Single Drug Adaptive Therapy

on Hormone Refractory Breast Cancer

by

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of the Requirements for the Degree
Master of Science

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ABSTRACT

Adaptive therapy utilizes competitive interactions between resistant and sensitive cells by keeping some sensitive cells to control tumor burden with the aim of increasing overall survival and time to progression. The use of adaptive therapy to treat breast cancer, ovarian cancer, and pancreatic cancer in preclinical models has shown significant results in controlling tumor growth. The adaptive therapy model comes from the integrated pest management agricultural strategy, predator prey model, and the unique intra- and inter-tumor heterogeneity of tumors. The purpose of this thesis is to analyze and compare gemcitabine dose response on hormone refractory breast cancer cells retrieved from mice using an adaptive therapy strategy with standard therapy treatment. In this study, we compared intermittent (drug holiday) adaptive therapy with maximum tolerated dose therapy. The MCF7 resistant cell lines to both fulvestrant and palbociclib were injected into the mammary fat pads of 8 weeks old NOD/SCID gamma (NSG) mice which were then treated with gemcitabine. Tumor burden graphs were made to track tumor growth/decline during different treatments while Drug Dose Response (DDR) curves were made to test the sensitivity of the cell lines to the drug gemcitabine. The tumor burden graphs showed success in controlling the tumor burden with intermittent treatment. The DDR curves showed a positive result in using the adaptive therapy treatment method to treat mice with gemcitabine. Due to some fluctuating DDR results, the sensitivity of the cell lines to gemcitabine needs to be further studied by repeating the DDR experiment on the other mice cell lines for stronger results.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation for each committee member's contribution to this thesis. Thank you Dr. Maley for allowing me to continue as a graduate researcher in this lab, supporting me in the review and analysis of this project despite the obstacles that were encountered, and providing input and revision to improve the thesis. I want to sincerely thank Sareh Seyedi for taking the time to not only supervise and teach me the protocols, but for also contributing, editing, and reviewing my thesis throughout the entire process. I would also like to thank Dr. Blattman and Dr. Anderson for his and her revisions to my thesis and for providing me with insight on this project.

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CHAPTER 1

INTRODUCTION

Cancer is a group of diseases with uncontrollable growth and spread of abnormal cells that have affected people across the world for centuries without a cure and with little success in effective and long-lasting treatments (American Cancer Society, 2018). Cancer causes about 1 in every 6 deaths with 9.8 million deaths by cancer in 2018; making it the second leading cause of death worldwide (World Health Organization, 2018). Breast Cancer, with equal cases to lung cancer, is the most common cancer with 2.09 million cases and 627,000 deaths in 2018 (World Health Organization, 2018). Therapeutic resistance and therapeutic toxicity present as the most significant obstacles in achieving durable cancer therapy (Conti, 2021).

Current cancer therapies are designed to administer the maximum tolerated dose (MTD) of the drugs with the aim of killing a maximum number of cancer cells (Gatenby et al., 2009). The use of a relatively static approach to diminish ever-evolving cancer cells limits the tumor response by neglecting tumor resistance. Tumor resistance to therapy is caused by the temporal and spatial heterogeneity in cancer (Gatenby et al., 2009). Following the Norton-Simon model, tumor therapies often fail due to the evolution of resistant clones once therapy has begun (Gatenby et al., 2009). Highly proliferative cell phenotypes are favored in the evolution and natural selection of tumor and cancer cell growth, leading to rapid tumor growth (West et al., 2020). The adaptive therapy model strives to maintain a controllable stable tumor burden. This is done by allowing a significant population of treatment-sensitive cells to survive to suppress the

proliferation of the less fit chemoresistant populations (Gatenby et al., 2009; West et al., 2020; Conti, 2021).

Therapeutic resistance is the most significant complication in oncology. Current cancer treatments utilize drugs at the maximum tolerated dose with the goal of killing as many cancer cells as possible. This approach gives way to therapy-resistant cells that consequently cause the death of the patient. Integrated pest management (IPM) was developed by pest managers who faced the same challenge of toxicity and resistance in using pesticides. IPM does not simply work to eradicate pests but instead has the goal of controlling resistant pests by keeping some sensitive pests at a non-damaging level to the economy of the crops (Cunningham, 2019; Conti, 2021).

The adaptive therapy strategy inspired by Bob Gatenby (Moffitt, Florida) from the IPM agricultural strategy utilizes the competition between resistant and sensitive cells while also keeping some chemo-sensitive cells in the minimum threshold of cancer (Barzman et al., 2015). Adaptive therapy utilizes the competitive interactions between drug-sensitive and drug-resistant subclones. Adaptive therapy, developed by Bob Gatenby, is distinguished by its focus on evolutionary effects rather than molecular properties of cancer cells implemented in past treatments (Ibrahim-Hashim, 2017). Instead of utilizing the maximum dose of the drugs during the treatment, the adaptive therapy strategy focuses on adjusting treatment through adjusting dosage and, or drug holidays (on and off treatment) (Gatenby et al., 2009). There are challenges that have come with this evolutionary-based treatment of cancer cells that continue to be reviewed and altered within treatment studies (Conti, 2021).

Adaptive therapy studies have shown substantial results in the cell lines of breast cancer, ovarian cancer, and prostate cancer (West et al, 2020; Zhang et al., 2017). The treatment of MCF7 estrogen receptor-positive (ER+) and MDA-MB-231/luc triple-negative breast cancers in a mouse mammary fat pad using paclitaxel, implemented the adaptive therapy model through regular application of the drug, dose-skipping, and progressively using a smaller effective dose of the drug (Minton et al., 2016). In this study, the tumor was treated with intensive treatment with regular drug application. Variable dosing algorithms allowed initial tumor control to be reached and maintained with the administration of progressively smaller drug doses. Continual lower drug doses resulted in prolonged progression-free survival while having control of tumor volume. These results were also seen in notable studies where adaptive therapy extended time to progression (TTP) in-vivo ovarian and breast cancer treatments (Strobl et al., 2020). In-vivo experimental evidence for ovarian cancer supported the breast cancer results that once tumor control is achieved, it can be maintained with progressively lower drug doses (Minton et al., 2016). In the first clinical trial linking adaptive therapy to metastatic castrate-resistant prostate cancer, the use of androgen deprivation therapy showed results of an increase in TTP of 27+ months and a reduction in cumulative drug use of 47% (Strobl et al., 2020). Prostate cancer is most often treated with androgen deprivation therapy (ADT) to target cancer cells' reliance on testosterone (You et al., 2017). After an initial response to ADT, cancer cells become resistant within a few months to a year, progressing to metastatic castrate-resistant prostate cancer (mCRPC) (You et al., 2017). The success in treating prostate cancer in terms of an evolutionary-based therapy has created interest in researching the use of adaptive therapy in other cancers. The

substantial evidence of success seen in studies using adaptive therapy methods to treat breast cancer comes with urgency in heightening the positive results in increased tumor shrinkage, TTP, and stabilization and survival (Conti, 2021).

