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Obesity enhances breast cancer risk, metastasis, and response to immunotherapy

By

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CHAPTER 6

When your immune system helps cancer grow: A story of obesity and breast cancer

Communicating Science to a broader audience as part of the Wisconsin Initiative for Science Literacy Program

To the reader:

I decided to conclude my Ph.D. dissertation with a chapter dedicated to you, a non-expert in the field of obesity-associated breast cancer, so my work can reach a broader audience. This chapter is brought to you thanks to the Wisconsin Initiative for Science Literacy (WISL) at UW-Madison. This chapter gives you a non-technical explanation of my thesis entitled “Obesity enhances breast cancer risk, metastasis, and response to Immunotherapy.”

Science communication is an important part of being an effective scientist. Our work cannot benefit you if you are not able to access the information. In recent years, with the widespread use of the internet and multiple media sources, it is very easy for scientific information to be misrepresented, unbeknown to the person enjoying that content. As scientists, we often focus on the gritty details of what questions we are trying to answer. Most of our day-to-day communication about our science is with other scientists that have similar expertise to our own. We sometimes forget how to communicate with non-scientists. It is an art to communicate research, specifically biomedical research in my case, to a non-expert. Science is its own language, and it takes years to learn. However, it doesn’t have to be inaccessible to you.

During my Ph.D., the COVID-19 pandemic highlighted a failure of scientific communication to the public by scientists. This in turn resulted in deaths that were likely preventable. With improved scientific education and communication, the public will be able to know how to protect themselves from the latest public health threat, as well as help communicate to others how to do the same. I know everyone cannot fall in love with science as much as me, but I hope everyone can find a trusted friend in science,

and is able to understand, enjoy, and benefit from when she reveals her exciting new secrets.

Introduction:

Many families have been affected by breast cancer. You may be familiar with the pink folded ribbon, a recognizable symbol for breast cancer awareness, but you may not know what health factors could increase your chances for developing this disease. Two of those risk factors, which were the focus of my dissertation, are breast density and obesity. Some women that have high breast density may become obese in their lifetime. It is unknown how these risk factors together might affect breast cancer risk. Not only do higher breast density and obesity affect the likelihood of developing breast cancer, but they also can affect how breast cancer grows and spreads.

Cancer is an overgrowth of the body's own cells until they form a **tumor,** or mass. Breast cancer is an overgrowth of breast cells, or **epithelium**. In humans, obesity has been shown to increase both breast cancer tumor size and the cancer's ability to spread, or **metastasize**, to the lungs. Cancer metastasis is often fatal for cancer patients. It is currently unknown why obese patients have larger tumors at diagnosis, and more metastasis to the lung. Women with dense breasts also have worse overall outcomes when they develop breast cancer. The first aim of my work was to test if breast density and obesity together would accelerate risk and tumor progression more than one risk factor alone. Second, I focused on obesity only to understand why these patients may have more metastasis in the lungs. Thirdly, I investigated why obese patients have better responses to some types of cancer therapy.

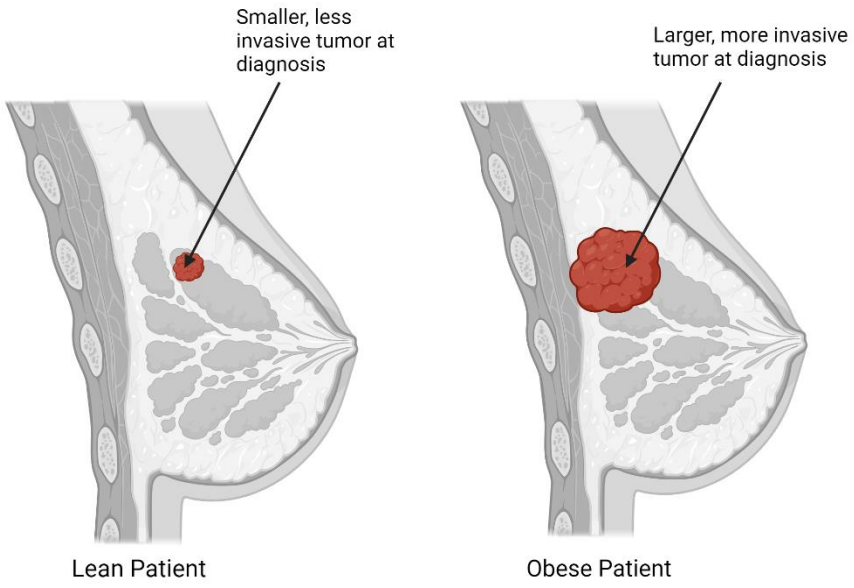

Figure 6-1 Obese patients frequently have larger, more invasive breast tumors at the time of diagnosis: Shows an example of a tumor at the time of diagnosis in lean women and a tumor at diagnosis in an obese patient. Obese women at the time of diagnosis frequently have larger tumors that are more invasive. Invasive tumors have spread farther into the surrounding breast and eventually into the chest.

What is breast density?

Breast density is an increase in glandular tissue and structural support fibers, like collagen, in the gland. **Glandular tissue** includes the epithelium, ducts and lobes of the mammary gland. The rest of the mammary gland mostly consists of fat. The mammary gland has many fibers that provide structural support to the gland, including **collagen.** Collagen is one of the main structural support fibers in the mammary gland. It is not completely understood how increased collagen contributes to breast cancer risk. In tumors, collagen can structurally help tumors grow. Breast density is thought to make it more difficult for doctors to see early breast cancer on a mammogram. Fat is black on a mammogram, while glandular tissue, collagen, and breast cancer are white on this

scan. Mammary density blocks the visualization of small breast tumors on scans because it shows up as the same color. Although high breast density may contribute to breast cancer being missed on scans, evidence also suggests that increased glandular tissue, collagen and other fibers may change the breast environment to promote cancer.

