6-8-2016

Preclinical Study for Targeted Breast Cancer Therapy

Katie Bond
Santa Clara Univeristy

Lauren McCormick
Santa Clara Univeristy

Jordan Karroll
Santa Clara Univeristy

SANTA CLARA UNIVERSITY

Department of Bioengineering

I HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER MY SUPERVISION BY

Katie Bond, Lauren McCormick, and Jordan Karroll

ENTITLED

PRECLINICAL STUDY FOR TARGETED BREAST CANCER THERAPY

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

BACHELOR OF SCIENCE
IN
BIOENGINEERING

Zhiwen Zhang — 06/07/2016

Thesis Advisor — date

06/08/2016

Department Chair — date

PRECLINICAL STUDY FOR TARGETED BREAST CANCER THERAPY

By

Katie Bond, Lauren McCormick, and Jordan Karroll

* Each author has made equal contribution.

SENIOR DESIGN PROJECT REPORT

Submitted to
the Department of Bioengineering

of

SANTA CLARA UNIVERSITY

in Partial Fulfillment of the Requirements
for the degree of
Bachelor of Science in Bioengineering

Santa Clara, California

Spring 2016

ABSTRACT

Cancer is a devastating disease which affects millions. For this reason, many scientists are working to develop drugs that can specifically target cancer cells within the body. Preclinical trials are an important phase in the drug development process, as they allow scientists to determine whether or not a drug candidate may be viable for human use. Targeted therapies can be achieved by using monoclonal antibodies as well as small molecule drugs. In this study we compared the effects of two control monoclonal antibody drugs, “mAb-1” and “mAb-2,” with one small molecule drug, “Drug S,” on the MDA-MB-231 human breast cancer cell line in a xenograft SCID mouse model. All treatment groups containing Drug S exhibited the same trends as the negative control, mAb-1, suggesting Drug S is an ineffective tumor-reducing treatment.

ACKNOWLEDGEMENTS

We would like to give thanks to the following people for their generous help in making our senior design project possible.

Dr. Zhiwen Zhang acted as our project advisor and provided us with invaluable advice and guidance throughout our project.

We would also like to thank the employees of Bayside BioSciences for allowing us to work with them this year and for their willingness to teach. Dr. Ying Liu graciously granted us access to the lab space and helped us to hone our laboratory techniques. Dr. Frank Xie assisted us in our project and answered our many questions.

We greatly appreciate Ms. Jayshree Ullal for her kind donation of $2,500 to us for our work on cancer. The work that we have done was in this honor.

Lastly, we would like to thank the SCU School of Engineering for providing us with the necessary resources, as well as allowing us to participate in the Senior Design Conference.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

mAb - Monoclonal Antibody
IP - Intraperitoneal
PO (*per os*) - Oral dosing
QD (*quaque die*) - Once a day
BID (*bis in die*) - Twice a day
SCID - Severe combined immunodeficient
GLP - Good Laboratory Practice
IACUC - Institutional Animal Care and Use Committee

Chapter 1: Introduction

1.1 Background and Motivation

Cancer continues to be one of the leading causes of death, accounting for one in seven deaths throughout the world, and one in every four deaths in the United States. There are many contributing factors which may cause cancer, including external factors such as smoking and diet, and internal factors such as genetics. Cancer occurs when cells begin to grow too quickly. This rapid proliferation soon begins crowding out and destroying the body's healthy cells. Most cancers form lumps or growths called tumors. These lumps can be characterized as being benign, noncancerous, or else malignant, cancerous. Treatment for cancer aims to reduce or eliminate these tumors.[1]

Our study is specific to breast cancer, which ranks second among cancer-causing deaths in women, with one in eight women being diagnosed with breast cancer in her lifetime. Treatment of breast cancer usually involves either breast-conserving surgery by surgically removing tumors and the surrounding tissue or mastectomy (surgical removal of the breast). Treatment can also include radiation, hormone therapy and chemotherapy. Some targeted therapies are available, but pharmaceutical companies are constantly discovering new and improved therapies that specifically target cancer cells.[1]

1.2 Project Overview

We spent a year working in the labs of Bayside BioSciences, a pharmaceutical contracting company. Their primary projects involve preclinical testing, both *in vitro* and *in vivo*, for various pharmaceutical products. Over the course of this project we analyzed the effects of potential cancer therapies in the preclinical stage of the drug development process. Our work began with *in vitro* experiments to grow the MDA-MB-231 breast cancer cell line. After proliferating the MDA-MB-231 cells we injected them into breast tissue of mouse models to induce tumor growth *in vivo*. Following tumor growth, we injected the test compounds to determine their efficacy and safety within a mouse model. We evaluated the results in the context of control tests and drug

cocktails obtained from these experiments to ascertain the viability of moving the compound further through preclinical testing.

1.3 Problem Statement

Current cancer treatments do not have specific targeting abilities. Pharmaceutical companies are creating drug candidates for this purpose in order to develop a more effective cancer treatment. For our senior design project, we conducted a study to determine the efficacy of three drug candidates in combating the growth of human breast cancer cells in immunodeficient mice.

1.4 Significance

Preclinical testing is an important aspect of progressing a drug from the developmental stage to market. Before a drug can be administered in clinical trials it must go through a series of preclinical trials to ensure the safety of clinical trial patients. In the event that these drugs are efficacious they could serve as a novel alternative to current cancer therapies. A combination of these drugs could provide a highly specific targeted therapy to eliminate cancer cells while conserving noncancer cells. If the tests we conduct produce favorable results, the tested drugs may continue into higher level pre-clinical trials.

1.5 Literature Review

MDA-MB-231 Cell Line

The MDA-MB-231 breast cancer cell line has been used in numerous cancer studies to test the efficacy of cancer treatments. This cell line is often used to model metastasis, the migration of cancer cells to other parts of the body. Through gene regulation, the MDA-MB-231 cell source also allows for a variety of efficacious breast cancer tumor models. Study procedures utilizing MDA-MB-231 cells can more accurately represent human breast cancer conditions when injected into the mammary fat pads of immunodeficient mice. The use of an orthotopic injection site allows studies to successfully model the growth and spread of MDA-MB-231 cancer cells because the environmental conditions and hormone availability is representative of that in a human model. The use of immunodeficient mice is advantageous as it removes the host

specimen's ability to attack the foreign cancer cells. This allows the MDA-MB-231 cells to grow at a faster rate and more quickly model the effects of cancer therapies.[2]

Antibody Drugs

Antibodies are proteins that help the immune system identify foreign substances. Monoclonal antibodies are manufactured using a type of cell called a hybridoma, which multiples, producing a continuous supply of the antibody. Monoclonal antibody-based treatment of cancer is one of the most successful therapeutic strategies for tumor reduction.[3] These antibodies are used to target specific antigens, or protein targets on the surface of cancer cells. The FDA has approved several monoclonal antibody products. Trastuzumab is a humanized antibody that has been proven effective in combination with chemotherapy for patients with the human epidermal growth factor receptor 2/*neu* positive breast cancer.[4] Additionally, hundreds of monoclonal antibodies are currently at different stages of clinical trials. In one study, the expression of ganglioside GD2 was discovered on breast cancer stem cells. This is an ideal potential target for anti-tumor immunotherapy due to its high expression on cancer cells and restricted expression on normal tissue. In this study four anti-GD2 antibodies have been developed and tested in the clinic: 3F8, hu3F8, ch14.18 and hu14.18.[5] Future studies of tumor cells and the molecular pathways which are involved in the development of human cancers could help in identification of more cell surface antigens. This could provide additional therapeutic targets for more effective monoclonal antibody based products.

