathway can lead to proliferation and lack of apoptosis of cancer cells. PI3K is controlled by the main PI3K heterodimer, made up of subunits p85 and p110, of which the former regulates the activation of the latter when upstream growth factor receptor tyrosine kinases (RTSs) are activated. PI3Ks lead to activation of AKT, and is important in cancer cell survival and growth. Studies show that AKT can activate the ER pathway independent of estrogen, leading to proliferation of cancer cells regardless of the availability of estrogen (Carnero, 2008). The MAPK/ERK pathway is a signaling cascade that sends a signal from the receptor on the surface of the cell into the DNA in the nu-

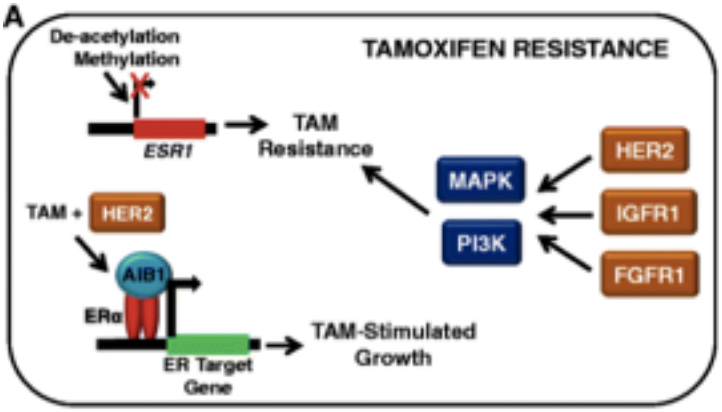

Figure 4: Mechanisms of tamoxifen resistance through MAPK and PI3K pathways, and when bound to coactivators (AIB1) (Adapted from Hayes and Lewish-Wambi, 2015)

cleus of the cell. The signaling is relayed by a kinase-mediated phosphorylation cascade. This pathway contributes to cancer when one of the proteins in the pathway becomes mutated and are either permanently phosphorylated or dephosphorylated, causing the protein to either remain "on" or "off". The PI3K/AKT and the MAPK/ERK pathways greatly contribute to tamoxifen resistance due to the upregulation of growth factor signaling pathways, and increase proliferation and decrease apoptosis (Cargnello, et al., 2011).

5.2 AIB1 Coactivator

Another mechanism of endocrine therapy resistance to tamoxifen is mediated by Amplified in breast cancer 1, or AIB1, and other coactivators in breast cancer. Tamoxifen, as used in endocrine therapy, is an antagonist, blocking estrogen receptors in breast cancer cells so estrogen cannot bind. But at times, AIB1 will act as a coactivator protein—as shown in Figure 3—and will contribute to agonistic activities of tamoxifen, leading to tamoxifen resistance and tamoxifen-dependent cell proliferation, especially in the presence of HER2 signaling. Because of this research that discovered the agonistic properties of tamoxifen when expressed with AIB1, the coactivator is considered an oncogene whenever overexpressed (Hayes, et al., 2015).

5.3 Dysregulation of Cytochrome P450

A third mechanism of resistance to tamoxifen is due to the dysregulated metabolism of the drug. When tamoxifen enters the body during hormone therapy, enzymes CYP2D6 and CYP3A4 in cytochrome P450 in the liver convert tamoxifen into 4-hydroxytamoxifen and endoxifen, its normal and active metabolites that have a much greater affinity to inhibit estrogen-dependent proliferation than nonactive tamoxifen. These metabolites allow for the proper treatment, but when the cytochrome P450 proteins—specifically CYP2D6—changes its form, it can

cause poor metabolic activity, which results in worse clinical outcomes of tamoxifen treatment, and more resistance toward the drug. In a study with 80 women with breast cancer who were taking tamoxifen for adjuvant hormone therapy, it was found that the concentrations of endoxifen was significantly lower for those with a defective CYP2D6 gene compared to those with both functional proteins (Rodriguiez-Antona, et al., 2006).