In this experiment the goal was to harness this evolutionary approach to treat hormone-refractory breast cancer, MCF7 cell line that have evolved to be resistant to fulvestrant and palbociclib, using gemcitabine in a preclinical model.

Synergistic treatment of metastatic breast cancer has been tested with the drugs palbociclib and fulvestrant. However, resistance to these two drugs in breast cancer was found due to clonal evolution response (O'leary et al., 2018).

Palbociclib is a selective inhibitor of cyclin-dependent kinases 4 and 6. CDK4 and -6 regulate the transition from G1 to S cell phase and are critical drivers of oncogenesis in some tumors (Cadoo et al., 2014). Palbociclib inhibits the ability of CDK4 and -6 to phosphorylate RB, preventing continued division. Fulvestrant is an oestrogen receptor (ER) antagonist that competitively inhibits binding of oestradiol to the ER, and blocks and accelerates the degradation of ER protein leading to complete inhibition of oestrogen signaling through the ER (Osborne et al., 2004). The combination of a CDK 4/6 inhibitor, a drug that blocks cancer cell growth beyond the G1 phase, and an ER modulator such as fulvestrant, inhibits a tumor cell's power of division and growth; cellular senescence (Elmi et al., 2019; Conti, 2021).

There are three major changes in driver genes that cause resistance and future tumor cell proliferation. RB1 mutations, driver mutations in growth factor receptors and signal transduction pathways, as well as the evolution of ESR1 mutation, were seen in

patients treated with palbociclib and fulvestrant. The RB1 mutation was found to be a mechanism of resistance to CDK 4/6 inhibition. These mutations were absent at the start of treatment and were identified in patients after being treated in combination with palbociclib and fulvestrant (O'leary et al., 2018; Conti, 2021).

The predator-prey model in ecology has been used to study the tumor-immune interactions in oncology (Kareva et al., 2021). This model can be used in this context with cancer cells acting as the "prey" for the immune system and the immune cells as the "predator" (Kareva et al., 2021). There are discrepancies when applying tumor-immune interactions to the predator-prey model, with key distinctions allowing for unique ways to increase the effectiveness of cancer therapies. Theoretical models are especially helpful for oncologists when navigating evolving cancer cells, growing or shrinking tumors, and a changing immune system that causes immunotherapy resistance. Using the predator-prey model has led to the immunotherapy goal of increasing immune cell efficiency against tumor cells potentially with the use of tumor vaccines, checkpoint inhibitors, and genetically engineered immune cells (Kareva et al., 2021). This is due to the realization of the importance of predator efficiency in eliminating the prey using structure and mechanisms.

The intra- and inter-tumor heterogeneity within a single person's cancer and between various tumors after metastasizing allows for cancer therapy resistance (Gedye and Navani, 2022). The abundance of mutations within cancer including multiple driver mutations and non-silent exon mutations make cancers complex enough to evolutionarily adapt and evade targeted therapy (Gedye and Navani, 2022). Adaptive therapy purposefully interrupts effective treatment in order to delay resistance, unlike current

therapies that continue treatment until progression. The adaptive therapy model utilizes on/off dosing to delay selection of treatment resistant tumor cell populations. The use of on and off treatment in heterogeneous tumor populations competing for limited substrates using different evolutionary survival strategies returns to equilibrium; delaying selection of treatment resistant tumor subpopulations and therapy resistance (Gedye and Navani, 2022). There are a few challenges that come with the use of adaptive therapy including the lack of understanding of the temporal fluctuations in resistance that change with selection pressure (Gedye and Navani, 2022). These challenges are studied in pre-clinical work to identify an effective evolutionary therapy.

The goal of this study was to analyze and compare gemcitabine dose response on hormone refractory breast cancer cells retrieved from the mice in an adaptive therapy strategy with standard therapy treatment. Specifically, we tested whether the drug gemcitabine can control the MCF7 cancer cell line which is resistant to palbociclib and fulvestrant.

Gemcitabine inhibits the synthesis and cell proliferation while promoting apoptosis in cancer cells (Zheng et al., 2014). When gemcitabine is inside the cell, the drug is rapidly phosphorylated. The phosphorylated gemcitabine inhibits ribonucleotide reductase that produces deoxynucleotides required for DNA synthesis and repair (Plunkett et al., 1995). Gemcitabine nucleotide is then locked into DNA with exonucleases unable to remove gemcitabine, inhibiting further DNA synthesis (Plunkett et al., 1995; Conti, 2021).

Drug dose response curves are used to identify and compare resistant and sensitive cells. This is done in cell culture through high control of the drug concentration

and drug administration in order to measure the inhibition of tumor cell growth (Mayer and Janoff, 2007). The effects of combined drugs can be seen in DDR experiments through synergistic or antagonistic relationships at different ratios of the drugs (Mayer and Janoff, 2007). Drug resistant cell lines can be predicted through DDR curves as well as the predictive value of drug sensitivity dependent on drug combination and disease type (Florento et al., 2012). Sensitive cell lines are identified through low viability at low drug concentrations while resistant cell lines are identified through high viability at higher drug concentrations. The results of a DDR curve give drug concentrations that will inhibit cell viability at different levels of drug treatment (Florento et al., 2012; Conti, 2021).

CHAPTER 2

MATERIALS AND METHODS

Analyzing the Dose-Response of tumor of those mice under treatment with chemotherapy drug (gemcitabine)

1. Drug Dose-Response Curve experiment and analysis on resistant MCF7 cell lines to fulvestrant and palbociclib

The DDR protocol for the resistant MCF7 cell lines to fulvestrant and palbociclib was carried out by Sareh Seyedi. Human breast cancer cell lines, MCF7 cell lines (ER+), have been evolved to be resistant to fulvestrant and palbociclib. In order to obtain resistant cell lines, the MCF7 cell lines ($1e+10^7$ cells) were cultured in a hyperflask with RPMI 1640 media, 500 ml +10% FBS and treated for one month with IC70 (First two weeks), IC80 (third week), and IC90 (last week) of the drugs. The media of the hyperflask changed once per week with the addition of drugs to the media. When changing the media, the media was poured into an empty bottle by holding the hyperflask at an angle followed by adding 100ml of PBS to wash the flask. Finally, the fresh media with drugs was poured into the flask.