Small Molecule Drugs

Small molecule drugs often work as inhibitors of receptor tyrosine kinases (RTK's). During tumor growth and formation these RTK's are often deregulated, allowing cancer cells to grow abnormally quickly. The small molecule drugs, by acting as ATP mimetics, are able to inactivate the proteins in the kinases, which in turn slows the growth.[6] Through targeting kinase activity, studies have shown it is often the case that normal cells are able to survive inhibition of the kinase activity whereas tumor cells cannot, providing a selective targeting mechanism.[7] One benefit through using small molecule drugs is they are capable of passing through plasma

membranes.[6] This allows them to interact within the cytoplasm through both receptors and intracellular signaling molecules. Small molecule drugs offer a specificity in targeting cancer cells without harming normal cells that provides many opportunities for potential cures.[7]

1.6 Team Management

We worked with Bayside BioSciences, a pharmaceutical contracting company, under the instruction of Dr. Ying Liu and Dr. Frank Xie. Our professional partnership with Bayside BioSciences required that we sign a nondisclosure agreement to protect the company's intellectual property. In addition to this partnership, we were under the advisement of Dr. Zhiwen Zhang of Santa Clara University's Bioengineering Department.

1.7 Budget

We received a generous grant of $2,500 from Ms. Jayshree Ullal's SITA Foundation Dean's Fund. These funds went towards the purchase of SCID mice, cell culturing media, and other supplies. All other expenses were covered by Bayside BioSciences.

1.8 Timeline

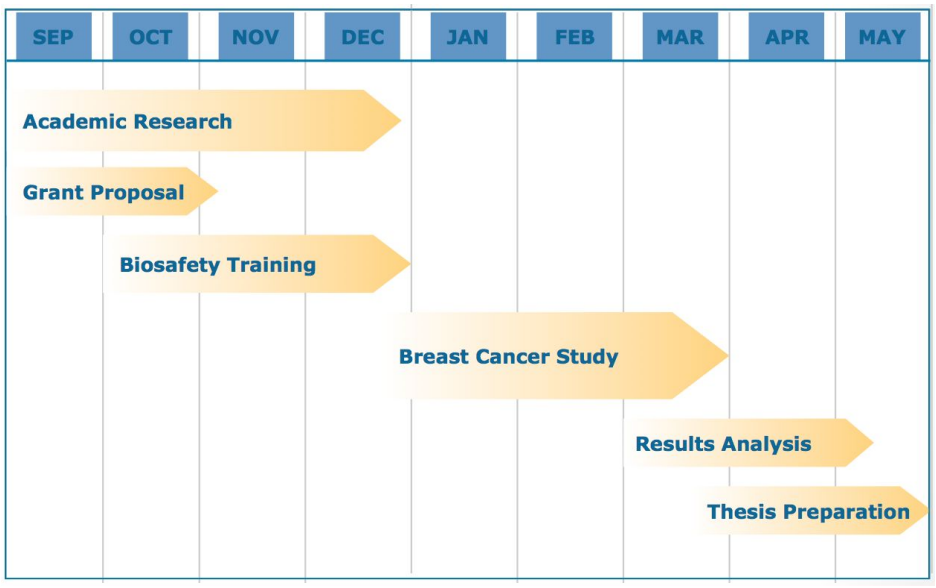

Figure 1.1: Project timeline

During the fall quarter of 2015 we were educated in good laboratory practices through laboratory and biosafety training at Bayside BioSciences. We observed and aided in the data collection of numerous tumor reducing efficacy studies. During this time we also practiced mammalian cell culturing technique. In the winter quarter of 2016 we conducted our study. This included dosing and collecting weight and tumor measurements. During the spring quarter of 2016 we finished analyzing our data and prepared for our senior design presentation and thesis.

Chapter 2: Constraints

2.1 Current Methods

There are two categories of treatment methods for breast cancer. Local therapies, such as surgery and radiation therapy, target the site of tumor growth. Systemic therapies, including chemotherapy, hormone therapy, and targeted therapy, are not specifically applied, and therefore may affect more than just the cancer cell target. Surgical removal of breast tumors can be as simple as a partial mastectomy, when the tumor and tissue around it is removed, and as extreme as a total mastectomy, when all of the breast tissue and sometimes the lymph nodes are completely removed. Surgical removal of the breasts is an invasive procedure which can be difficult for women to decide to do. Additionally, while surgery may physically remove all cancerous tissue, there may be some remaining cancer cells requiring continuing radiation or hormone treatments.[8] Chemotherapy is a commonly used therapy involving small molecule drugs administered intravenously or orally, and subsequently circulates throughout the body. This method targets cancer cells by inhibiting specific steps in the process of cell division, causing apoptosis, or cell death. Common negative side effects of chemotherapy, including loss of appetite and low blood count, can leave the patient feeling sick and weak during treatment. Since chemotherapy is a systemic therapy, it also risks causing permanent damage to noncancerous cells and can therefore risks organ failure.[9] Our study uses a targeted therapy involving a combination of a small molecule drug and a monoclonal antibody. The small molecule drug functions by releasing molecules which target signaling pathways within cancer cells to cease cell growth and/or initiate apoptosis. Similarly, monoclonal antibodies are signal proteins which can bind to specific receptor proteins on the outside of cancer cells, prompting the cell to take in the signal protein. Once the signal protein is inside the cell, the drug attached to it will be released and will then kill the cell. This combination of targeted treatments allows for a more specific response and results in fewer mild side effects than chemotherapy drugs alone.[10]

2.2 Engineering Standards and Realistic Constraints

Ethics

Due to the fact that our project was done in the preclinical phase of drug development, our testing was done using *in vivo* models. Our models, therefore, had to be constructed using live mice, which may raise some ethical concerns. In order to address these concerns, we were educated through both the Institutional Animal Care and Use Committee (IACUC) as well as Good Laboratory Practice (GLP). The IACUC sets guidelines that outline rules and regulations for animal testing and biosafety. GLP training ensures ethical procedures are taken as well as the protects the uniformity, consistency, and reproducibility of results. Together, the IACUC and GLP training allowed us to successfully complete our project while maintaining a paramount level of ethical practice. In addition to the training we underwent, the laboratory we worked in went through periodic veterinary checkups and inspections to confirm the animals were being treated ethically through proper housing, diet, and care.

Another ethical consideration we had to take involved the confidentiality of the study. Since Bayside Biosciences is a contracting company, they work with third party clients. Due to this fact, we had to maintain the anonymity and confidentiality of the client's classified information. In order to protect this classified information, we could not disclose the identity of the compounds used in the study.

Health and Safety

We also had to take into consideration the health concerns for both the consumers of the eventual product as well as the researchers conducting the study. In terms of the consumer, we had to make sure our research on the drug was precise and accurate to protect their well being. In order to guarantee their safety, we aimed to provide a factually accurate study, look at the side effects of the drugs being tested, and create usage warnings as they pertain to pregnancy, age, other illnesses, and concurrent medications the consumer might be taking. Through providing the consumer with this knowledge, they can use the drug as intended. The drug we tested would have to go through more testing before it reached the consumer. Our study was in the preclinical phase so it is only tested for safety in animals in this stage, following this it would be tested in humans and then available to the consumer.

