

## **Induction of Tumor Immunity By The Use Of The HER2 Protein**

### **Introduction**

All cancer, no matter what the type, is the result of abnormal cell proliferation. While normal body cells grow, divide, and die in an orderly fashion, cancer cells continue to divide uncontrollably. During the early years of a person's life, normal cells divide more rapidly, and this process slows once an individual becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries. In contrast, cancer cells continue to grow and divide, outlive normal cells and continue to form new abnormal cells.

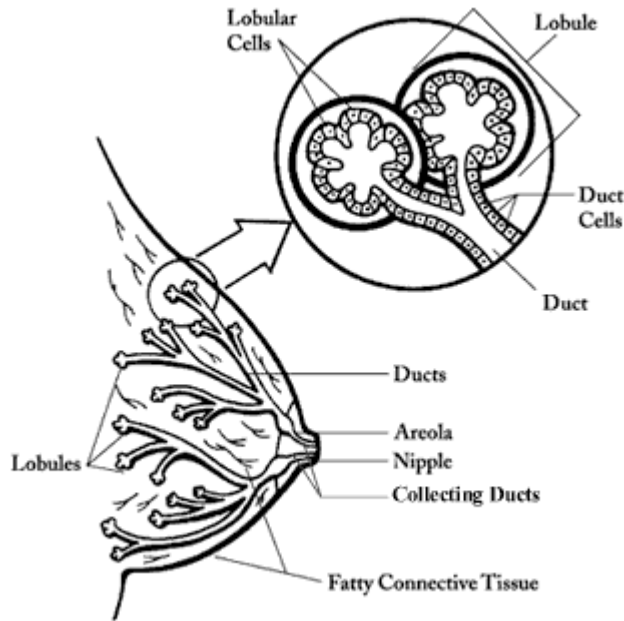
Cancer cells develop because of damage to DNA. While the body can typically repair such damage, the inability to do so results in cancer. Because of the relationship between DNA and cancer, it is possible for cancer to be inherited. However, generally a person's DNA becomes damaged by exposure to something in the environment, such as smoking. Cancer can result in tumors, although they are not always formed. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow.

Often, cancer cells travel to other parts of the body where they begin to grow and replace normal tissue; a process known as metastasis. Regardless of where a cancer may spread, however, it is always named for the place it began. For instance, breast cancer that spreads to the liver is still called breast cancer. Benign tumors do not spread to other parts of the body and are, with very rare exceptions, not life threatening.

Different types of cancer can behave very differently. For example, lung and breast cancer vary in that they grow at different rates and respond to diverse treatments. This is why patients need specialized treatments in order for it to be effective.

Cancer is the second leading cause of death in the United States. Half of all men and one third of all women in the United States will develop cancer during their lifetime, and millions are currently living with cancer or are in remission. The risk of developing most types of cancer can be reduced by changes in a person's lifestyle, such as quitting smoking and eating a better diet. The sooner a cancer is found and treatment begins, the higher the survival rates.

Breast cancer is the development of a malignant tumor from normal cells of the breast. It is the most common type of cancer in females worldwide. Although lung cancer is the most fatal cancer in women, breast cancer is the second most deadly. While it is commonly associated with females, males can also get breast cancer because both sexes' breasts are composed of the same tissue. The following figure depicts the structure of the breast:



[http://www.cancer.org/docroot/CRI/content/CRI\\_2\\_4\\_1X\\_What\\_is\\_breast\\_cancer\\_5.asp](http://www.cancer.org/docroot/CRI/content/CRI_2_4_1X_What_is_breast_cancer_5.asp)

The female breast consists of many lobules, or milk producing glands. The tiny tubes that carry the milk from the lobules out to the nipple are known as ducts. The most common types of breast cancers develop in these structures of the breast and the two most common types are Ductal carcinoma, which accounts for about 65-90% of all cases, and lobular carcinoma which accounts for close to the other 10%.

There are many risk factors that are associated with getting breast cancer. Epidemiological and etiological risk factors include age, alcohol consumption, and environmental causes. Genetics also play a role in the development of breast cancer. Two autosomal dominant genes, BRCA1 and BRCA2, have been linked to the rare familial form of breast cancer. People in families expressing mutations in these genes have a 60% to 80% risk of developing breast cancer according to Robbins Pathological Basis of Disease. If a mother or a sister was diagnosed with breast cancer, the risk is approximately 2-fold higher than those women without a familial history. Levels of hormones like estrogen, androgens and estradiol can all be contributing factors to breast cancer development. Late onset of menopause can increase the risk by about 4% each year that menopause is late. Other factors that increase risk of breast cancer include use of contraceptive pills before the age of 20, being over the age of 30 during first pregnancy, excessive amounts of fat intake, and even history of benign breast disease.