The drug dose-response curve (DDR) method was used to confirm and compare the resistant cell lines' sensitivity with the parental cell line. DDR curves are made to measure how sensitive cell lines are to specific drugs. In this experiment, the MCF7 cell line sensitivity to fulvestrant and palbociclib was measured in a DDR curve (both parental sensitive MCF7 cell lines and MCF7 resistant cell lines to fulvestrant and palbociclib). The viability of the cells was measured using cell titre glo. Cell titre glo

releases ATP from live cells, which when quantified under a plate reader, values are given corresponding to live cells under each condition. In this model, a 96 well plate was used with 2 controls (media without drug and media plus the solvent of each drug) and 10 treatments (drugs with different concentrations, 30uM, 40uM, 50uM, 60uM, 70uM, 80uM, 90 uM, and 100uM), 8 replicants per treatment.

Equal amounts of cells were plated on all wells on Day 1. After 24 hours, the cells were treated with drugs with concentrations as mentioned above. The cells were incubated under the drugs for at least one doubling time of the cell lines, which for these cell lines was 48 hours. At the end of the experiment, an equal amount of cell titre glo was added to each well (100ul). The wells were then incubated for five minutes and measured with a luminescence value of 570 nm. These values were then normalized and inputted into GraphPad Prism to construct a drug dose-response curve.

The mice handling was carried out by Sareh Seyedi. First the hormone-refractory MCF7 cell lines that were tagged with luciferase were injected into the mammary fat pads of NOD/SCID gamma (NSG) mice, 8 weeks old and $3e+10^6$ cells per mouse. When harvesting $3e+10^6$ cells/mouse, the cells were first washed with PBS for 15 minutes to remove any trace of FBS from the cells. Then an accutase (cell detachment solution) was added to the cells and incubated in an incubator (37°C, 5% CO2) for 10 minutes. Next, equal amounts of media (RPMI 1640) +10% FBS were added to the cells to neutralize the accutase, then the cells were collected by pipetting and centrifuged for 5 minutes, 1100 RPM. After centrifugation, the excess supernatant fluid was removed and the cells were resuspended in a suitable volume of media for counting using the automated counter cell machine. Finally, the centrifugation was repeated to collect the cells, and this time all

supernatant fluid was removed and the pellet of cells was mixed with 1:1 matrigel: PBS for having 3e+10^6 cells/100ul. Matrigel was used to augment the tumor growth for our cancer cell lines. The hormone-refractory cells have been injected into the mammary fat pads of NOD/SCID gamma (NSG) mice.

For the MCF7 xenograft model, 17b-estradiol 90-day release pellets, 0.36 mg per pellet were implanted on the dorsal region of the mice on the day of cell injections in order to grow human breast cancer cells that are estrogen receptor-positive. These cell lines that evolved to be resistant to fulvestrant and palbociclib were treated with gemcitabine (50 mg/kg IP injection two days per week) as a maximum tolerated dose (MTD).

In this experiment we compared the intermittent adaptive therapy strategy with standard therapy. In the intermittent adaptive therapy strategy, the maximum tolerated dose of the drug was used and when the tumor burden ever fell below 50% of its value at the start of treatment, we stopped the treatment. If it ever rose above the initial tumor burden, we started the treatment again. The endpoint of the study was when the tumor reached to 3e.10^9 based on the luminescence or 2000 mm^3 based on the caliper or when monitoring, there were any signs of disseminated disease, such as pulmonary metastasis, indicated by difficult labored breathing in the mice.

Our groups

1. Control: No treatment = vehicle control (4 mice)
2. Control: gemcitabine continuous maximum tolerated dose (MTD) (6 mice)
3. Gemcitabine intermittent treatment (6 mice)

No treatment:

Because gemcitabine is dissolved in saline, our no treatment control consists of injection with the same volume of saline that is used in the maximum tolerated dose condition. The bioluminescence machine (Xenogen) was used to take in-vivo imaging. Measurements via bioluminescence were taken 3 weeks after the cell injection and were repeated twice per week. For in-vivo imaging, before taking the photo, the D-luciferin (150 ug/g) was injected into the mice via intraperitoneally (IP) method, and in-vivo imaging was done during 10-15 minutes of the injection.

2. Drug Dose-Response Curve experiment and analysis on cell lines that have been retrieved from the mice under gemcitabine treatments

Sareh Seyedi harvested the cell line retrieved from the mice. After harvesting the tumors from the mice, the cell lines have been retrieved from the tumor. The tumor sliced and diced into the smallest pieces and then 7 ml of the digest media (6ml collagenase + 10 ml TrypLE Express + 34 ml 1X sterile PBS) were added to the plate and incubated for 30 minutes. After incubation the digest media and cells were separated from any remaining tissue and transferred into a conical tube. Then another 3 ml digest media was added to the remaining tissue and sliced them again followed by adding 7 ml digest media and incubating the plate in a tissue culture incubator for another 30 minutes. Next, we collected media and cells from the dish and added them to the same conical tube we had. We were able to use a cell strainer to aid in the separation of tissue chunks from the media. Finally, we plated the cells in a petri dish with complete media that contained Normocin at a 1:500 ratio and put it in the incubator to grow. Then cell lines were frozen and kept at -80 °C (modified by Lisa Abegglen).

For this DDR experiment Aviona Conti was trained by Sareh Seyedi, then Aviona Conti completed the DDR protocol with the help and supervision of Sareh Seyedi. In this experiment we used cell lines that we could retrieve from groups of gemcitabine intermittent adaptive therapy, gemcitabine standard therapy and control (no treatment). We could use one cell line from each group and compare them with parental MCF7 resistant cell lines that we injected into the mice.

The frozen cell lines were plated in petri dishes with complete media (RPMI 1640 media, +10% FBS). The media of the petri dishes was changed every three days and passaged at 75% - 80% of confluence. During passaging the spent media was removed and the cells were washed with PBS (stand in PBS for 10 minutes in order to help to remove trace of FBS to help in detaching cell lines). The PBS was removed and Accutase was then added to the cells. The plate was then placed inside the incubator until the cells detached (about 5-10 minutes). Once the cells detached, equal amounts of complete media were added, washed, and pipetted to bring all the cells into suspension. The cells were transferred into a tube and centrifuged for 5 min at 1100 rpm. The media was then removed and the pellet re-suspended in a suitable volume. The drug dose-response curve (DDR) method was used to confirm and compare the treated cell lines' sensitivity with the parental cell line.