As for the researchers, we determined the biggest threat to our health and safety during this project was through the handling of the animals and the sharp objects. The mice we were working with could be unpredictable and move quickly while we were working with them. This brought the concern of using sharp objects, like needles and scalpels, near them. The mice could also bite us if not handled properly. To minimize this safety concern we went through biosafety training which taught us the safest methods for handling the mice and sharp objects.

Economic

The economic aspect was a significant concern for our project. In order to create a successful drug, the drug must be cost efficient and accessible to the general public. To do this a balance must be found in which the drug is affordable, yet at the same time capable of bringing the pharmaceutical company profit. While it may be legal to inflate the price of a potentially life saving drug, it is not ethical.[11] From this we realized there is also a distinction to be made between what is legal and what is ethical.

Manufacturability

The process behind developing new medicines is complicated, expensive, and time consuming (Figure 2.1). On average, it takes at least 10 years for a new medicine to complete the journey from initial discovery to the marketplace.[12] Additionally, it costs approximately 2.6 billion dollars to develop a drug that gains market approval.[12] Once a drug has been discovered, it is sent through preclinical trials to test its safety on animal models. If the preclinical trial is successful, the drug candidate may move on to clinical trials, where the drug is assessed further for safety and efficacy in humans. After clinical studies, the FDA will review the drug and approve it if applicable. After FDA approval for market, the company continues to monitor the drug for unexpected side effects and additional data.[12]

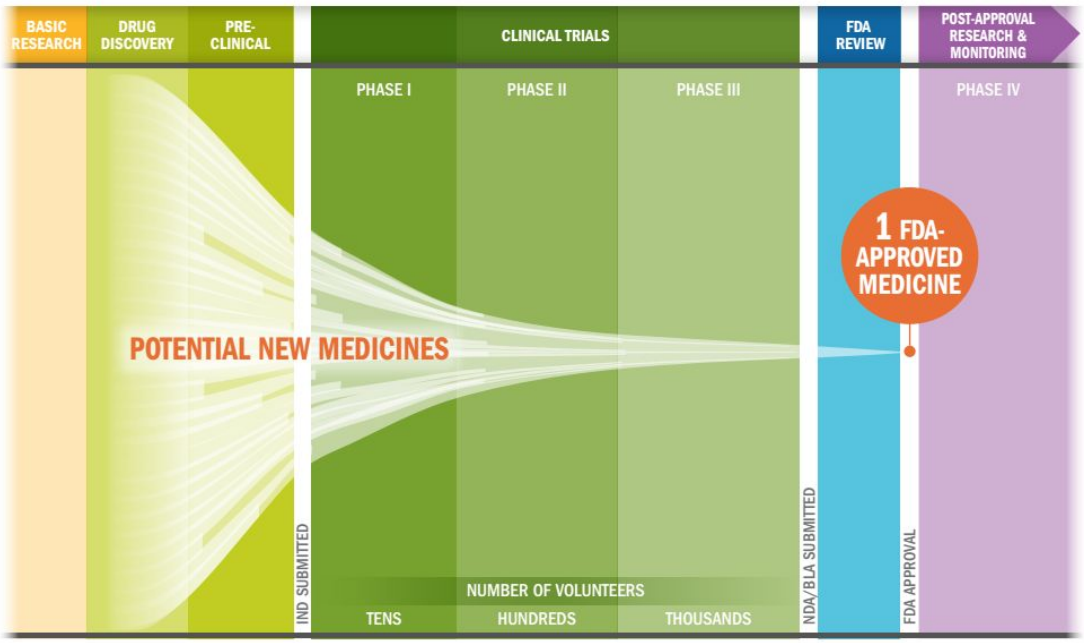

Figure 2.1: Drug development process
Source: Pharmaceutical Research and Manufacturers of America

The processes for which monoclonal antibody drugs and small molecule drugs go through are similar, however they differ in their difficulty of manufacturing. Currently, mAb's are more difficult to design and produce due to their larger size and complexity. Furthermore, with mAb's, they require an antibody generation step which increases the cost significantly. The cost to manufacture a mAb is also much higher due to their high specificity for targeted therapies.

Social

On a social level, we had to consider the possibility that while it is legal to test on animals, it may not be ethical from some perspectives. We also had to keep in mind the possibility for abuse or misuse of the potential drug. As preclinical drug testers, we can only go so far to ensure the consumer is assuming ethical responsibility for their welfare. Because side effects differ among patients, any drug should be taken under physician supervision. However, once out of a physician's care, it is the responsibility of the patient to follow the dosing instructions. This is where the possibility of an overdose arises. Also, a patient may become frustrated with the length

of time it takes to cure their disease, so they might attempt increasing their dosage with the hope it would cure them more quickly.

Chapter 3: Design Description

3.1 Format of Study

Table 3.1: Active compounds corresponding to group number

Group	Active Compounds	Sample Size
Group 1	Vehicle Only	5
Group 2	mAb 1	5
Group 3	mAb 1 + mAb 2	5
Group 4	mAb 1 + mAb 2 + Drug S	6
Group 5	mAb 2 + Drug S	5
Group 6	mAb 2	5
Group 7	Drug S	5
Group 8	mAb 1 + Drug S	6

This study includes 8 groups, each containing a different combination of three different drugs (Table 3.1). Each treatment arm had between five and six mice, for a total of 42 mice in the study. The first group was a control, containing only the vehicles with no treatment, which acted as a baseline to which we could compare other groups and eliminate results that may be due to the vehicle rather than the drug itself. The rest of the groups were created to provide every combination possible of the three drugs, including each drug on its own and a cocktail of all three drugs together. By looking at these different drug combinations, the goal is to determine a which will provide the highest specificity to target the cancer cells. This is especially important because while we are injecting one type of cancer cell in our *in vivo* mouse model, human tumors tend to have much greater variation in cancer cell type, so it is difficult to target them all. It is therefore important to look at these drugs in combination so we can find a treatment that will provide the optimum benefit.

3.2 Drug Types

Vehicles

For each drug, we had a corresponding vehicle used in place of the active drug if it was not being administered to the treatment group. A vehicle is a chemically inactive substance or structure used as a medium to deliver a drug. An ideal drug delivery vehicle should be non-toxic, biocompatible, non-immunogenic and biodegradable.[13]

Monoclonal Antibody

The first drug type we used were monoclonal antibodies (mAb), which acted as our positive and negative controls for the study. Therapeutic antibodies have recently become one of the most successful and important strategies in treating cancer patients. An antibody is a protein with specialized binding regions which are able to target specific receptors, antigens, on cancer cell surfaces, inhibiting the cell by one of three mechanisms (Figure 3.1).[14]