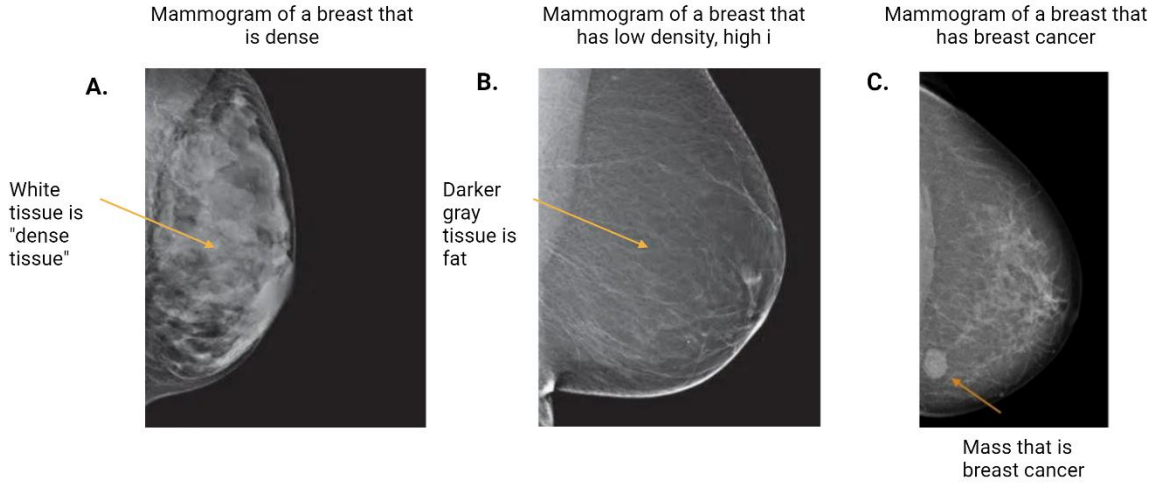

Figure 6-2: Breast density on a mammogram can make it challenging to identify early breast cancer lesions. Breast density is thought to consist of increased glandular tissue taking up a majority of the area of the breast. Breast density also consists of increased structural support fibers like collagen. Collagen and glandular tissue appear white on a mammogram, while fat appears dark gray. **(A)** Shows a mammogram of a dense breast. **(B)** Shows a mammogram of a low dense/fatty breast and **(C)** Shows a mammogram of a breast with a large breast cancer mass. A low-density breast consists mostly of fat. The breast appears mostly dark gray with a few white areas appearing, representing collagen and glandular tissue. Early and small breast tumors will be easier to see in breasts with low density. The last mammogram shows a breast with a large tumor in the lower left corner (C). The tumor shows up on the mammogram as white, similar to dense issue. The tumor is present in a breast with some density. Hopefully you can appreciate if this tumor was smaller and within a more dense breast like in (A), the tumor would be difficult to identify. Images are from the American Cancer Society https://www.cancer.org/cancer/types/breast-cancer/screening-tests-and-early-detection/mammograms/breast-density-and-your-mammogram-report.html and *Nature* *https://www.nature.com/articles/s41598-020-77053-7*

Obesity

Obesity is defined as having a body mass **index (BMI) of ≥30 kg/m^2.** BMI is calculated by taking a person's weight divided by the square of a person's height in meters. It is not always an accurate measurement to determine if someone is obese. However, it is still used in humans today to determine a person's level of obesity. More accurately, obesity is defined as an excess of adipose tissue in the body, often caused by an excess caloric intake. Obesity affects the mammary tissue by increasing the size and amount of fat cells in the gland. These fat cells are called **adipocytes**. These adipocytes are large cells that store fat in the body. As a person becomes more obese these cells expand and become stressed. They will eventually die from this stress. Macrophages, a cell that is part of our **immune system,** helps "**clean up**" cells when they die.

The immune system is made up of many types of cells, including macrophages, that protect our bodies from bacteria, viruses and fungi. Immune cells can also protect our bodies from cancer. In fact, they play an important role in preventing and fighting cancer. However, sometimes immune cells can promote the development of cancer. Fat cells that die cannot remain where they are, they need to make room for new cells. Multiple macrophages will surround a dying adipocyte and start to "eat" the adipocyte. We can visualize macrophages doing this under a microscope. We call these structures of macrophages and adipocytes **crown like structures (CLS)**. However, a lot of CLS in a mammary gland is **not** considered normal. In lean people, we can see very few CLS because adipocytes are a healthy size. These CLS can be present before cancer develops, and also after. In general, CLS are thought to possibly increase breast cancer

risk because they are signs of **inflammation.** However, a direct link between CLS and breast cancer risk has not been identified.

Inflammation is an increase in immune cells and **cytokines** that activate or turn off immune cells. Cytokines are substances that are produced by immune cells, to communicate to other immune cells to help fight infections and cancer. Sometimes immune cells will be activated and produce inflammation cytokines when it is unnecessary. Chronic inflammation increases the risk for cancer. We do not want our immune system to be overly active all the time, instead we only want the immune system active when we have an illness, are responding to vaccines, or other immune therapy.

In obesity, inflammation often is high, even if a person does not have an infection or cancer. Macrophages are one of these cells that cause inflammation, and although they are cleaning up dying fat cells, they can produce proteins that promote the growth of epithelium. Macrophages are around **mammary ducts,** in addition to forming CLS (crown like structures). Mammary ducts are part of the glandular tissue in the breast and help transport milk during lactation. In obesity, there is more macrophage-driven inflammation in the mammary gland. Inflammation is a hallmark of cancer, and these macrophages could increase risk. However, a direct link between inflammation in the mammary gland and breast cancer has not been identified. This is a current area of research, as other labs try to identify how macrophages contribute to breast cancer risk.

Inflammation also results in cells in the breast producing extra collagen or structural support fibers. Too much collagen could increase breast cancer risk, and can later help early cancer cells spread to other parts of the gland, and eventually other

organs. Like high breast density, obesity causes an increase in collagen production in the breast. Specifically, we have seen increased collagen around mammary ducts. Mammary ducts are lined with epithelium, and these are the cells that will eventually turn into cancer if they are damaged. As part of my research, I quantify macrophages and collagen to try to measure inflammation and breast density.

Breast density can also increase macrophages and breast inflammation. It is unknown if both obesity and breast density together may further breast cancer risk or further increase both collagen deposition and inflammation.

Obesity effects on tumors in the breast

Think of the tumor as an ecosystem. When talking about the cells, cytokines, and structural support fibers, like collagen, we often call this the **tumor microenvironment (TME).** Multiple cell types are found within the tumor. Remember, a tumor is an overgrowth of our own cells because cells have lost their ability to control their growth. A breast cancer tumor is made of mostly abnormal breast epithelium. However, immune cells are incorporated into the tumor as well, cells like macrophages. Breast tumors are also surrounded by adipocytes or fat cells. These fat cells can affect how the tumor grows, and they function differently in obesity. Macrophages have been shown, in some studies, to be increased around and within breast tumors. Macrophages are thought to contribute to tumor growth. In fact, more macrophages within mammary tumors can be associated with worse outcomes for patients.