Drug Dose-Response Curve experiment and analysis:

DDR curves are made to measure how sensitive cell lines are to specific Gemcitabine in the same procedure as explained before. In a 96 well plate we had two controls (media without drug and media plus the solvent of drug) and 10 treatments (drugs with different

concentrations, 10uM, 20uM, 30uM, 40uM, 50uM, 60uM, 70uM, 80uM, 90uM, and 100uM), 8 replicants per treatment.

Equal amounts of cells were plated in all wells on Day 1. After 24 hours, the cells were treated with drugs with concentrations as mentioned above. Then cells were incubated under the drug for at least one doubling time. At the end of the experiment, an equal amount of cell titre glo was added to each well (100ul). The wells were then incubated for five minutes and measured with a luminescence value of 570nm. SpectraMax M5 with SoftMax Pro 6.2.2 software was used to measure the luminescence values. These values were then normalized and inputted into GraphPad Prism to construct a drug dose-response curve.

CHAPTER 3

RESULTS

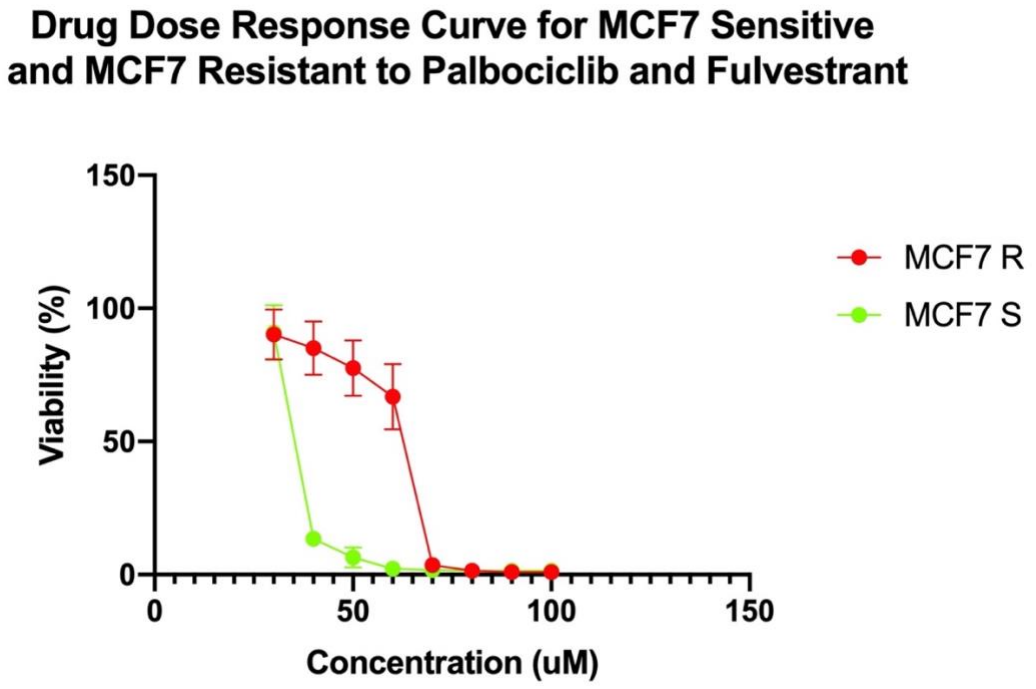
1. Analysis Drug Dose-Response Curve of resistance MCF7 cell lines to fulvestrant and palbociclib

Based on figure 1 and 2 we showed that we were able to get resistant cells to the combination of fulvestrant and palbociclib. The MCF7 R is the resistant cell line shown in the red curve in figure 1 and the MCF7 S is the sensitive cell line shown in the green curve in figure 1. MCF7 R cell lines have an IC50 value of 62.65 uM which means at this inhibitory concentration, 50% of the cells are viable or in the other words, these drugs inhibit the growth of 50% of the resistant cells at a concentration of 62.65 uM. In comparison to that, MCF7 S cell lines have an IC50 value of 13.13 uM, showing that the combination of these two drugs is able to inhibit the growth of 50% of the cells at a lower concentration (Conti, 2021).

In figure 2 the normalized values of the raw data used in the DDR curve were also used in this bar graph to compare the viability of the sensitive cell line to the viability of the resistant cell line at different concentrations. The bar graph shows significant differences between the cell lines at the concentrations of 40 uM, 50 uM, and 60 uM. This significant difference was also shown in the p-values of the different cell lines at each concentration. This is because the p-values for these concentrations were below the significance level of 1×10-5. The concentrations of 30 uM, 70 uM, 80 uM, 90 uM, and 100 uM were not significantly different between the cell lines because the p-values were above the significance level. These p-values and t-test confirm that we successfully

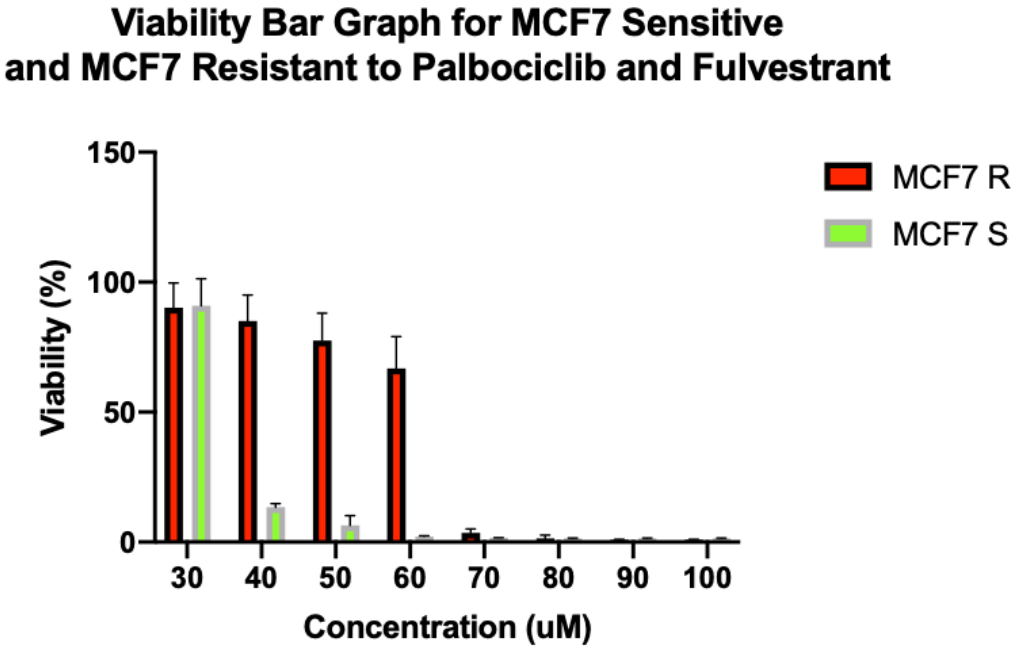
obtained a resistant cell line because the cell lines viability at these concentrations were significantly different (Conti, 2021).