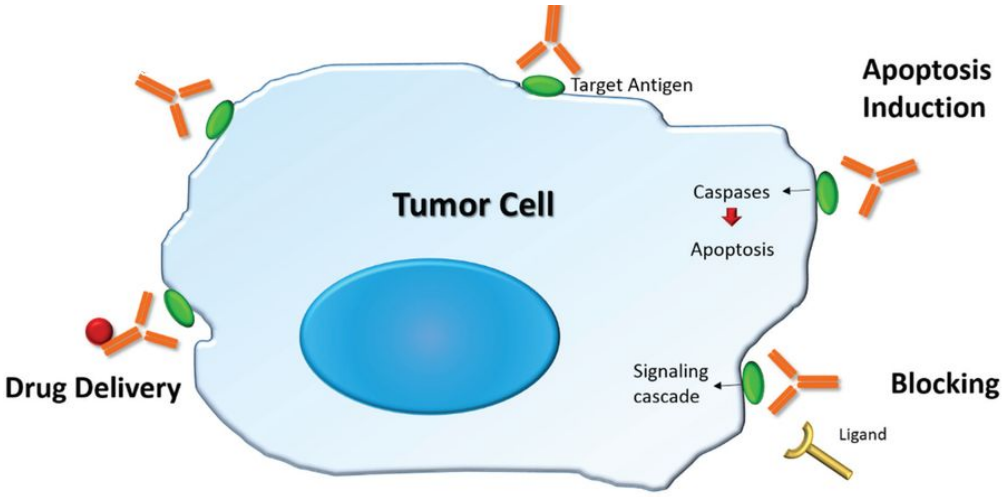

Figure 3.1: Functions of therapeutic mAb drugs

Source: Loureiro et al.

The first way mAbs can inhibit the tumor cell is that it can initiate the release of enzymes such as caspases, which causes apoptosis, or cell death. The antibody can also bind with the antigen to block a different protein, or ligand, from binding to the cell. This prevents an internal signaling cascade, thereby hindering cell processes important to cell replication. Lastly, the antibody may be carrying a drug compound to the binding site, releasing it into the cancer cell and allowing it to take effect.[15]

Pharmaceutical companies can create these drugs by first identifying surface proteins specific to the cancer cell, and then by creating a monoclonal antibody which is able to target that protein. However, mAbs are very large (~150,000 daltons) (Figure 3.2), and this causes problems with orally administration because the antibodies cannot pass through the epithelial membrane of the

digestive tract to get into the circulatory system.[14] Instead, mAb drugs must be administered via intravenous injection of intraperitoneal injection. For our study, intraperitoneal injection was utilized because it is much easier to perform on mice and has a similar effect to intravenous injection.

Small Molecule

The second drug we used in our design was a small molecule drug, which was the experimental drug for the preclinical study. Whereas monoclonal antibodies are very large, small molecules are, as their name suggests, much smaller in size (~10 daltons)(Figure 3.2).[16] The benefit of using small molecule drugs is that, due to their size, they have high tissue permeability and are therefore capable of passing through the epithelial membrane in the digestive tract to target other cells in the body.[17] Because of this, small molecule drugs may be dosed orally, which is much more convenient for the consumer.

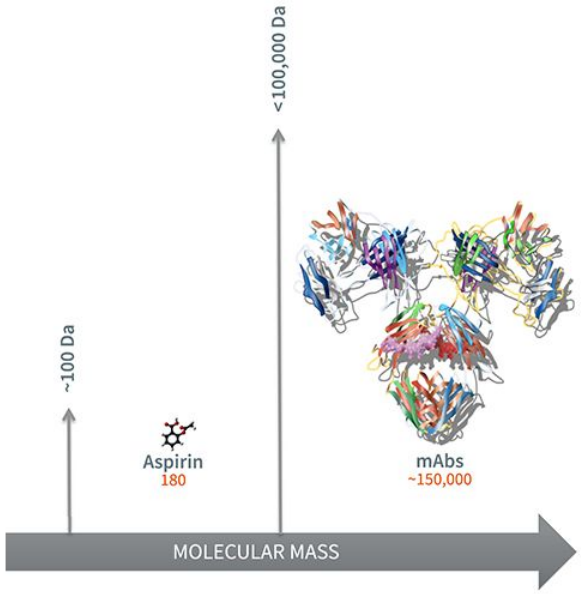

Figure 3.2: Size comparison between small molecule and mAb drugs

Source: Amgen

Rather than targeting receptors on the outside of the cell, these drugs penetrate cancer cell membranes and bind to proteins inside the cell, altering or inhibiting internal cell pathways, and

consequently damaging the cell. Unfortunately, since small molecules are able to permeate many different membranes, they are less specific in the cells that they target relative to antibodies.[17]

The way a pharmaceutical company determines a small molecule drug to use in a study is very different from a monoclonal antibody. The typical method of choosing a small molecule drug to test is by running preliminary *in vitro* tests on thousands of different small molecules from a library of identified compounds. Then these choices are narrowed down to a select few which show potential efficacy for a trial.[17]

Chapter 4: Materials and Methods

4.1 Research Model

For our study, we first created a xenograft cancer model. Xenograft refers to the transplantation of cells or tissues from one species to another. In our case, this was demonstrated by the culturing of human breast cancer cells *in vitro* and the implantation of those cells in mice, where they were then given different drugs to determine their efficacy on reducing tumor size (Figure 4.1). This process is a common research technique for variations of preclinical oncology research.[18]

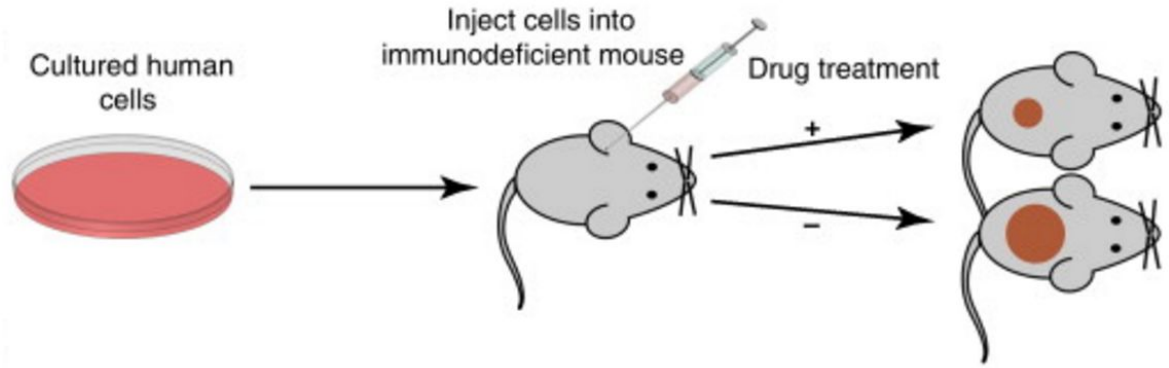

Figure 4.1: Drug testing in xenograft models

Source: Roper

For our research, the type of mice we used were severe combined immunodeficient (SCID) mice. Specifically, CB17 SCID mice, which have a compromised immune system due to impaired or absent T and B lymphocytes. Lymphocytes are subtypes of white blood cells made in the bone marrow that account for a critical part of the immune system. T cells are involved in cell-mediated immunity, and B cells are responsible for humoral immunity by creating antibodies.[19] When T and B lymphocytes are absent or impaired, this prevents the mice's immune system from rejecting the foreign xenograft cells, allowing them to grow uninhibitedly within the mice.