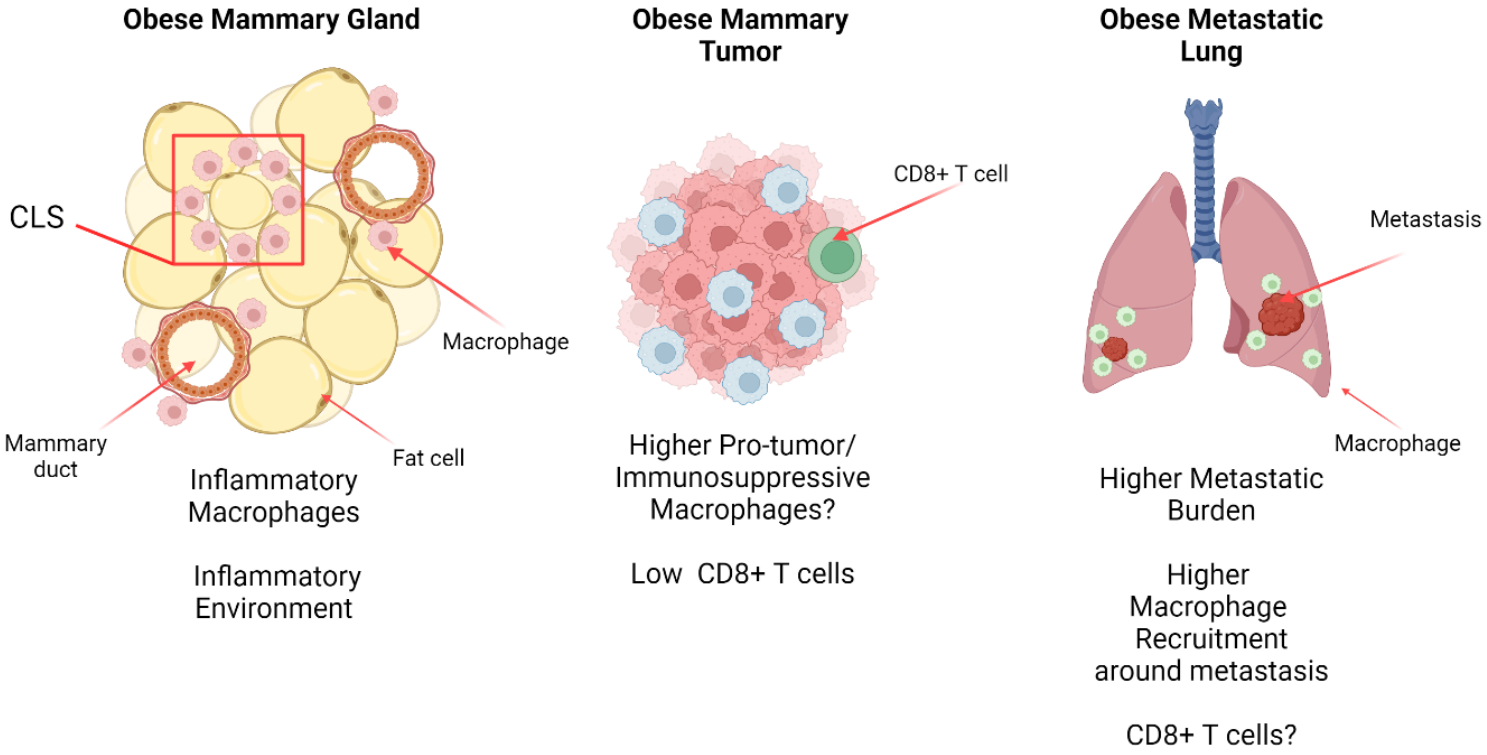

Figure 6-3: Obesity changes the immune system in the mammary gland, mammary tumor, and in the lung. (A) Obesity increases inflammation in the mammary gland before cancer occurs. This inflammation can increase breast cancer risk. Inflammation in the mammary gland is mostly driven by macrophages that have been recruited to the gland to "clean up" or "eat" dying or dead fat cells. Macrophages will also be present around ducts and are increased in obesity. **(B)** Macrophages in tumors are sometimes thought to be immunosuppressive or "pro-tumor." They can suppress other cells like CD8+ T cells from recruiting into the tumor. Obese mammary tumors have been shown to have less CD8+ T cells in mice. Therefore, these cells cannot kill tumor cells and shrink tumors. **(C)** Breast cancer can spread to the lung. These masses or small tumors are called metastasis. My lab has shown there are increased macrophages physically around metastasis in obese mice. However, it is unknown if, in the lung, these macrophages are pro- or anti-tumor and if they suppress CD8+ T cells like we see in tumors.

The tumor microenvironment, or TME, is complicated. Sometimes increased levels of certain types of cells are beneficial to killing tumor cells and thereby shrinking tumors, however, sometimes immune cells can suppress other parts of the immune system from killing cancer cells. In normal tissue, we generally do not want many

immune cells, like macrophages, because of the associated inflammation. Macrophages can either promote tumor growth or support tumor killing. Although we don't fully understand the phenomenon, there are more macrophages within tumors in patients with worse outcomes (tumors that grow fast), and we believe these macrophages promote tumor growth by suppressing other cells. Therefore, in obesity, macrophages could be causing other cells to be excluded or "turned off" in tumors.

In fact, in our mouse experiments in my lab, we have shown that **CD8+ T cells** can be excluded from tumors in obese mice. CD8+ T cells are a cell that can directly kill tumors with "toxic" proteins. Macrophages in obesity may be playing a role in suppressing important cells like CD8+ T cells from entering the tumor. In tumors from lean mice and people, this occurrence has been well studied, however, it is uncertain if this also occurs in metastasis.

Remember, metastasis is the spread of breast tumor cells to other organs, like the **lungs**. The lungs are a common site for metastasis to grow. Obese patients are known to have more metastasis and have a higher risk for metastatic spread. Why obese patients have more metastasis is **unknown**.

Many studies have looked at how macrophages change the mammary gland to possibly increase breast cancer risk and affect its growth and spread. However, it is unknown how macrophages could be aiding growth of breast cancer in the lungs under obese conditions. In previous work in Dr. Lisa Arendt's Lab, we saw an increase in macrophages surrounding metastasis in obese mice, with macrophages in direct physical contact with metastasis. This contact may create a barrier for CD8+ T cells, preventing them from reaching and killing the tumor cells. In addition, macrophages may

produce signals that impede CD8+ T cell recruitment or **exhaust** CD8+ T cells by sending too many activating signals. We thought that these macrophages may be increasing metastasis growth by impairing CD8+ T cells ability to kill breast cancer cells in the lung. However, it is currently unknown if this is true, especially when patients are also obese.

CD8+ T cells

CD8+ T cells are lymphocyte immune cells that directly attack cancer cells . They kill cancer cells by identifying them with a **receptor called the T-cell receptor (TCR), a protein on the cell's surface that identifies other proteins**. CD8+ T cells can only kill cells they are designed to recognize. For example, a CD8+ T cell that is programmed to kill a breast cancer cell will not kill a liver cell.

All of our cells express proteins known as **antigens** that are specific to their cell type. Antigens act like “tags” that signal to other cells, like cells in the immune system, what they are. Antigens can tell immune cells “I am not normal; I am a cancer cell” or “I am a cell infected with a virus.” Macrophages can pick up these antigens from dead cancer cells and **present** them to CD8+ T cells, activating those T cells to kill surrounding live cancer cells.

When T cells are activated, they begin to produce their own cytokines and cancer killing proteins. Remember that cytokines are how immune cells communicate to help fight infections and cancer. CD8+ T cells with low cytokine production may be **exhausted.** CD8+ T cells become “tired” or **exhausted** from killing tumor cells. The second aim of my thesis was to identify if CD8+ T cells are exhausted in obese lungs

before or after metastasis, which may explain why obese patients have more metastatic burden.