Figure 1



Note: The percentage of viability of both MCF7 R and MCF7 S cell lines on different concentrations of fulvestrant and palbociclib. This curve was made by Sareh Seyedi and Aviona Conti.

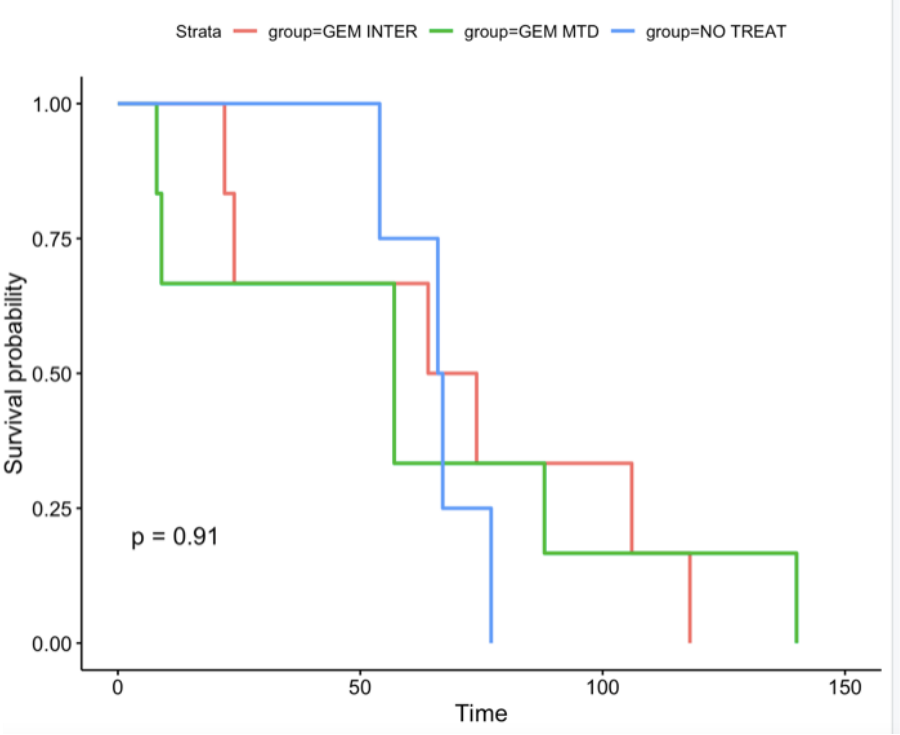
Figure 2



Note: The percentage of viability comparing both MCF7 R and MCF7 S cell lines on different concentrations of fulvestrant and palbociclib. At the concentrations of 40 uM, 50 uM, and 60 uM the cell lines are significantly different. This graph was made by Aviona Conti.

2. Analyzing and comparing the *Drug Dose-Response Curve of* cell lines that have been retrieved from the mice under gemcitabine therapy (intermittent and standard) and no treatment with parental MCF7 R cell lines

Figure 3



Note: Kaplan-meier curve for comparison of the survival probability among gemcitabine intermittent therapy, gemcitabine standard therapy and control (no treatment). This curve was made by Sareh Seyedi.

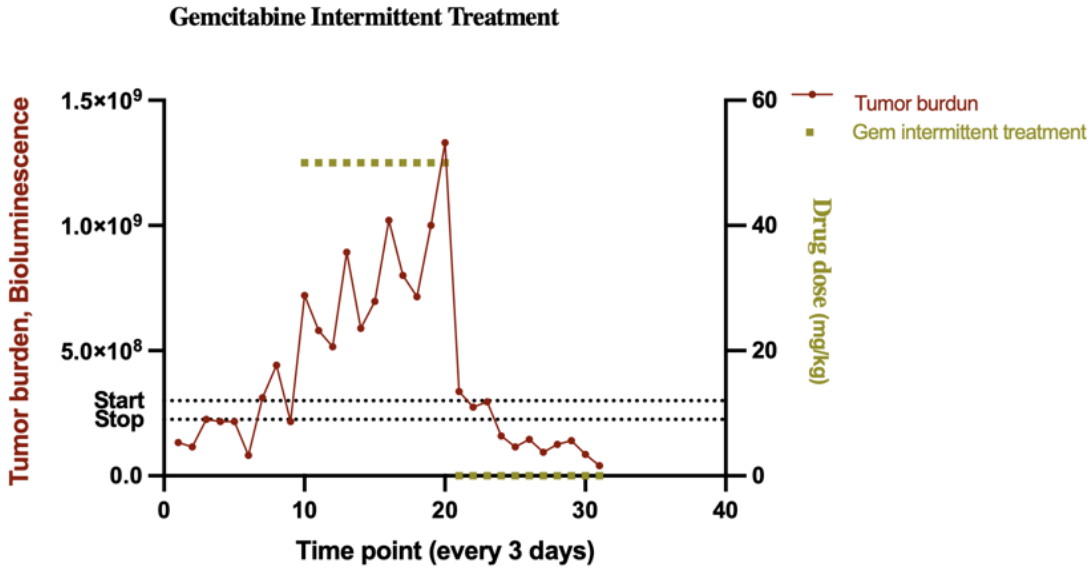
After euthanizing all the mice, we analyzed their survival probability using COx regression and Kaplan-meier curve. As we can see in figure 3 there is no significant difference in progression free survival between the gemcitabine intermittent therapy and gemcitabine standard therapy.

Below we also show the tumor burden changes during the treatment. We had 6 mice per treatment arm and 4 mice for the control group. But here since we could just work on one mouse per group we provided the tumor burden of that mice along with the

result of its DDR.