Severe combined immunodeficient mice are very sensitive to infection by a wide range of normal pathogens, opportunistic pathogens and commensal organisms. This means that the mice

had be housed and maintained in a separate room from the other animals in the lab to create a maximum barrier for infection. Food, water, bedding, cages and anything else that came into contact with the mice were irradiated, or sterilized, beforehand. Every time the cages were changed or the mice were handled, it was all done in a sterilized laminar flow hood. Careful handling and attention to detail was essential to housing SCID mice.[20]

4.2 MDA-MB-231 Cell Line

The xenograft cells we used came from the MDA-MB-231 cell line originating from the breast tissue of a 51 year old female. This cell line was derived from a metastatic site which means that this cell line is often used in the study of metastatic cancer.[21]

Figure 4.2: Cancer cells injected into the mammary gland

Source: Russell

Prior to implantation, the cells were grown in culture and were passaged several times. Cell passaging, or splitting, is a technique that enabled us to keep the cells alive and growing under cultured conditions until the mice were ready for implantation. At that point, the cells were isolated from the cell culture and resuspended in Matrigel. Matrigel is a liquid matrix containing support proteins and growth factors which help the foreign breast cancer cells attach and differentiate within the mice.[22]

For this study, mice were given an anesthetic and were then injected with a specific volume of the cell solution containing approximately 2 million cells per injection. Most tumor xenografts are injected subcutaneously on the backs of the mice. However, to more accurately model the hormones and other factors that play a role in breast cancer development in humans, our xenograft injections were made on the underside of the mice, in the mammary fat pads between the skin and muscle (Figure 4.2).[23] The mammary fat pad model is currently the preferred location of transplanting human breast cancer cells. Using this model, the tumor cells can be tracked as they move through the blood and lymphatic vessels within the mammary gland and metastasize, or spread, to other organs.[23]

4.3 Dosing Regimen

Table 4.1: Treatment arms and corresponding dosing techniques

Active Compounds	Intraperitoneal		Oral
Vehicle Only	Vehicle 1	Vehicle 2	Vehicle S
mAb-1	mAb-1	Vehicle 2	Vehicle S
mAb-1 + mAb-2	mAb-1	mAb-2	Vehicle S
mAb-1 + mAb-2 + Drug S	mAb-1	mAb-2	Drug S
mAb-2 + Drug S	Vehicle 1	mAb-2	Drug S
mAb-2	Vehicle 1	mAb-2	Vehicle S
Drug S	Vehicle 1	Vehicle 2	Drug S
mAb-1 + Drug S	mAb-1	Vehicle 2	Drug S

Once the xenograft cells were implanted into the mice, the tumors grew for two weeks until they were approximately 100-150 mm^3 in size. Mice with tumors that were either too large or too small and were dismissed from the study. The mice were then shuffled into groups based on this measurement to ensure that a range of tumor sizes were represented, while maintaining a common average of about 150 mm^3 for each group. Once the mice were shuffled, eight treatment arms were created to test the different drug therapies.

4.4 Dosing Schedule

Our dosing schedule consisted of three drugs, two monoclonal antibodies, “mAb-1” and

"mAb-2", as well as a small molecule drug, "Drug S". The monoclonal antibodies acted as our controls in the study. mAb-1 represented the negative control, meaning that it is ineffective at reducing tumor size. mAb-2 represented the positive control because it demonstrates the trend of effective tumor reducing treatment. Drug S, the small molecule drug, is our experimental drug, which we will compare to the negative and positive controls, as well as a control group containing no active treatment.

As can be seen in Table 4.2, mAb-2 or its vehicle was dosed once per day, every day except Thursday. Drug S or its vehicle was dosed twice per day, every day except Thursday. On Thursdays, when mAb2 and Drug S were not administered, mAb-1 or its vehicle was dosed instead. The dosing amount for each drug is based on weight and was calculated based on the expected weight of a 20 gram mouse. The dosing volume for Drug S was approximately 200 microliters, whereas the dosing volume for mAb-1 and mAb-2 was approximately 90 microliters.

Table 4.2: Dosing Schedule

Drug	Frequency	Specifications	Administration
Vehicle 1 or mAb-1	1x/week	Thursday Only	IP
Vehicle 2 or mAb-2	1x/day	Except Thursday	IP
Vehicle S or Drug S	2x/day	Except Thursday	PO

Dosing frequency (Table 4.2) is determined based on the pharmacokinetics property of the drug. In general, small molecules have a much shorter half-life in animals or human beings, ranging from a few minutes to a few hours. mAbs generally have a much longer half-life in vivo, ranging from hours to days. Half-life refers to the time it takes for the concentration of a substance, such as a drug, to be reduced by one-half. This is the duration of the action of a drug. This explains why Drug S, as a small molecule drug, is dosed twice per day. Monoclonal antibodies can vary

in half-lives, explaining why mAb-2 must be dosed every day, while mAb-1 is dosed only once a week. The longer the half-life of a drug is, the less frequently it must be dosed.

4.5 Data Collection

The first quantitative measurement used was body weight. These measurements began for each treatment group on the first day of dosing, and continued to be recorded multiple times a week as specified by the client.

Average body weight for the SCID mice we tested is expected to be between 18 and 20 grams. Changes in this expected body weight can be a good indicator of drug toxicity. A significant body weight drop of 15% or greater, as specified by the client, over the course of the study is indicative of a toxic drug and treatment is stopped.

To quantify our data, we also measured tumor size over the course of the study. Tumor measurements were taken twice per week, on Tuesday and Friday, using a digital caliper (Figure 4.3).[24] A caliper is a device used to measure the distance between two opposite sides of an object. Digital calipers allow for the measurement to be recorded electronically when connected to a computer. To measure the tumors, we first measured the length (L) of the tumor, then the width (W). These values were sent to the computer where they were used to calculate the total tumor volume according to the following formula:

$$\frac{(L*W^2)}{2} \qquad \text{(eq. 1)}$$

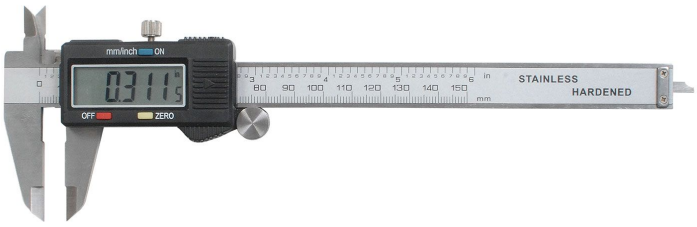

Figure 4.3: Digital caliper for tumor volume measurements
Source: "Precision Digital Caliper"

Measurement by digital caliper is currently the accepted method in industry. In order for this method to be viable, the same individual must consistently take each measurement to ensure precision of data. This allows the data to be accurately analyzed for relative tumor growth.[25]

Chapter 5: Results

5.1 Body Weight

As seen in Table 5.1 each group was within the expected range of average body weight at the start of the study. All but Groups 2 and 3 ended the study still within the expected range. Groups 2 and 3 had the greatest decrease in body weight over the course of the study. Group 2 exhibited a 11.8% decrease and Group 3 exhibited a 14.5% decrease, so mAb-1 alone and the mAb-1/mAb-2 combination are not considered to be toxic treatments.