The HER2 surface protein receptor will be of significant importance because it plays a major etiological role in the development of breast cancer. This HER2 receptor is also known as ErbB-2 receptor because it belongs to a group known as the epidermal growth factor receptor family. The HER2 receptor can be found on the surface of the cell membrane and is associated with the receptor tyrosine kinases or RTKs. The RTKs are key regulators in normal cellular processes and, they also play a major role in the maturation and development of many types of cancers, especially breast cancer. Normally the HER2 receptor is involved in the process of cell growth and differentiation.

Following numerous studies, scientists believe that HER2 is an orphan receptor and there is no epidermal growth factor family of ligands that are capable of activating it.

The HER2 receptor is known as a proto-oncogene which is a normal gene that has the capability of becoming an oncogene caused by mutation or overexpression. When these proto-oncogenes are activated, they become specific oncogenes (also known as tumor inducing agents). The proto-oncogene or the HER2 gene becomes overexpressed, eventually leading to breast cancer.

It is known that about 25-30% of breast cancers are caused by this overexpression of the HER2's protein product and the amplification of the gene itself. When this occurs, the prognosis is serious and there is an increased risk that the disease will persist. Other types of cancers that are associated with these developments include ovarian cancer and stomach cancer.

The medication Herceptin can be effective in combating the increased expression of the receptor. Developed by Genentech, Herceptin kills cancer cells carrying excess HER2. Even though Herceptin was approved as a breast cancer treatment by the U.S. Food and Drug Administration in September of 1998, until only recently has one known how it interacted with the receptor. Since HER2 positive breast cancers tend to be more aggressive than other types of breast cancer, and they also do not respond very often to hormone treatment, Herceptin is used to target the HER2 in attempts to slow cancer growth and result in a reduction of the metastatic tumor size. This drug may either be given by itself or it can be combined with chemotherapy. Either way, studies have shown this treatment to reduce the recurrence of breast cancer by up to 50 percent. The HER2 gene is continually being studied in order to improve the knowledge about breast cancer.

Many studies have attempted to show why the HER2 receptor behaves so differently from its relatives HER1, HER3 and HER4. Dan Leahy, a professor of biophysics, determined the structure of the HER3 gene in his laboratory last summer. All four of these HER relatives have a similar sequence of their building blocks; it is the excess HER2 that leads to uncontrolled cell growth resulting in breast cancer.

Like its relatives, the HER2 receptor is stuck in the cell membrane, partially outside the cell, and partially inside. The extracellular part, whose structure the scientists determined, is the receptors "on switch." Through this region, HER receptors join into pairs to become fully active and trigger events that eventually result in cell division.

Another experiment, lead by Hyun-Soo Choo, involved a team of researchers that grew crystals consisting of the extracellular region of the HER2 protein. They then exposed these crystals to radiation and eventually were able to interpret the data and figure out a structure for the protein.

Comparisons of HER family structures reveal that a few key changes in the sequence make all the difference for HER2. Specifically, HER2 doesn't need to be "opened" before it can pair with another HER, which is why extra HER2 can cause cancer, and why no small molecules or ligands have been found that bind to it. Numerous studies are being done in order to gain a better understanding of how this HER2 receptor can be manipulated in order to create a vaccine for breast cancer.

## Description of Vaccine

The type of vaccine investigated was the subunit vaccine with a bacterial adjuvant. The protein used was the HER2 surface protein receptor. This protein is present in abundance on breast cancer cells as mentioned above. The receptor protein will be purified. The adjuvant that will be used is a modified Bacille Calmette-Guérin (BCG) with the HER2 protein inserted into the membrane. BCG is an attenuated live strain of the bovine tuberculosis bacillus that is used for the generation of human immunity to *Mycobacterium tuberculosis*. BCG will elicit a very strong response because it has lipopolysaccharide on its surface. Macrophage receptor CD14 will come into contact with the LPS and release tumor necrosis factor alpha to induce inflammation. TNF alpha is primarily secreted by macrophages and dendritic cells. Its primary effect is vascular dilation through out the body. This causes increased blood flow especially to the sight of infection. This promotes the accumulation of leukocytes which will mount the primary immune response. Several chemokines will be included with the vaccine to help upregulate the immune response. Anti-CTLA-4 antibodies will be included in the initial dose of the vaccine to prevent the inhibition of the CTLs. CTLA-4 works to stop tumor immunosurveillance and attenuate the efficacy of cancer vaccines. IL-15 is a known stimulatory factor of CTLs and Th1 cells and it will be included in the administration of the vaccine. It is important during the period immediately following the administration of the vaccine that the immune system be as free of inhibition as possible without inducing auto-immunity.