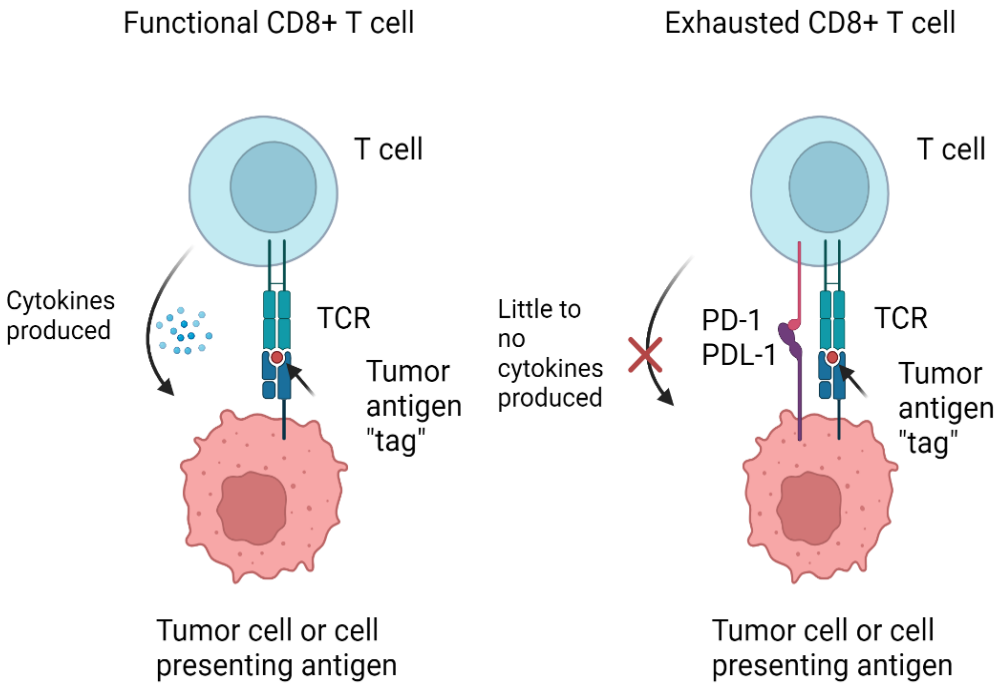

Figure 6-4: T cells can become exhausted when killing tumor cells. When CD8+ T cells interact with tumor antigens or “tags” with their T cell receptor (T cell uses this to recognize the tumor), they produce cytokines (anti-cancer proteins) to kill cancer cells. However, when exhausted CD8+ T cells interact with tumor antigens, they cannot produce cytokines, and tumor cells survive. This is because these CD8+ T cells express PD-1 and it binds to a PD-L1 positive cell (tumor cell or cell presenting antigen).

When CD8+ T cells are activated, they raise a “flag” or **receptor** on their surface known as programmed cell death-1, or **PD-1,** after they start killing cancer cells. When they receive an activation signal by tumor cells or cells like macrophages, they produce more PD-1. When PD-1 binds to its “partner” **PD-L1, it acts like pressing the “off” button, causing** CD8+ T cells to become exhausted and dampening their ability to kill

cancer cells. This can eventually cause CD8+ T cells to die (Figure 6-4). Oncologists can block this interaction so CD8+ T cells can stay “on” and continue to clear cancer cells.

How we can reduce tumor promoting macrophages and keep CD8+ T cells “on”

Doctors that treat cancer, known as **oncologists**, use drugs and other agents to improve the immune system’s ability to fight cancer, a strategy called **immunotherapy**. Popular immunotherapies target cancer-promoting macrophages and exhausted CD8+ T cells. Depleting macrophages from breast tumors has been shown to reduce tumor size. We can deplete macrophages using **colony stimulating factor-1 receptor (CSF-1R)** blocking agents to inhibit macrophage recruitment to tumors and metastasis. Decreasing macrophages can also increase CD8+ T cells in the tumor, improving the body’s immune response, and shrinking tumors, in part because CD8+ T cells are able to come in contact with the tumor cells. However, these newly recruited CD8+ T cells can still become exhausted and express PD-1. This is why a combined anti-CSF-1R and anti-PD-1 approach is promising for the removal of breast cancer metastasis.

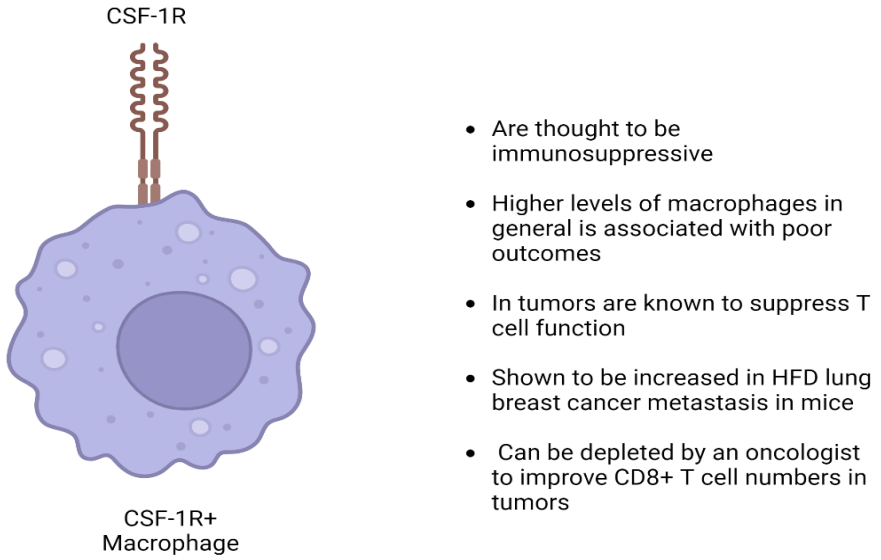

Figure 6-5: Macrophages express colony stimulating factor-1 receptor (CSF-1R). Macrophages express CSF-1R that can affect their recruitment and development in tumors. Blocking this receptor with anti-CSF-1R, removes macrophages from the tumor, overall improving CD8+ T cell numbers and activity.

When it works, blocking PD-1/PD-L1 causes tumors to shrink and metastasis to be cleared. However, it does not always work for some patients. One reason for this is that CD8+ T cells sometimes cannot get into the tumor to fight it. The therapeutic combination of depleting macrophages and blocking PD-1 results in reduced tumor sizes compared to one therapy alone in many cancers.

To summarize, breast tumors with high macrophages and low CD8+ T cells have an overall poorer prognosis (Figure 6-6A). When macrophages are reduced in the tumor, CD8+ T cells are increased to the tumor (Figure 6-6B). These CD8+ T cells begin to shrink the tumor, but CD8+ T cells become exhausted or “tired” and increase their

expression of PD-1 (Figure 6-6C). An oncologist can block PD-1 to keep the newly recruited CD8+ T cells turned “on,” shrinking tumors or metastasis further (Figure 6-6D).