Table 5.1: Average body weight (g) and percent body weight decrease

Active Compounds	Day 3	Day 4	Day 7	Day 8	Day 9	Day 11	Day 15	Day 22	% Decrease
Vehicle Only	19.2	19.6	19.4	19.8	18.8	18.9	18.9	18.7	2.6%
mAb-1	19.6	19.9	19.6	19.6	19.1	18.9	18.9	17.5	11.8%
mAb-1 + mAb-2	20.2	21.3	19.9	19.7	19.2	19.1	19.1	17.6	14.5%
mAb-1 + mAb-2 + Drug S	19.2	19.5	19.0	19.3	18.7	18.9	18.6	18.5	4.3%
mAb-2 + Drug S	18.3	19.8	19.0	19.5	19.2	19.5	18.9	19.0	-3.8%
mAb-2	18.5	19.9	19.1	19.9	18.8	19.1	18.4	18.8	-2.2%
Drug S	18.8	19.8	19.8	20.1	19.6	19.3	18.6	18.9	-0.6%
mAb-1 + Drug S	20.5	21.3	20.6	20.5	20.1	20.0	19.8	19.9	4.4%

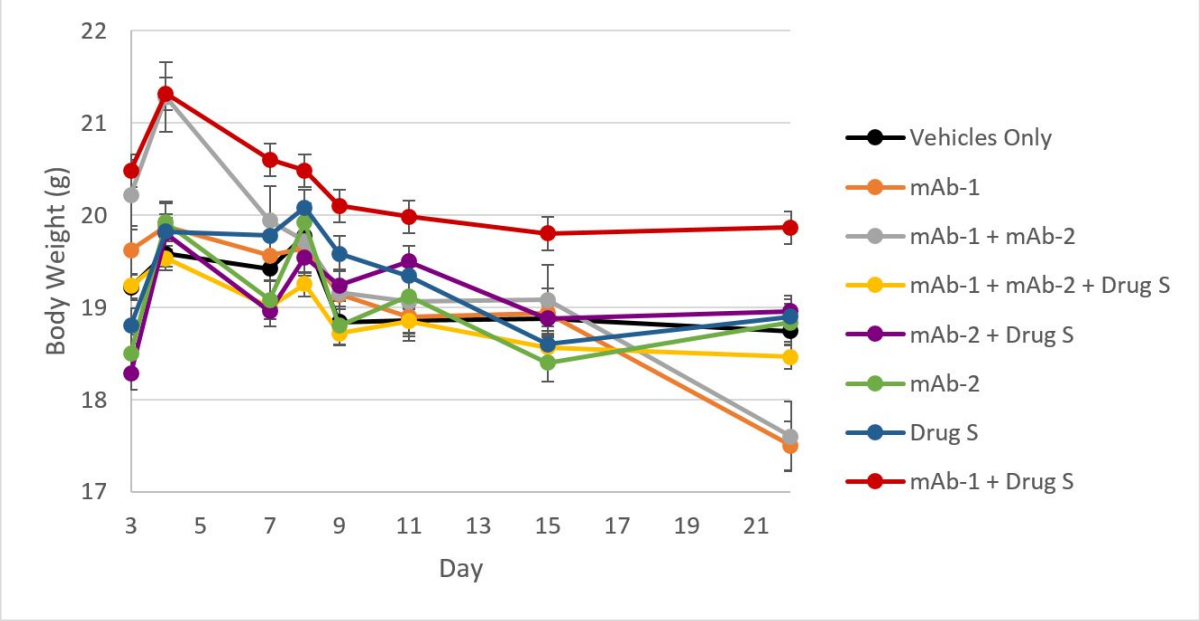

Figure 5.1: Average body weight measurements for all groups

Figure 5.1 shows fluctuations during the first few days of measurement. This is due to the time of day during which the mice were weighed. These mice tend to weigh one gram heavier in the

morning than in the evening. There is a general up and down trend common to all of our experimental groups, suggesting that they may have been measured in the evening on Day 3 while they may have been measured in the morning on Day 4. This can sometimes occur when there are other experiments in the lab that have higher priority at a certain time.

5.2 Tumor Size

Table 5.2: Average tumor volume (mm^3) and percent tumor volume increase

Active Compounds	Day 1	Day 3	Day 8	Day 11	Day 15	Day 18	Day 22	% Increase
Vehicle Only	163.4	166.0	251.5	384.1	514.1	626.3	930.0	469%
mAb-1	141.7	219.0	280.4	372.0	468.0	720.1	916.9	547%
mAb-1 + mAb-2	142.9	182.2	312.1	323.6	394.9	532.6	608.6	326%
mAb-1 + mAb-2 + Drug S	159.5	211.3	330.5	386.1	560.3	707.4	1105.2	593%
mAb-2 + Drug S	159.9	215.1	351.8	471.8	538.8	631.5	972.7	508%
mAb-2	144.9	158.5	320.5	426.7	378.6	473.7	540.8	273%
Drug S	172.3	328.6	387.2	548.1	613.5	785.6	1141.9	563%
mAb-1 + Drug S	143.3	215.9	229.5	419.0	477.8	611.6	1019.6	612%

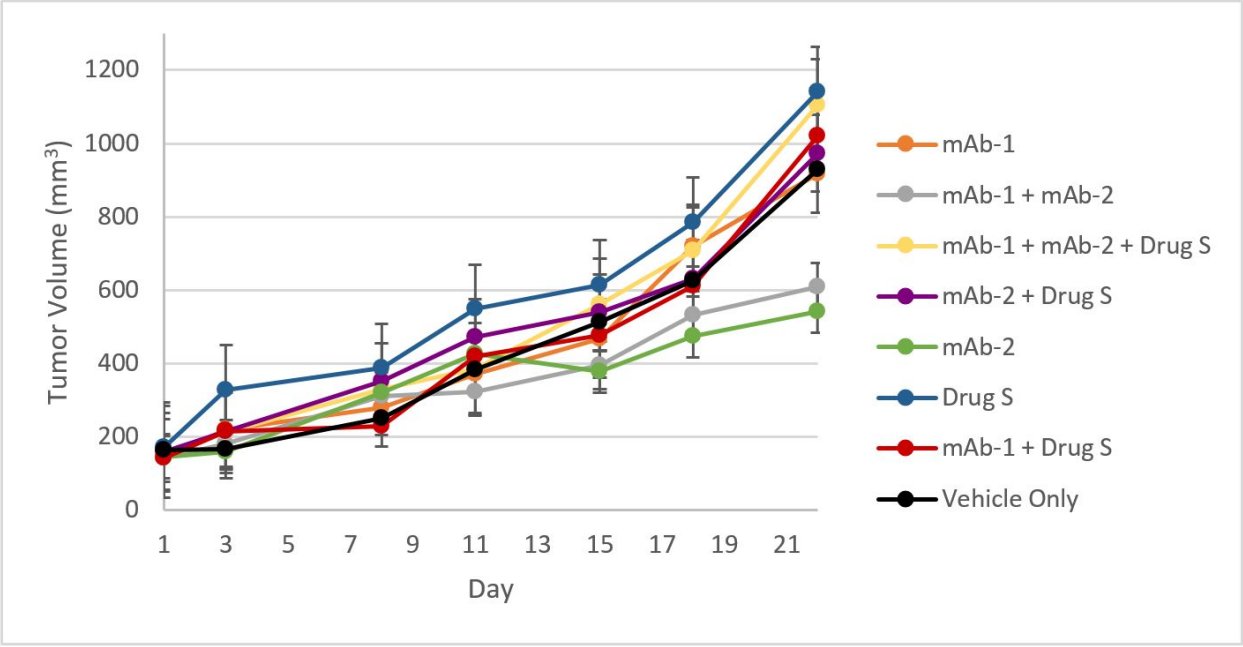

Figure 5.2: Average tumor measurements for all groups

Controls

Our negative control, Group 2, demonstrates the trend (Figure 5.2) of a non-tumor reducing treatment with an exponentially increasing curve and 547% increase in tumor size (Table 5.2). Group 1, the vehicle only group, follows the trend of the negative control, which is consistent with what we expected for untreated tumors. Group 6 is the positive control and demonstrates the trend (Figure 5.2) of an effective tumor reducing treatment due to the decreased rate of tumor growth (273% increase, Table 5.2) relative to the negative control. Group 3 contains both the positive and negative control, and exhibits a similar trend of tumor expansion to that of the positive control alone (Figure 5.2).