A. Intermittent adaptive therapy

Figure 4

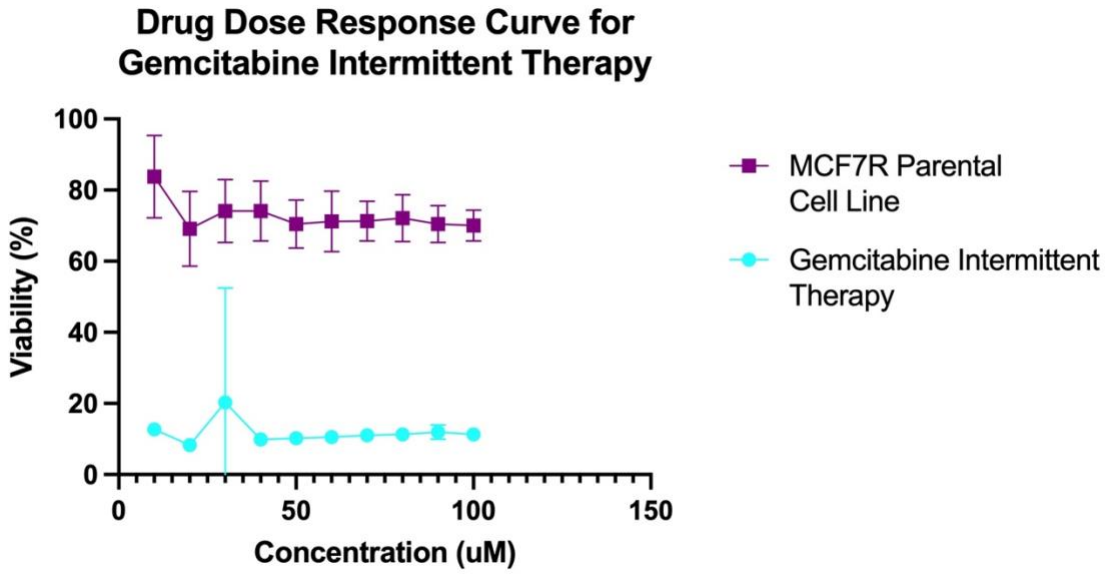


Note: The tumor burden for the mouse treated with gemcitabine intermittent treatment. The tumor burden increased and peaked on day 20 and then declined until 0 tumor burden. This graph was made by Sareh Seyedi.

As mentioned in the method section, in this treatment strategy therapy, the maximum tolerated dose of the drug is used and when the tumor burden ever falls below 50% of its value at the start of treatment, we stop the treatment. If it ever rises above the

initial tumor burden, we start the treatment again. In figure 4 the treatment started when the tumor burden reached above the start point ($3e*10^8$) and it continued at the maximum tolerated dose until we saw a 50% reduction in tumor burden, then the treatment stopped. The important point here is that after reduction in the tumor burden it didn't return to the start size and more surprising in the absence of the drug it continued to decline until we had to euthanize the mice due to loss of weight and hunching. Below we analyzed the sensitivity of the cell line that we retrieved from the tumor, to gemcitabine. Figure 5 shows the sensitivity of this cell line to different concentrations with a bit of fluctuation (less viability in 20uM concentration and more resistant in 30uM concentration).

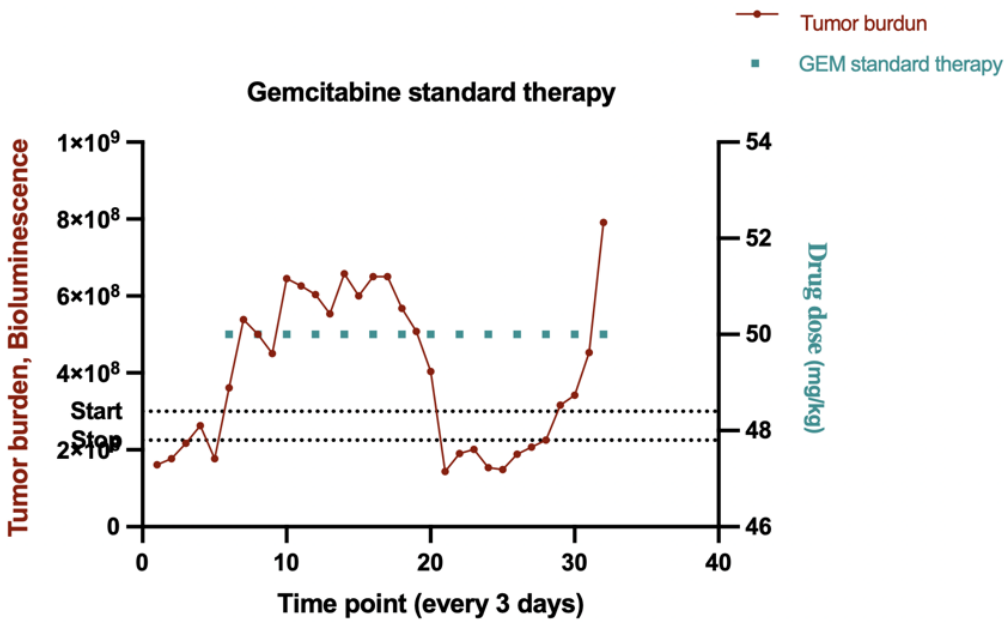
Figure 5



Note: The percentage of viability of MCF7 gemcitabine intermittent therapy cell lines on different concentrations of gemcitabine. This curve was made by Aviona Conti.