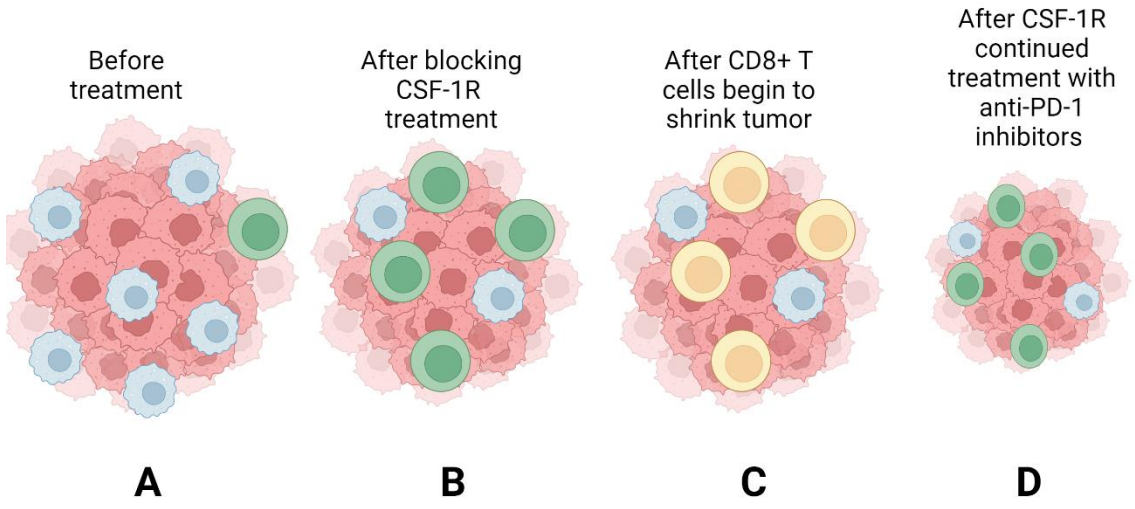

Figure 6-6: How anti-CSF-1R and anti-PD1 may shrink breast tumors (A) Shows a tumor prior to treatment. Macrophages, in blue, are high, and suppress CD8+ T cells (green). (B) When macrophages are depleted with anti-CSF-1R, CD8+ T cells increase in the tumor. These CD8+ T cells are active and functional and produce cytokines (anti-tumor proteins) to aid in the anti-tumor immune response. (C) As CD8+ T cells kill tumor cells and recognize more and more tumor antigen “tags” they become tired or exhausted. Exhausted T cells (yellow) will express PD-1 and lose their ability to kill cancer cells. (D) After blocking PD-1 and continuing to keep macrophages low with anti-CSF-1R, CD8+ T cells are able to be turned back “on” and become activated. These CD8+ T cells are able to continue to kill cancer cells and overall shrink the tumor.

Surprisingly, obese patients have longer overall survival on therapies targeting PD-1/PD-L1 binding on CD8+ T cells compared to lean patients. This is shocking because in obese mouse models, and as shown in some human studies, CD8+ T cells are low in the tumor. However, the mechanism behind this better response in patients with obesity is unknown. With obesity increasing macrophages around metastasis in the lung, and clinical evidence that obese patients respond better to PD-1/PD-L1 targeted

therapy, I believe that obese patients with metastasis might respond better to a combination approach targeting macrophages and exhausted CD8+ T cells.

To test these questions, I used mice to model how obese and lean patients with metastasis may respond to these therapies. The last aim of my thesis was to investigate if obese mice will have reduced metastasis to the lung on a dual macrophage, CD8+ T cell targeted therapeutic approach, or if targeting macrophages or CD8+ T cells alone will be more efficacious. Lastly, if responses are different than in lean mice, why?

How we study breast cancer risk and progression in the lab:

Mice are an important tool used in biomedical research to study diseases like cancer and can help us answer questions about cancer progression. There is a lot we do not know about the body, so using an animal model helps us mimic as closely as possible what might happen in you or me. Many factors affecting cancer growth involve the whole body, or "system." We call these factors **systemic** effects. One systemic factor is our immune system. Immune cells are in our tissues, blood and lymph system, surveying and killing pathogens, including cancer cells. Cancer metastasis involves cancer cells surviving leaving the primary tumor (in the mammary gland, in this case), traveling in circulation in the body, and entering secondary organs, like the lungs. Mouse models allow us to mimic this process as closely as possible to what would happen in a cancer patient.

In my thesis work, I used different diets to induce obesity. Lean mice received a low-fat diet, and obese mice received a high-fat diet. I utilized a mouse model where mice are born with abnormal mammary epithelium that is genetically altered to form

multiple mammary tumors. These mice will begin to form tumors from birth. These models are useful for us to study the early stages of breast cancer (Figure 6-7). I used these genetically altered mice to study early and late tumor formation under conditions of obesity and breast density. Later, to study later stage metastatic breast cancer, I inject tumor cells into the mammary gland so I can remove the tumors surgically (Figure 6-8). This is to mimic when tumors are removed in breast cancer patients before their metastasis is treated.

Mammary glands are composed of many ducts that are lined with a single layer of epithelial cells. When you look at mammary glands under a microscope, you are looking down the center of the mammary duct, like looking down an innertube. The center of the duct is referred to as the **lumen**, a hollow space that allows milk to flow out of the mammary gland during lactation. As these cells begin to divide, they form additional layers, filling the lumen of the duct. In the normal mammary gland, these cells do not fill the lumen, and this space is empty until a woman would begin to lactate after giving birth. In cancer, epithelial cells continue to divide, the entire lumen will be filled, and the lumen is no longer visible. Eventually the structure of the duct cannot withstand the rapidly dividing cells. The now cancerous epithelium breaks the outer structure of the duct and continues to divide and fill up the surrounding gland (Figure 6-7). Eventually these mice will form multiple tumors that metastasize to the lung. This is how tumors progress in genetically engineered mice. These genetically engineered mice can also be fed a HFD (high-fat-diet) to induce obesity. Thus, we can observe how these early tumor stages are affected by obesity.

As I previously mentioned, breast density can include a buildup of “structural support fibers,” known as collagen. To model breast density we used mice that lack the ability to break down collagen in the mammary gland. In normal mice, and in you and I, collagen is broken down and rebuilt as tissue adapts to changes. However, the bodies of these mice can’t break collagen apart into pieces; instead, the collagen forms a dense network in the mammary gland. We can cross collagen-dense with mice that form mammary tumors to model breast cancer under high density conditions. These mice can also be fed a HFD so we can model obesity and breast density together.