Receptivity of Drug S

All groups containing Drug S exhibit trends similar to those of the negative control and vehicle only groups, suggesting Drug S is not an effective treatment for MDA-MB-231 tumors (Figure 5.2). All groups containing Drug S had a greater than 500% increase of tumor size. When Drug S was put in combination with the negative control in Group 6, we observed the greatest percent increase in tumor growth (612%, Table 5.2) among all the treatment groups. Therefore there was no tumor reducing benefit in this combination of mAb-1 and Drug S.

When Drug S was combined with the mAb-2 positive control in Group 8, tumor growth followed the negative control trend, rather than that of the positive control. Similarly, our drug cocktail containing mAb-1, mAb-2, and Drug S (Group 4) follows the trend of the negative control tumor growth trend, suggesting Drug S reduced mAb-2's effectiveness to that of a non-tumor reducing treatment (Figure 5.2).

Chapter 6: Discussion

6.1 Toxicity

Although none of our treatment arms reached the 15% body weight decrease (Table 5.1) that indicates drug toxicity, Group 3 was bordering this limit, and should therefore not be discounted. The solvent used to carry the mAb drug compounds, dimethyl sulfoxide (DMSO), may have caused this drop in body weight. DMSO is a solvent commonly used to solubilize compounds to facilitate drug delivery. Although used in numerous studies, injected DMSO can induce apoptosis of cells by interfering with their plasma membrane compositions.[26]

All of the treatment groups containing mAb-1 demonstrated a decrease in body weight, while all others (excluding Group 1) exhibited an overall increase in body weight. This suggests that the concentration of DMSO used in mAb-1 formulations may have contributed to the greater body weight disruptions in Groups 2 and 3. While it is comparably effective to the positive control, the mAb-1 and mAb-2 combination (Group 3) had potentially toxic effects. Therefore, the positive control alone should be considered for use above the combination of the two controls. Future studies should be conducted to reevaluate the composition of these treatment groups' compounds.

6.2 Effects of Drug S on Controls

When Drug S was given in combination with the mAb-1 or mAb-2 control, the treatment was less effective than the vehicle only group. Group 8, which contains mAb-1 and Drug S, was least effective at reducing tumor growth (Table 5.2). These non-tumor reducing effects may be due to negative drug interactions. We can be certain it is Drug S which causes these negative interactions because of the different treatment arms we analyzed. We can see that when mAb-1 and 2 are combined, they do not seem to have any negative drug interactions, so the treatment continues to be effective (Figure 6.1).

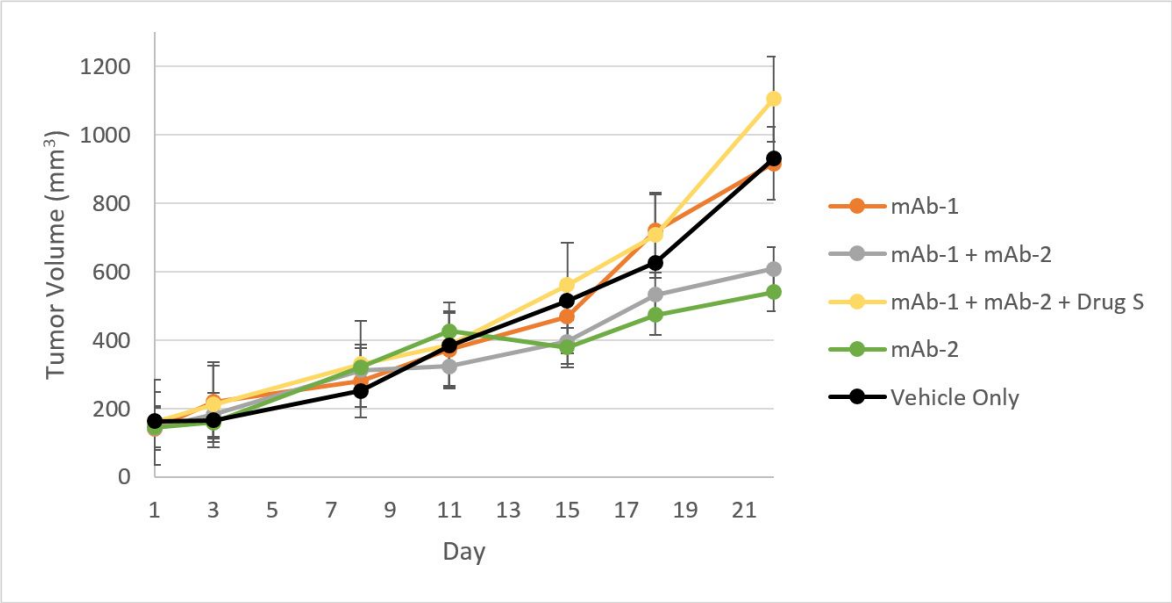

Figure 6.1: Average tumor measurements for controls and cocktail

Small molecule drugs work by interfering with cellular pathways within the cell; mAb's, on the other hand, targets receptors on the outside of the cell, causing a cascade which disrupts the cell's natural pathways (See Chapter 3.2). When mAb-2, our positive control, bound to the outside of the cancer cell, it had either initiated or disabled a cellular pathway leading to apoptosis. This mechanism slowed tumor growth in Group 6.

In Group 8, when mAb-2 was paired with our small molecule, Drug S, it is likely that the mechanism used by Drug S interfered with the signaling pathway usually affected by mAb-2. This interference therefore prevented mAb-2's usual tumor reducing effects.

6.3 Metastasis of MDA-MB-231 Tumors

By the end of the study, many of the MDA-MB-231 tumors had metastasized to the lungs and heart. From our literature research we have found that the MDA-MB-231 is an aggressive cell line commonly used to model metastasis, or the spread of cancer to other areas of the body, in xenograft cancer studies.[27] This cell line has high levels of autophagy due to increased permeability of mitochondrial pores. This autophagy in the MDA-MB-231 cell line allows for increased rates recycling of old proteins and cell debris to fuel the anabolic needs of the cancer

cells.[28] This results in faster and more efficient proliferation and spread of the cancer cells.

Chapter 7: Summary and Conclusions

7.1 Summary

Our study demonstrated the effects of small molecule and mAb drugs on MBA-MB-231 tumors in CB17 SCID mice. Treatment groups containing small molecule Drug S did not prove to be effective tumor reducing drug candidates. The positive control, mAb-2, alone was the most effective treatment as it reduced the rate of MDA-MB-231 tumor growth by 72% (Table 5.2) when compared to the vehicle only group (Group 1). Even when in combination with mAb-2, Drug S facilitated the greatest percent increase in tumor size among the treatment arms; therefore, Drug S is an inefficacious breast cancer treatment.