5.4 Overexpression of FOXA1 and IL-8

The overexpression of the Forehead box protein A1 (FOXA1) gene and increased production of IL-8 are two changes in the cell that attribute to a newer mechanism that can potentially explain the resistance to hormone therapy. This was determined in ER+ breast cancer cell lines that were initially responsive to hormone therapy, and developing new cell lines that were resistant to tamoxifen therapy. The genetic differences between the resistant and non-resistant cell lines were compared, and it was determined that there was more FOXA1 protein in the resistant ones than the parental, non-resistant ones. This is because FOXA1 promotes the expression of other genes, and the overexpression of FOXA1 activates overexpression of genes that have the potential to cause metastasis, for example, ones that cause cell development and proliferation. One of the genes that is activated by the overexpression of FOXA1 is IL-8, which allows for cell survival. Once FOXA1 protein expression was reduced in the resistant cells, they became susceptible to tamoxifen therapy, perhaps because of the decrease in the IL-8 expression, and therefore, increased apoptosis (Fu, 2016).

5.5 Overexpression of NFκB

NFκB, a protein complex which regulates transcription of DNA and cell survival, has been linked to cancer and endocrine therapy resistance. NFκB can also promote proliferation

and block apoptosis at times, through crosstalk with ER. Many studies have shown a correlation between increased NFκB and tamoxifen resistant cells, although it is not yet confirmed which of the many theories is correct in determining why an increase of these proteins can causes resistance. NFκB p50-p65, a heterodimer, was found as the most abundant form, and resistant cells exhibit an upregulation of the dimer, promoting more proliferation and less apoptosis. There are also increased phosphorylation levels of p65 at serine-536, which is important for proper function and activity of the molecule, but also leads to tamoxifen resistant cells. Tumor associated macrophages, or TAMS, are involved in the initiation and progression of tumor cells, and about 50% of tumors in some patients are made up of TAMs. Studies have shown that increased macrophage presence in breast tumors lead to increased resistance and decreased overall survival, compared to cancer cells with no TAMs. TAMs make up an important immune cell signaling protein, or cytokine, called tumor necrosis factor alpha, or TNFα, which leads to the activation of NFκB. Following TNFα stimulated proliferation in ER+ cell lines, NFκB p65 is phosphorylated at serine-536, which has been found to significantly increase tamoxifen resistant cells in the body (Dixon, 2014). More recently, scientists found that TNFα and another cytokine, interleukin 1 beta (IL1β), activate phosphorylation of serine-305 as well, which can change the conformation of the estrogen receptor. This will not only reverse tamoxifen effects on breast cancer cells, but will also increase invasive properties of the cancer cells (Stender, et al., 2017).

5.6 Cyclin D/CDK6

The next mechanism of endocrine therapy resistance is related to the cell cycle and activation and expression of the genes that drive it. To fully understand this mechanism, it is important to know background on the cell cycle and its stages. The cell cycle refers to the process in

which a cell is induced to divide to produce two daughter cells. In order for a cell to divide, it must first make a copy of its DNA, or genetic material, and to segregate the replicated chromosomes into two daughter cells. There are many genes and proteins that allow for this cycle to occur, and ensure that each step happens consistently at the proper time and place. Eukaryotic cells have two phases within the cycle, interphase—where the cell grows and makes a copy of its DNA—and the mitotic, or M phase—chromosome and cell separation into two daughter cells. Interphase has three subdivisions: the G1 phase, where the cell grows and copies organelles, the S phase, where another copy of the DNA is made and the centrosome is duplicated, and the G2 phase, where the cell continues to grow and prepare itself for mitosis. The mitotic phase occurs immediately after G2, and this is when the cell divides its chromosomes and cellular components and forms two new cells (Hunt, et al., 2011).

Much of the evidence for endocrine resistance is found in the upregulation of positive regulators and the downregulation of negative regulators in the G1 phase progression, which blocks the anti-proliferative effects of endocrine therapy. One very important protein synthesized during the G1 phase that drives the G1/S phase transition is cyclin D, a member of the cyclin protein family involved in regulating the cell cycle. Cyclin D activates and regulates cyclin-dependent kinases 4 and 6 (CDK4/6), and the cyclin D/CDK4/6 complex plays a major role in the cell cycle and its progression. Growth factors stimulate the pathways that induce cyclin D production, and cyclin D cannot be produced without them. Once a sufficient amount of cyclin D has been made and activated, it binds with the CDK4/6 complex to continue the cell cycle, because cyclin D alone has no effect on G1/S without CDK6, and vice versa. After the complex has formed, it phosphorylates Retinoblastoma protein (Rb)—which had been previously bound

to the E2F transcription factor. The phosphorylated Rb, which becomes pRb, can now separate itself from the E2F, and the transcription factor moves on and activates the proteins in the next stages of the cycle. Overexpression of cyclin D causes an increase in active complexes that drive the progression of the cell cycle, leading to tamoxifen resistance (Dixon, 2014).