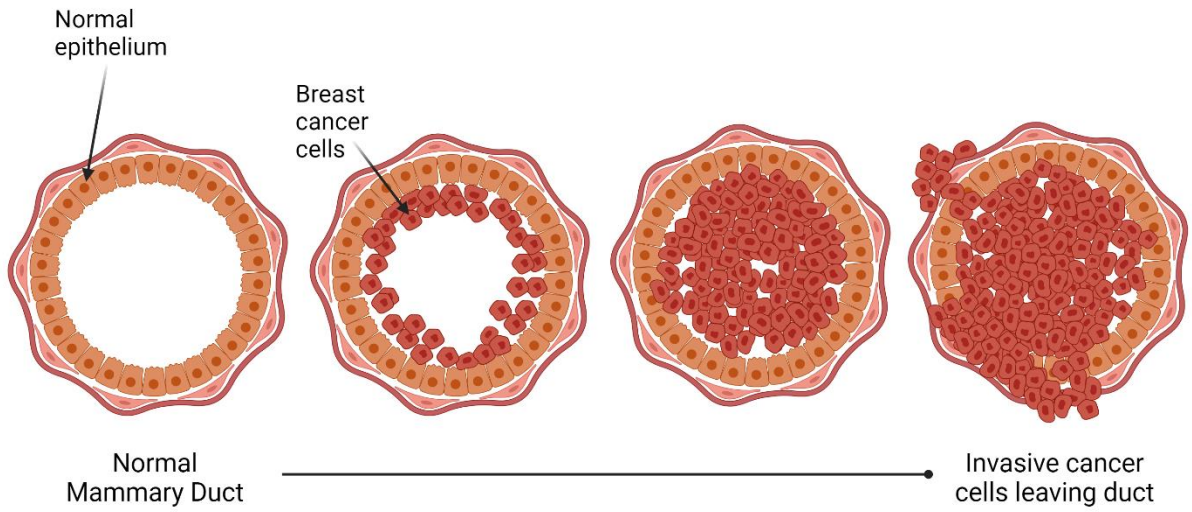

Figure 6-7: The progression of breast cancer. Normal epithelium lines the mammary duct. Cancer cells begin to fill the lumen of the duct until they invade into the surrounding mammary gland.

After mice become obese, I can induce a mammary tumor by injecting mammary tumor cells directly into the mammary gland on each flank of the mouse. Two tumors will then grow and form noticeable lumps under the skin on each side of the mouse. Once one tumor reaches 0.5 cm in size, tumors can be surgically removed from the mice. I removed the tumors in our mice to mimic when there is a resection (removal) of breast cancer in women. Although women have their breast tumors removed, cancer

cells still might be present throughout other parts of the body. The cells may grow and form metastasis. We used this model over a genetically engineered tumor model because it allows us to remove tumors from mice (Figure 6-8). Women often have their tumors removed prior to some types of therapy so it is important to try to model this in our mice.

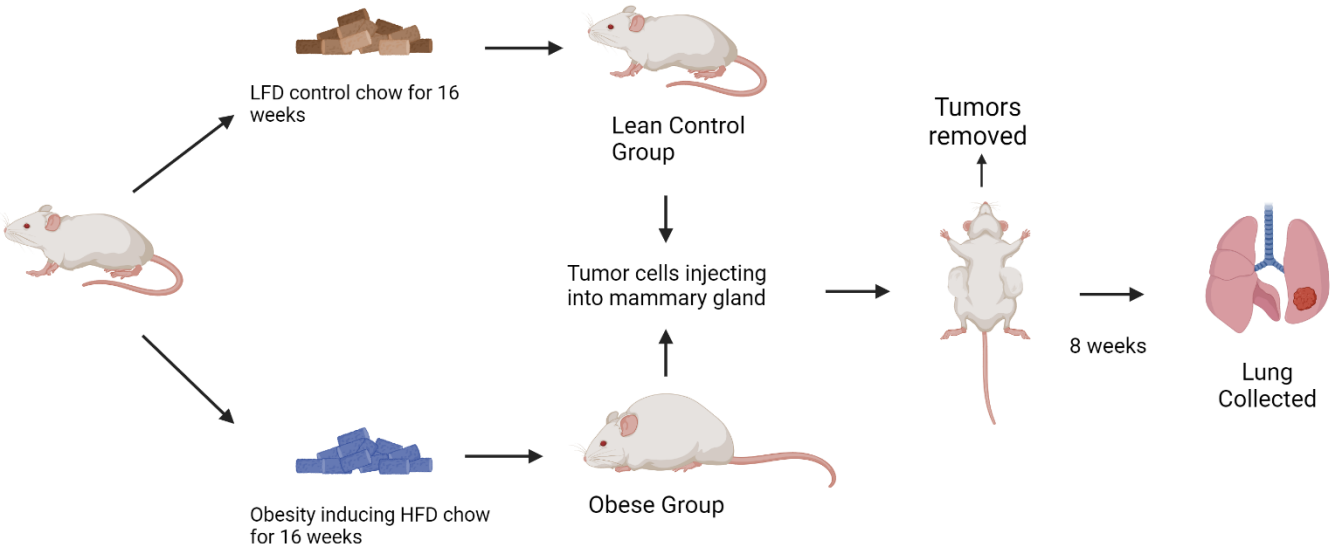

Figure 6-8: Methods for inducing breast cancer metastasis in obese mice. Female mice were fed a low-fat diet or high-fat diet for 16 weeks. Once mice fed a high-fat diet were obese, tumor cells (mammary cancer cells) were injected into the mouse's mammary glands. These cells were then left to grow in lean and obese mice. At a certain size, I removed the tumors surgically from the mice to mimic when women have their tumors removed. I then waited another 8 weeks for metastasis to grow in the lung before I analyzed the metastasis and immune cells in each group. This is a great way to model obesity-induced breast cancer in mice.

The main results of my PhD

Obesity and breast density on breast cancer risk and progression

My work revealed that in mice without mammary tumors, breast density and obesity increased macrophage-driven inflammation. Mice with high breast density and obesity had more CLS (crown like structures, or macrophages around dying adipocytes), indicating more adipocyte death and total inflammation within the gland. I also saw increased collagen around ducts in these mice compared to control mice. CD8+ T cells were reduced in the mammary gland of obese mice, but not in lean mice. This indicates obesity specifically decreases CD8+ T cells, potentially causing the immune system to miss the development of early mammary tumor stages.

Evaluating tumor-bearing mice, I did not see differences in tumor progression in the mammary glands of mice with high density breasts, obesity, or both risk factors combined. However, there were more macrophages around tumors in mammary dense mice, obese mice, and mice with both risk factors combined. CD8+ T cells were only reduced in tumors of obese mice, with or without increased breast density. Lean mice with dense mammary glands did not have less CD8+ T cells, like what I saw in mice

without cancer. Low CD8+ T cells in tumors and higher macrophages around the edges may increase cancer metastasis (Figure 6-9).