B. Standard therapy

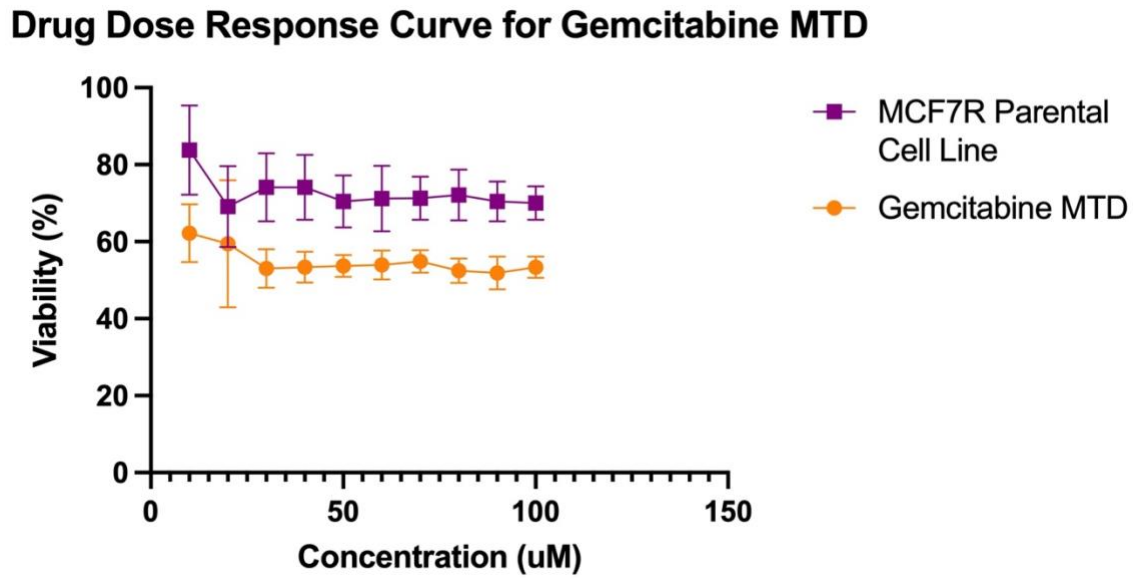
Figure 6



Note: The tumor burden for the mouse treated with gemcitabine standard therapy MTD. This graph was made by Sareh Seyedi.

In the standard therapy, when the tumor reached above the start point we applied the maximum tolerated dose of the drug continuously until we had to euthanize the mouse, which in this case we had to euthanize due to loss of weight. Figure 6 shows that in the standard therapy we had a significant decline in the tumor burden but later due to continuously applying the treatment we saw a sharp increase in tumor burden. This is due to putting continual selective pressure on the tumor and selection for resistant cells.

Figure 7



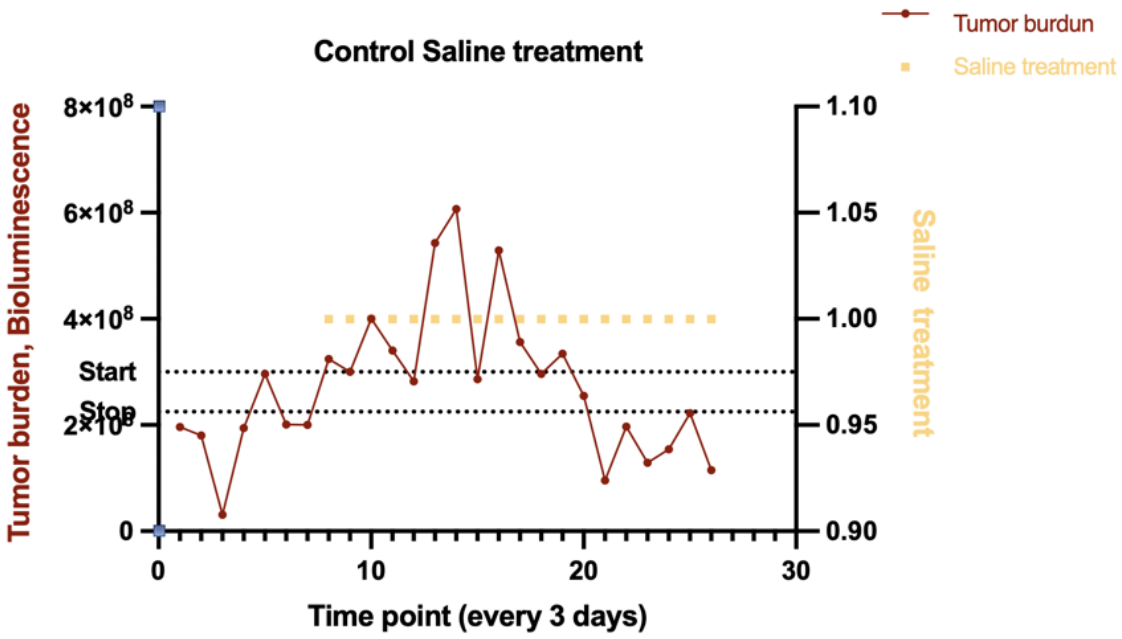
Note: The percentage of viability of MCF7 gemcitabine MTD cell lines on different concentrations of gemcitabine. This curve was made by Aviona Conti.

As a result of the dose response of the cell line from standard therapy, there is a gradual decline in the viability of cell line by increasing the concentration of the drug but then there is a gentle increase in the viability of cell line followed by a small fluctuation.

Overall, this cell line is more resistant in all different concentrations compared to the intermittent therapy. However, there is still a fluctuation in different concentrations.

C. Saline treatment (control)

Figure 8



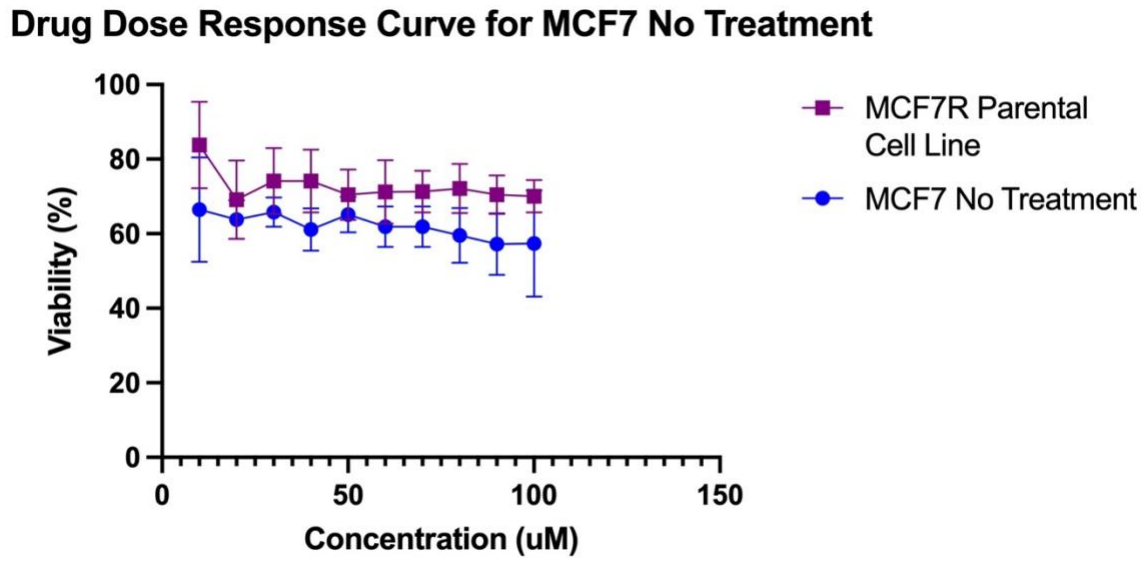
Note: The tumor burden for the control mouse treated with saline treatment. This graph was made by Sareh Seyedi.