The macrophage also releases interleukin-8 which acts upon other phagocytes such as neutrophils. It draws neutrophils to the infection site. Also released by the macrophage is IL-6. These act upon lymphocytes to increase their effectiveness in producing an immune response. It also acts on hepatic cells to induce the production of acute phase protein. Acute phase proteins include mannose binding protein, c-reactive protein, and fibrinogen. Mannose binding protein will provide a useful response since human cells do not display mannose on their surface. Mannose is present on BCG so the mannose binding protein will assist in identifying the bacteria as not self. This promotes phagocytosis. C-reactive protein binds phosphocholine, a surface constituent of BCG, opsonizing the bacteria and assists in phagocytosis. IL-1 is also released and stimulates the division of bone marrow cells. IL-12 is another cytokine produced by macrophages. It stimulates the adaptive immune response by helping to differentiate naïve T-cells. IL-12 stimulates the growth and function of T-cells. It also mediates enhancements of the cell killing activity of NK cells and CTLs. This will stimulate the lysosomal digestion of ingested pathogens. This digestion will allow for the presentation of the HER2 protein to CTLs by professional antigen presenting cells like macrophages and dendritic cells. It increases the production of interferon gamma and tumor necrosis factor alpha.

The chemoattractants stimulate the accumulation of leukocytes to the site of infection. The release of IL-12 causes t-cells to differentiate into Th1 cells. These cells can modify the immune response of several different types of leukocytes. Th1 cells induce longer lived CTLs. Naïve b-cells stick to the antigen or antigen pieces are taken up by the b-cells and presented on MHC class 2. Upon the presentation of the antigen on Class 2 MHC, the b-cell can interact with Th2 cells. When the Th2 cell binds to the MHC class 2, it releases CD40 ligand, IL-4, 5, 6, and 13 to stimulate the b-cell to differentiate into plasma cells and divide. Plasma cells are further stimulated to produce

antibodies against HER2. The antibodies produced first are the IgM and IgD which primarily act as b-cell receptors. IgM functions as a b-cell receptor and assists in complement activation as well as clumping together antigen-antibody complexes. IgG is produced later when the b-cell is stimulated to produce it by Th1. The function of IgG is to opsonize the HER2 to promote its phagocytosis, to neutralize the cell the HER2 is on, and to activate the complement cascade.

Memory to the HER2 vaccine will be generated by the inclusion of the several costimulatory molecules. These molecules include CD80, ICAM-1 and LFA-3. All of these induce Tcs to preferentially kill tumor cells. Additional boosters of immune efficiency will be given at later dates. These boosters will include IL-15 to increase the lifespan of Tcs and also increase the efficacy of the CTLs at killing tumor cells. It is important to administer these boosters after the initial immune response to the HER2-BCG has been completed to ensure that there are cells for the IL-15 to act upon. These boosters will be given intravenously to allow them to increase the lifespan of all the CTLs they encounter.

Following the primary immune response, immune memory is generated. Memory b-cells are produced from germinal center b-cells. This occurs in the lymph nodes where b-cells are selected for their affinity to the pathogen and affinity toward self MHC. Activated naïve t-cells differentiate into effector cells that mount the immune response and into memory t-cells that will persist after the infection has passed until a similar pathogen is encountered.

This will provide immunity against the subunit that was included in the adjuvant mixture.

### **Description of Immunity Assessment**

The subunit vaccine will be injected into the muscle. Since it contains the bacterial adjuvant it will undoubtedly elicit a large immune response increasing the number of macrophages that would take the HER2 surface protein of the cancer cells directly to the lymph nodes and activate the T cells as described in the Vaccine Description. This injection will be given as soon as the cancer cells are detected and the earlier the treatment begins, the less likely damage will result.