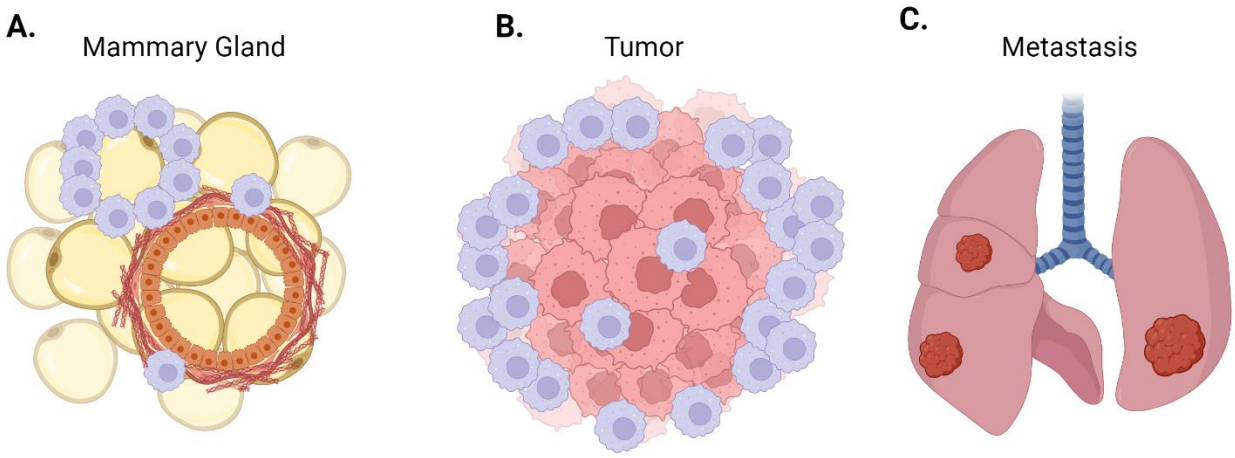

Figure 6-9: Results showed obesity and breast density together may increase breast cancer risk and metastasis to the lung. (A) In the normal mammary gland, obesity and breast density together increased crown-like structures (CLS), or macrophages around dying adipocytes, compared to one risk factor alone in young mice. Collagen was also increased around ducts of mice with both risk factors. This may correlate with a higher risk for breast cancer development. (B) Although we didn't find any differences with tumor size. Tumors from mice with both risk factors have more macrophages surrounding the tumor edge. This may lead to more invasive tumors and worse prognosis. (C) In the lung, mice with both risk factors had more metastasis compared to mice with one or no risk factors. Therefore, women with breast density and obesity may be at higher risk for metastatic spread to the lung.

Lastly, measuring metastasis to the lung, mice with both risk factors had increased levels of metastasis in the lungs (Figure 6-9C). However, mice with just high mammary density or obesity alone did not have increased metastasis compared to our control mice. I found this surprising, as it shows that a combination of higher breast density and obesity could really promote cancer spread to the lungs. Overall, I concluded that **(1) a combination of breast density and obesity together may increase breast cancer risk beyond one risk factor alone. (2) During the**

progression of breast cancer, both risk factors together seem to promote metastasis to the lung, which may translate to an overall poorer survival outcome in patients with both conditions at the time of breast cancer development.

I discovered CD8+ T cells are more exhausted under obese conditions before metastasis

In my second project, I looked at how CD8+ T cells functioned in the lungs of obese and lean mice before and after metastasis. In non-tumor bearing mice, obesity increased PD-1+ (programmed cell death-1, a marker for exhaustion) in CD8+ T cells within the lungs. This indicated to me that they may be exhausted. Obesity also increased TCR (T cell receptor, what T cells used to recognize tumor cells) signaling within the lungs, which may indicate an over- activation of T cells, even before cancer is present. T cells also produced less cytokines in the lungs of obese mice. This data indicates that obesity exhausts T cells prior to tumor development in the lungs, which may render CD8+ T cells unable to kill cancer cells that make it to the lung early on .

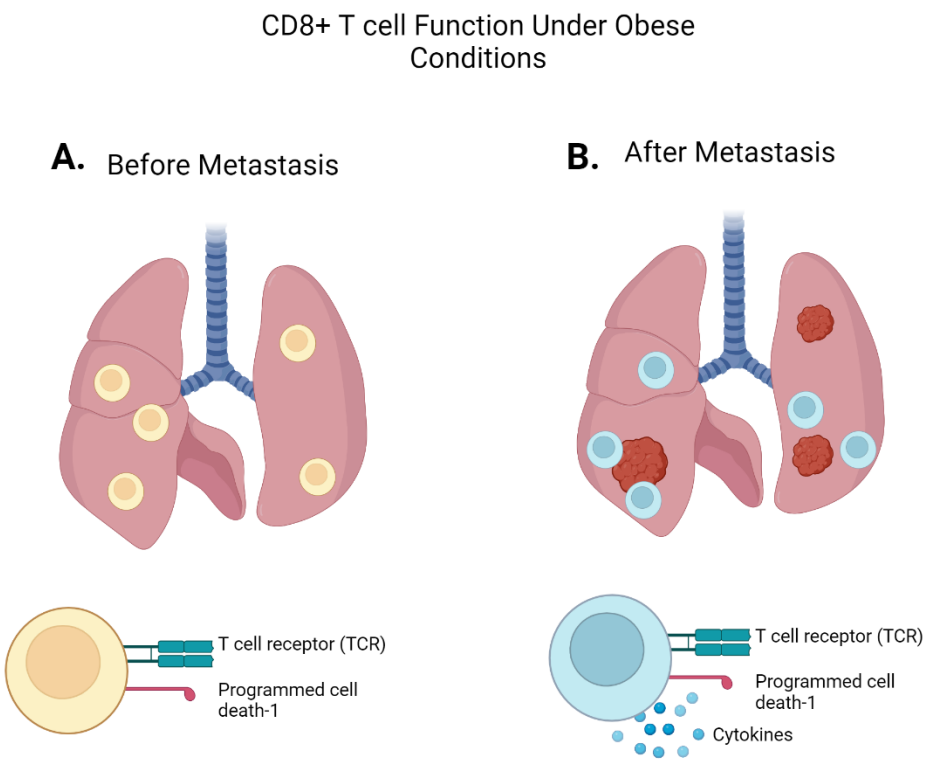

Figure 6-10: Obesity alters CD8+ T cell function in the lung. (A) Before metastasis, CD8+ T cells were more exhausted, failed to produce cytokines, and expressed PD-1 in obese mice compared to lean mice. This overall means these CD8+ T cells from obese lungs may be dysfunctional and therefore unable to clear early metastasis. (B) Obese mice had more metastasis than lean mice and also had more CD8+ T cells that expressed PD-1. However, in metastasis, obese CD8+ T cells were able to produce cytokines, which indicates that after metastasis CD8+ T cells are able to retain some function and may respond to anti-PD-1 inhibitors.