7.2 Future work

When we report our data to the client pharmaceutical company, we will suggest they attempt to alter their formulation of Drug S and conduct more *in vitro* testing prior to attempting more preclinical trials. If Drug S is no longer a viable option, we suggest searching for a small molecule drug that will work in conjunction with our positive control to amplify, rather than contradict, the signaling pathways needed to kill the cancer cells.

An additional consideration that the client may address in future research is using a different cell line. By the end of our study, many mice had tumors that had metastasized, affecting their lungs and other organs, making the cancer too difficult to treat. Perhaps if the client were to test Drug S on a less aggressive cell line, they may be able to better identify how it affects intracellular cancer cell pathways. Then Drug S may be strengthened so it can be used to treat more aggressive forms of cancer.

7.3 Conclusions

As we discussed before, this process of drug development takes many years and thousands of drug candidates to sift through. While Drug S did not prove to be an effective form of treatment due to the results of this study, our project was ultimately successful. From here, we can inform

the client of our findings so that they may reformulate Drug S and get us one step closer to finding a drug that will be able to combat this complex disease.

BIBLIOGRAPHY

[1] Siegel, Rebecca, and Ahmedin Jemal. "Cancer Facts & Figures 2015."*American Cancer Society, Inc.* (2015): n. pag. Web. 13 Oct. 2015.

[2] Kocatürk, B., Versteeg, H. H. Orthotopic Injection of Breast Cancer Cells into the Mammary Fat Pad of Mice to Study Tumor Growth.. *J. Vis. Exp.* (96), e51967, doi:10.3791/51967 (2015).

[3] Scott, Andrew M., James P. Allison, and Jedd D. Wolchok. "Monoclonal Antibodies in Cancer Therapy." *Cancer Immunity* 12 (2012): 14. Print.

[4] Modjtahedi, H., S. Ali, and S. Essapen. "Therapeutic Application of Monoclonal Antibodies in Cancer: Advances and Challenges." *British Medical Bulletin* 104.1 (2012): 41-59. Web.

[5] Ahmed, Mahiuddin, and Nai-Kong V. Cheung. "Engineering Anti-GD2 Monoclonal Antibodies for Cancer Immunotherapy." *FEBS Letters*588.2 (2013): 288-97. Web.

[6] Imai, Kohzoh, and Akinori Takaoka. "Comparing Antibody and Small-molecule Therapies for Cancer." *Nature Reviews Cancer Nat Rev Cancer* 6.9 (2006): 714-27. Web.

[7] Zhang, Jianming, Priscilla L. Yang, and Nathanael S. Gray. "Targeting Cancer with Small Molecule Kinase Inhibitors." *Nature Reviews Cancer Nat Rev Cancer* 9.1 (2009): 28-39. Web.

[8] "Surgery for Breast Cancer." *Surgery for Breast Cancer*. American Cancer Society, 15 Jan. 2016. Web. 26 Jan. 2016.

[9] Burns, C. Patrick and Vaena, Daniel A. "Chemotherapy and Other Antineoplastic Drugs."*AccessScience*. McGraw-Hill Education, 2014. Web. 5 Feb. 2016.

[10] "Targeted Therapy for Breast Cancer." *Targeted Therapy for Breast Cancer*. American Cancer Society, 15 Jan. 2016. Web. 26 Jan. 2016.

[11] "Economic Impact." *PhRMA*. Pharmaceutical Research and Manufacturers of America - See More At: Http://www.phrma.org/economic-impact#sthash.a2okaDQM.dpuf, n.d. Web. 31 May 2016.

[12] PhRMA. "Biopharmaceutical Research & Development: The Process Behind New Medicines." (n.d.): n. pag. *PhRMA*. Pharmaceutical Research and Manufacturers of America. Web.

[13] "Drug Delivery Systems: Getting Drugs to Their Targets in a Controlled Manner." *National Institute of Biomedical Imaging and Bioengineering*. National Institutes of Health, n.d. Web. 27 May 2016.

[14] Wang, W., EQ Wang, and JP Balthasar. "Monoclonal Antibody Pharmacokinetics and Pharmacodynamics." *Journal of Pharmacokinetics and Pharmacodynamics* 84.5 (2008): n. pag. Nature Publishing Group. Web.

[15] Loureiro, Liliana R., Mylène A. Carrascal, and Ana Barbas. "Challenges in Antibody Development against Tn and Sialyl-Tn Antigens." *MDPI*. Biomolecules, 2015. Web. 27 May 2016.

[16] Amgen. "The Power of Biologics." Amgen Biosimilars. Amgen Inc., n.d. Web.

[17] "Small Molecule versus Biological Drugs" *Generics and Biosimilars Initiative*. GaBI, 29 June 2012. Web.

[18] Roper, Jatin, and Kenneth E. Hung. "Priceless GEMMs: Genetically Engineered Mouse Models for Colorectal Cancer Drug Development." *Trends in Pharmacological Sciences* 33.8 (2012): 449-55. Web.

[19] Parry, Nicola. "Functional Difference Between T Cells & B Cells." *Education*. Hearst Seattle Media, LLC, 2016. Web. 27 May 2016.

[20] Yeadon, Jim. "What Type of Housing Conditions Do Immunodeficient Mice Need?" *The Jackson Laboratory*. The Jackson Laboratory, 2016. Web. 27 May 2016.

[21] "MDA-MB-231 (ATCC® HTB-26™)." *ATCC®*. ATCC, 2014. Web. 27 May 2016.

[22] "Matrigel Matrix." *Matrigel Matrix | Extracellular Matrix | Corning*. Corning Incorporated, 2016. Web. 27 May 2016.

[23] Russell, Tanya. "Understanding Breast Cancer through Innovative Laboratory Models." *Aegis*. Aegis Creative Communications, Inc., 26 Nov. 2013. Web. 27 May 2016.

[24] "Precision Digital Caliper″ *Precision Digital Caliper*. Parts Express, 2016. Web. 27 May 2016.

[25] Ayers, Gregory D. et al. “Volume of Preclinical Xenograft Tumors Is More Accurately Assessed by Ultrasound Imaging than Manual Caliper Measurements.” *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine* 29.6 (2010): 891–901. Print.

[26] Galvao, J., B. Davis, M. Tilley, E. Normando, M. R. Duchen, and M. F. Cordeiro. "Unexpected Low-dose Toxicity of the Universal Solvent DMSO." *The FASEB Journal* 28.3 (2013): 1317-330. Web.

[27] Fantozzi, Anna, and Gerhard Christofori. "Mouse Models of Breast Cancer Metastasis." *Breast Cancer Research Breast Cancer Res* 8.4 (2006): 212. Web.

[28] Tu, Yi-Fang, Benny A. Kaipparettu, Yewei Ma, and Lee-Jun C. Wong. "Mitochondria of Highly Metastatic Breast Cancer Cell Line MDA-MB-231 Exhibits Increased Autophagic Properties." *Biochimica Et Biophysica Acta (BBA) - Bioenergetics* 1807.9 (2011): 1125-132. Web.