The first step for a patient would be to determine the progress of the cancer so far. This would be accomplished by injecting the HER2 protein into a mouse that would then produce antibodies to the antigen. The antibody forming cells of the mouse would then be taken from the mouse's spleen and the anti HER2 antibody forming B cells would be isolated. To determine which cells produce the anti HER2 antibody, single cells in the culture could be cloned and tested using an ELISA test to determine if the anti HER2 antibodies are formed from each cloned cell culture. This ELISA test would be run by placing HER 2 protein in the wells of a well plate and washing away any that do not stick. The wells are then filled with unrelated protein such as powdered milk to cover any open sites in the wells. A culture of each cloned antibody cell would then be placed in separate wells and allowed to bind to the HER2 protein if the antibody is specific for it. Anti mouse gamma chain specific antibodies extracted from a rabbit (which were created by injecting a rabbit with mouse IgG and removing these antibodies after the rabbit has generated a response to the foreign protein) will then be linked with enzyme and placed in the wells. If the antibodies in a well are specific for the HER2 protein then the Anti

mouse antibodies would bind to the Fc region of the mouse IgG antibodies and remain on the wells after washing. Then the chromogenic substrate would be added and react with the enzyme on the anti mouse antibodies present to result in a colored solution. A colored solution would signify the presence of the mouse HER2 specific antibodies and those monoclonal antibody producing cells could be further cultured to produce a large amount of anti HER2 antibodies. These antibodies would then be covalently coupled with a radioactive isotope such as <sup>131</sup>I and injected into the human cancer patient. Since the antibodies are all monoclonal they are specific for the HER2 protein on the breast cancer cells and would bind to the protein if present. To detect whether the antibodies bound, imaging devices such as X-ray photography or MRI can be used. The location and intensity of the bound antibodies would help determine the progress of the breast cancer. In addition, this test could be run over the course of the treatment to test the efficacy of the vaccine.

As previously described the vaccine would produce a cellular response to the bacterial adjuvant and since the HER2 protein is attached to the bacteria, the body would also amount a response to the presence of HER2 protein which is found on the cancer cells. Although both T cells and B cells will be active in fighting the cancer cells, the CD8 T cells will be most effective. This is because any HER2 specific antibodies released by B cells that come in contact with the cancer cells can have two main effects. The first is that the antibodies are attached to the HER2 protein on the cancer cells and Natural Killer cells interact with the antibodies through the CD16 receptor. This interaction causes the NK cell to kill the human cell coated with the antibody through a process called antibody-dependent cell-mediated cytotoxicity. The second effect is that the antibodies cover up the HER2 protein and form a barrier around the cancerous cells that won't allow T cells to kill the cell. This is obviously not a very effective system to rely on to destroy the cancerous cells. Therefore the T cells would need to be the main eliminator of the cancer.

During the immune response evoked by the vaccine, antigen presenting cells would take in and express the HER2 protein peptides on Class I MHC. This would activate CD8 T cells when naïve resting T cells come into contact with the APC. The active cytotoxic T cells would then circulate until they came into contact with the HER2 protein on the cancer cells. This would cause the CD8 T cells to bind to the cancer cells and secrete cytokines such as IL-2 to cause the proliferation of the T cell. Another main secreted cytokine would be TNF-alpha which causes cytolysis and cytostasis in cancer cells in vitro. In addition to the cytokines the CD8 T cell would secrete perforin and granzymes to kill the cancerous cell within 5 minutes of contact. These HER2 specific CD8 T cells are very important to the elimination of the cancer and their presence should be measured to assess the efficacy of the vaccine. This can be done by running an ELISPOT test for the cytokine TNF-alpha. This procedure includes coating a microwell plate with anti cytokine antibody and then incubating T cells from the patient's serum in the wells with the HER2 protein for 24 hrs. This would cause any T cells specific for the HER2 peptide to secrete the TNF-alpha, which would be bound to the anti-cytokine antibodies on the microwells. The cells are then washed off and biotinylated antibody is then added to sandwich the cytokines that bound to antibodies, before another washing. Avidin HRP followed by substrate is then added which would bind to the biotinylated antibody present and interact to cause the formation of colored spots. These spots would

indicate the production of the TNF-alpha cytokine, and the number and density of the spots directly correlates to the vaccines effectiveness in developing a T cell response toward the cancer cells HER2 protein.

Often in cancer, there is a remission which could then lead to a relapse. Therefore, in case of a remission, T cell memory for the HER2 protein would be important to prevent a relapse. In order to test the T cell memory for the cancerous protein, it would be important for the patient to receive another ELISPOT test for the HER2 protein after the cancer has been suppressed. This is important because without a strong T cell memory for the protein, the breast cancer could begin to develop again and spread without proper monitoring and treatment. If the EISPOT test results in an adequately strong response then the patient can be monitored less intensely and the vaccine would be highly efficient.

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