I showed that CD8+ T cells in obese mice with breast cancer metastasis express exhaustion markers like PD-1 but still can retain function

CD8+ T cells' exhaustion prior to tumor development allows cancer cells to evade the immune response in obese mice. Obese mice had more metastasis to the lung than lean mice, consistent with clinical data in humans and similar mouse studies. Contrary to my prediction, I found that CD8+ T cells were **not** decreased in obese mice lung

metastasis, though I did observe that PD-1 expression was higher, indicating possible exhaustion (Figure 6-10). However, CD8+ T cells from obese mice could still produce cytokines (the protein CD8+ T cells produce when activated, which helps kill cancer cells). Overall, this may suggest that in cases of lung metastasis, CD8+ T cells under conditions of obesity are able to retain some function. I concluded that **(1) Obesity impairs CD8+ T cell function to an exhausted-like state in the lungs of non-tumor bearing mice. (2) After metastasis, CD8+ T cells in the lungs of obese mice express PD-1, but they retain function.**

Out with the bad macrophages and in with the activated CD8+ T cells

I set out to test whether anti-PD-1 (a drug that blocks PD-1 from binding to PD-L1, keeping T cells “on”), anti-CSF-1R (a drug that blocks colony stimulating factor 1 receptor) depletion of macrophages, or a combination of both therapies would be better at removing breast cancer metastasis. I found that anti-PD-1 reduced metastasis in lean mice but not in obese mice. However, anti-PD-1 treatment in obese mice increased CD8+ T cells in the lungs. If CD8+ T cells are increased, I assumed that there would be more cells to clear metastasis from the lungs. Despite this increase, I suspected the CD8+ T cells were more exhausted. Indeed, other exhaustion markers were higher in these cells. What could be making them **exhausted** or **“turned off**?**”** I predicted it may be PD-L1+ (PD-L1 positive) macrophages! I saw that a population of cells, which includes macrophages, had higher PD-L1 expression! Therefore, macrophages may be decreasing anti-PD-1 response in obese mice

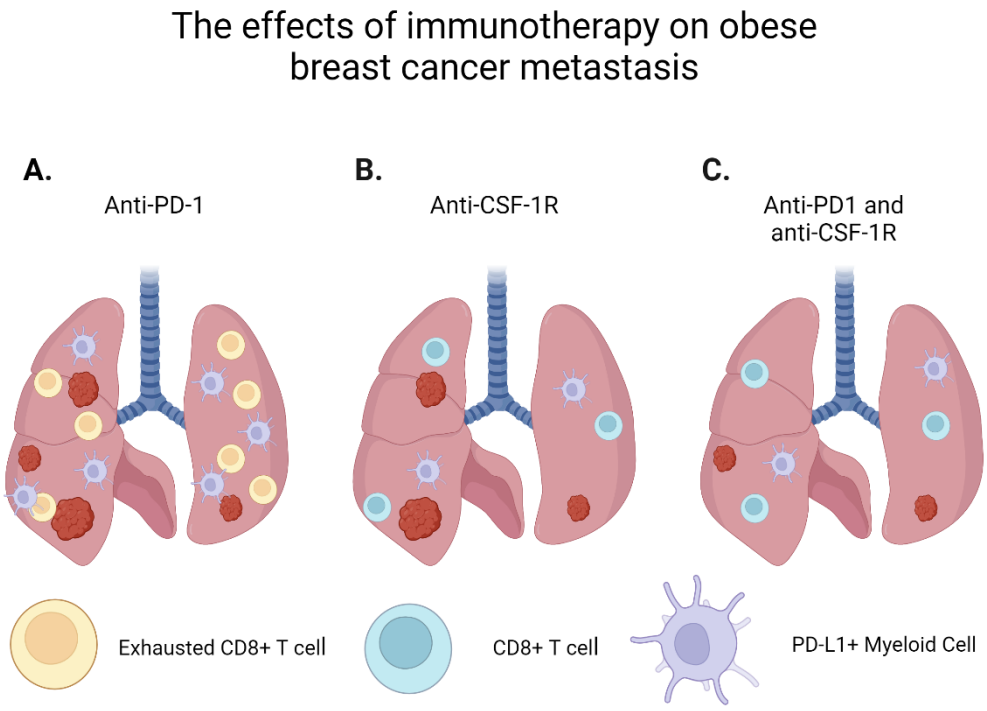

Figure 6-11: Obese mice responded better to immunotherapy. (A) Anti-PD-1 alone did not reduce metastasis in obese mice. It did increase the number of CD8+ T cells in obese lungs, but these CD8+ T cells were exhausted. In lean mice, CD8+ T cells were activated and were not increased. PD-L1+ Myeloid cells were also increased in obese lungs in response to anti-PD-1. Myeloid cells include macrophages! This might be why obese mice had a resistance to anti-PD-1 alone. (B) Macrophage depletion via anti-CSF-1R reduced PD-L1+ myeloid cells (probably macrophages) and reduced metastasis in obese mice. In lean mice, anti-CSF-1R did not reduce metastasis, suggesting macrophages are more tumor-promoting in obese metastasis. (C) Dual anti-PD-1 plus anti-CSF-1R was more efficient in obese mice, reducing metastasis and activating the immune system. Overall, macrophage depletion and dual therapy reduced metastasis more in obese mice compared to lean mice. This suggests that obese patients may benefit more from these therapies.

I found that anti-CSF-1R treatment increased activation of a population of cells, which includes CD8+ T cells, in lean mice. Therefore, depleting macrophages may improve T cell responses to cancer in lean mice. However, only in obese mice did depleting macrophages reduce metastasis. I also showed that only in obese mice did total immune cells increase. This was not seen in lean mice. It is possible that is why in lean mice, metastasis was not reduced.

PD-L1+ immune cells were reduced with anti-CSF-1R treatment only in obese mice, suggesting a reduction in cells that could exhaust CD8+ T cells. This also shows that macrophages are contributing to the PD-L1 expression in the lung. I also showed anti-CSF-1R treatment in obese mice increased PD-1 expression on CD8+ T cells (T cells could become exhausted!). This data suggested to me that combining anti-CSF-1R treatment with anti-PD-1 inhibitors could improve responses in obese mice. In fact, it did! I showed that a combination of anti-CSF-1R and anti-PD-1 reduced metastasis more in obese mice compared to lean (Figure 6-11). In this chapter we conclude **(1) Under conditions of obesity, responses to anti-PD-1 antibodies are limited. (2) Obese mice had more robust responses to anti-CSF-1R in the lungs, which was due in part to increased immune cells and decreased PD-L1+ macrophages (3) Obese mice also had more robust responses to a dual combination of anti-CSF-1R and anti-PD-1 antibodies within lung metastasis.**

Why this research matters:

My work showed that women with both high breast density and obesity may have a higher risk for breast cancer and could have worse overall survival. Identifying women with both risk factors may help diagnose women sooner and identify breast cancer patients that are at higher risk for metastasis. Additionally, investigating CD8+ T cells in the lung could identify new therapeutic targets for future patients. Lastly, exploring different patient demographics that have better responses to therapies already developed, like obese patients, helps us personalize cancer care, identify markers for therapy response, and save lives.