In normal cells, proper regulation of the cell cycle is necessary to ensure that enough cells are growing and dividing in the human body, and there are many different points of regulation within each pathway. Cyclin-dependent kinase inhibitors (CKIs) negatively regulate the CDKs. One example of a CKI is the protein p27, which binds to CDK6 and inhibits the binding of cyclin D, and the formation of the complex. This prevents the complex from phosphorylating Rb, and causes cells to arrest in the G1 phase of the cell cycle. However, one of the many growth factors found along the pathway phosphorylates p27, which inhibits it from binding to CDK6 and leaves it open to bind to cyclin D and activate the complex. Inhibitors are necessary to keep from overexpression and increased proliferation, and the downregulation of inhibitors show clear evidence to cancer and endocrine therapy resistance (Chu, et al., 2008). Along with high expressions of cyclin D and low expressions of CKIs like p27, endocrine resistance usually develops when there are high levels of anti-apoptotic proteins, like B-cell lymphoma 2 (BCL2), and low levels of pro-apoptotic proteins, like BCL2 antagonist killer (BAK), BLC2 interacting killer (BIK), and caspace 9 (Dixon, 2014).

6. Treatments of Endocrine Therapy Resistance

6.1 BOLERO-2 Trial

Endocrine resistance remains a constant challenge for patients and doctors alike when treating ER+ breast cancer. As previously shown, there are multiple mechanisms related to en-

docrine resistance, many of which are still being discovered. Still, recent treatment methods have arisen, giving hope to the patients that a successful treatment plan can be provided without the fear of resistance. One important mechanism that is being targeted for possible treatment is within the mTOR pathway. mTOR, or mammalian target of rapamycin, is a serine/threonine protein kinase found downstream of PI3K and AKT, as shown in Figure 5, and technically refers to two complexes, mTORC1 and mTORC2, which have very different functions. In regard to endocrine therapy resistance, mTORC1 is the more studied complex, and therefore, mTORC1 will hereby be referred to merely as mTOR in this paper. In the pathway, AKT is phosphorylated and activated, and that inhibits tuberous sclerosis complex 1/2, or TSC1/2, which binds to a G protein Rheb and activates it, converting Rheb-GDP to Rheb-GTP. Rhea-GTP activates mTOR, which activates and regulates proteins synthesis, proliferation, and suppresses apoptosis. mTOR complex consists of Raptor, mLST8, and proline-rich AKT substrate 40 (PRAS40). Because mTOR plays such an important role in proliferation and growth of cells within the PI3K/AKT pathway, an activating mutation or overexpression within the pathway can lead to the growth and development of cancer cells and tumor formation, as well significant endocrine resistance. A recent study, called the BOLERO-2 trial, has shown positive results when aiming to treat and prevent endocrine resistance through mTOR inhibitors. The mTOR inhibitor rapamycin (everolimus)

Figure 5: mTOR pathway and its upstream and downstream targets (Adapted from Malley and Pidgeon, 2016)

combined with the aromatase inhibitor exemestane were tested in postmenopausal patients with ER+ advanced breast cancer. FKBP12, or FK506 binding protein 12, binds directly to rapamycin and inhibits mTOR, limiting proliferation. Results showed that progression-free survival had increased with the inhibitor and the AI, compared to a placebo with the AI. Everolimus was previously an FDA-approved drug for other indications such as restinasis after angioplasty and immune suppression after kidney transplantation, paving way to approval for the breast cancer indication in July of 2012. For example, one trial showed that the addition of everolimus to letrozole in neoadjuvant setting showed a decrease in cancer growth compared to using letrozole alone. Another study found that tamoxifen with everolimus resulted in more progression-free survival than tamoxifen alone in postmenopausal, ER+ advanced breast cancer patients (Beaver, et al., 2013). Everolimus is taken orally once a day, and common side effects include infection, rash, nausea, and fever. Because there is a certain level of toxicity, it is important to discuss with a doctor the benefits of the drug before starting the treatment (chemocare.com).