In the control group we applied saline, the solvent of gemcitabine, after reaching the tumor to the start point until we euthanized the mouse. In this case the end point was when the tumor reached the end point size based on the caliper measurement (2000mm^3).

As figure 8 shows, there was first fluctuation in tumor burden followed by reduction in tumor size and the tumor burden kept fluctuating under the starting point until the mouse needed to be euthanized.

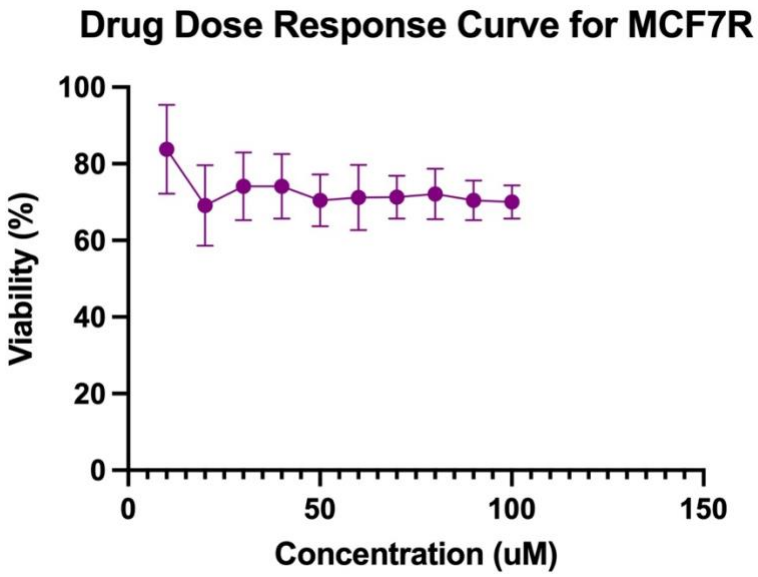
In the dose response curve of the cell line retrieved from this mouse, there is a declining trend in the viability of this cell line from lower concentration to higher concentration. However, surprisingly compared to the both cell lines that were under the treatment it is less sensitive to the gemcitabine.

Figure 9



Note: The percentage of viability of MCF7 no treatment control cell lines on different concentrations of gemcitabine. This curve was made by Aviona Conti.

Figure 10



Note: The percentage of viability of MCF7R parent cell lines on different concentrations of gemcitabine. This curve was made by Aviona Conti.

In figure 10 the MCF7R parental cell line shows a fluctuation trend in response to the gemcitabine at different concentrations. Surprisingly, it shows this cell line has more resistance to gemcitabine at different concentrations compared to all the other three cell lines retrieved from the mice.

CHAPTER 4

DISCUSSION

In this experiment, a resistant MCF7 cell line to palbociclib and fulvestrant was successfully obtained. The tumor burden graphs showed success in controlling the tumor burden with intermittent treatment. After the first reduction in tumor size the tumor did not return to its original size and continued to decline. Therefore, lower drug doses can be used under adaptive therapy once the tumor is controlled. We had several weeks off treatment for this mouse under intermittent therapy, and we saw continued decline in tumor size. In contrast, in standard therapy there was an increase in tumor burden due to continuous selective pressure that allowed for the resistant cells to survive and the tumor to grow. However, in single drug therapy with gemcitabine there are no significant differences in prolonging progression free survival. Moreover, we had toxicity issues in using this chemotherapeutic drug since we had to euthanize both mice due to the losing weight.

In comparison of all the DDR curves of all four cell lines, the MCF7 R parental cell line is more resistant to gemcitabine compared to cell lines from the no treatment group, standard therapy group and the intermittent therapy group respectively. Therefore, the cell line retrieved from the intermittent group was more sensitive to gemcitabine at the end of the experiment.

. The DDR curve for the standard therapy MTD showed more resistance than the intermittent therapy, but still fluctuated. These figures showed a positive result in using the adaptive therapy treatment method to treat mice with gemcitabine. The intermittent

therapy showed a stable tumor burden in the mice and sensitive, but fluctuating viability in the cell lines during different concentrations of the drug. Due to the time restraints in growing these cell lines, the viability was low before beginning the DDR protocol. This could be the cause of the fluctuating DDR results. The next step would be to repeat the DDR experiment on the other mice cell lines in order to produce more data from each group of mice to have stronger results.

CHAPTER 5

CONCLUSION

Our success in getting the MCF7 cell line resistant to palbociclib and fulvestrant enabled us to test an adaptive therapy strategy versus standard therapy with the chemotherapeutic drug gemcitabine. Although the tumor burden under adaptive therapy strategy declined and it was under control in the absence of the drug and based on the DDR curve, this cell line was more sensitive compared to the standard therapy. But based on the kaplan meier survival analysis there is no significant difference between these two strategies in using gemcitabine. More importantly, our results showed that this drug might be toxic because of the significant reduction in the weight of the mice under the treatment.

The challenges such as slow growth of the cell lines and low viability of the cell lines at the time of DDR experiment, could be factors in having the fluctuating DDR results. Repeating the DDR experiment on the other mouse cell lines and allowing them more time to grow will allow for stronger DDR results and a better comparison with their tumor burden graphs.

CHAPTER 6

CONTRIBUTIONS

Sareh Seyedi carried out the cell culture protocol to get the resistant MCF7 cell lines to fulvestrant and palbociclib and analyzed it. Next, she ran a pre-clinical experiment using the chemotherapy drug (gemcitabine) on NSG mice. At the end of the experiment she harvested the tumors from the mice and cultured them to retrieve the cell lines in Biodesign ASU.

Aviona Conti carried out the cell culture protocol to grow the cell lines and then use those cell lines in the Drug Dose-Response Curve experiment using gemcitabine with the help and supervision of Sareh Seyedi. She then analyzed the results of the Drug Dose-Response Curve experiment for each of the MCF7 cell lines using GraphPad Prism with the help and supervision of Sareh Seyedi.

The graphs under the Results section were made and analyzed by Aviona Conti with the help and supervision of Sareh Seyedi.

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