6.2 PALOMA-1 and PALOMA-3 Trials

A second more recently discovered method is using CDK4/6 inhibiters to combat endocrine resistance. The drug palbociclib was approved by the FDA in February 2015, when taken in combination with the AI letrozole. As previously mentioned, the CDK4/6/cyclin D complex helps regulate the cell cycle in G1 to S, as can be seen in Figure 6, and the complex is downstream of many proliferation signals. Palbociclib has the potential to overcome multiple mechanisms of endocrine resistance involving the CDK/cyclin D complex, including overexpression of CDK4/6, overexpression of cyclin D, and activation of anti-apoptotic proteins. The discovery of effective CDK4/6/cyclin D inhibitors began in the 1970's, when research showed the importance of the

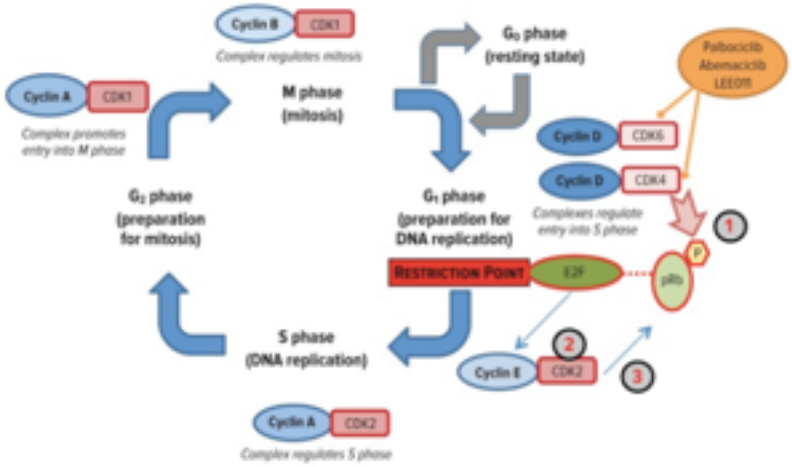

Figure 6: Cell Cycle Regulation via Cyclin-CDK complexes (Adapted from McCain, 2015)

complex as a regulator in the cell cycle, and continued in 1995, when the first trial of an inhibitor was being developed. After multiple tries and various forms of the drug, it was discovered that the drug worked best in ER+ breast cancer cell lines, and in 2009, the trial called PALOMA1 was launched, focused on postmenopausal women wither ER+, HER2- breast cancer who had not previously had treatment or surgery for the disease. Patients received a treatment of letrozole along with palbociclib, or letrozole alone, and results showed a progression-free survival of 20.2 months in patients who took both drugs, compared to 10.2 months with letrozole alone (McCain, 2015). Because of the success of PALOMA1, palbociclib became FDA approved, and researchers continued more trials, such as PALOMA3. PALOMA3 studied the effects of palbociclib and fulvestrant—a hormone therapy medication classified as a SERD—and fulvestrant alone in pre- and perimenopausal women with ER+ breast cancer who had undergone treatments that were not successful in halting the progression of the cancer. The trial was completed early due to the efficacy of the pairing of medication, and the two agents together have been approved by the FDA for ER+ breast cancer that has resisted endocrine therapy treatments (Finn, 2016). These successful trials proved that CDK6 inhibition is an effective method in treating endocrine therapy resistance.

7. Closing Remarks

These recent discoveries regarding the successes and failures of ER+ breast cancer treatment plans have allowed thousands of women to live longer and healthier lives, with options specifically targeted toward their type of cancer and their process of treatment. More patients are opting to try neoadjuvant therapy, and even with hormone therapy resistance, those patients are able to take advantage of the successes of the clinical trials BOLERO and PALOMA, either an mTOR inhibitor or a CDK/cyclin D complex inhibitor in combination with an aromatase inhibitor. Still, much is left to be learned about how breast cancer develops, and what can be done to properly treat those diagnosed with the disease. More and more work is done every day in order to learn as much as possible, to one day provide durable cancer treatment, and eventually find a cure.

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