

POLYAMINE TRANSPORT INHIBITOR EFFECTS ON PANCREATIC CANCER  
PROLIFERATION CELLS IN VIVO

by

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## ABSTRACT

Pancreatic cancer is a serious disease, one in which the survival rate over five years is less than 6%. (1) Often, malignant tumors will exhibit uncontrolled proliferation and it is postulated that they have high metabolic needs. One of the areas of interest in cancer metabolism is the unique need for large amounts of polyamines in order to sustain this uncontrolled proliferation. Polyamines are organic compounds that all cells need for proliferation and differentiation. (2) Cells obtain polyamines by manufacture of them directly or obtain them from the environment through their transport across the cell membrane. When cancer cells have limited access to polyamines they enter apoptosis, or controlled cell death. In animal models of cancer, cellular apoptosis can be tracked by measuring tumor weights as well as histological methods.

It is known that difluoromethylornithine (DFMO), a polyamine synthesis inhibitor, has shown some success in reducing tumor weights. (3) Cells deprived of the ability to manufacture polyamines will resort to transporting existing polyamines from the environment for their use. (3) It is believed that a polyamine transport inhibitor (PTI) can be designed and used in conjunction with DFMO to completely deprive cancer cells of polyamines and increase rates of apoptosis in tumor cells. Through this study the interaction of DFMO with newly identified PTI will be analyzed. A mouse model with tumor cells in the pancreas will give a picture of how cancer cells react to DFMO / PTI *in vivo*. The findings allow us to postulate how targeted compounds interact with protein signaling pathways that may be important for regulating response to inhibitors of polyamine synthesis and/or transport.

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## TABLE OF CONTENTS

LIST OF FIGURES .....	vi
LIST OF TABLES .....	viii
LIST OF ABBREVIATIONS/ACRONYMS/NOMENCLATURE.....	ix
CHAPTER ONE: INTRODUCTION.....	1
Problem Statement:.....	1
Hypothesis: .....	1
Specific Aim 1: .....	1
Hypothesis of Specific Aim 1:.....	2
Specific Aim 2: .....	2
Hypothesis of Specific Aim 2:.....	2
CHAPTER TWO: BACKGROUND .....	3
CHAPTER THREE: PRELIMINARY DATA.....	9
CHAPTER FOUR: MATERIALS AND METHODS.....	14
Tissue Preparation.....	14
Slide Preparation.....	14
Antibody Optimization .....	15
Slide Staining.....	16

CHAPTER FIVE: RESULTS AND ANALYSIS.....	18
Immunohistochemistry .....	18
CHAPTER SIX: FUTURE EXPERIMENT.....	22
CHAPTER SEVEN: CONCLUSION .....	27
APPENDIX: ANTIBODY DATASHEETS .....	29
LIST OF REFERENCES.....	33

## LIST OF FIGURES

Figure 1 : Polyamine Structures for the Three Polyamines Eukaryotes Require .....	4
Figure 2 : Polyamine Synthesis Mechanism (11) .....	5
Figure 3 : Polyamine Import through a Polyamine Transporter .....	6
Figure 4 : Polyamine Synthesis and Transport Pathway with Inhibitors Being Studied .....	8
Figure 5 : DFMO Percentages and Tumor Weight for the L3.6pl <i>In Vivo</i> Model (16) .....	9
Figure 6 : Untreated L3.6pl Tumor Histological Markers for Baseline Proliferation and Apoptosis (16).....	10
Figure 7 : 1% DFMO Treated L3.5pl Tumor Histological Markers for Potentially More Apoptosis and Less Cell Proliferation (16).....	10
Figure 8 : 3% DFMO Treated L3.6pl Tumor Histological Markers for Potentially More Apoptosis and Less Cell Proliferation (16).....	11
Figure 9 : Structures of DFMO (17), Trimer44NMe (7) and GW5074 (17) .....	11
Figure 10 : L3.6pl Ex Vivo Tumor Weights in Response to trimer44NMe (PTI) and GW5074 Treatments (16) .....	12
Figure 11 : Nude Mice with L3.6pl Derived Tumor Exhibit Anti-Growth Responsiveness to Single and Double Treatments Compared to Untreated Conditions (16) .....	13
Figure 12 : Decreased Phosphorylated c-Raf Immunohistochemistry Detected in GW5074 Treatment Groups .....	18
Figure 13 : c-Myc Immunohistochemistry Showed Decreased Expression that was Most Apparent in Response to GW5074 .....	19

Figure 14 : ODC Immunohistochemistry Exhibited Decreased Expression in Response to GW4074.....	20
Figure 15 : Composite Image of All Staining Images .....	21
Figure 16 : C57Bl/6 Mice with Panc02 Derived Tumor Exhibit Response to 1% DFMO alone and in combination with PTI and/or GW5074 (16).....	22
Figure 17 : Panc02 Tumors have Decreased Staining Against Ki67 Proliferation Marker in the Presence of Hematoxylin-Nuclear Stain (16) .....	23
Figure 18 : Panc02 Tumors have Decreased Staining Against c-Myc Nuclear Marker with in the Presence of Hematoxylin-Nuclear Stain (16) .....	24

## LIST OF TABLES

Table 1 : Dilutions Selected to Optimize Antibodies for Experimental Staining.....	15
Table 2 : Selected Antibody Dilution and Epitope Retrieval .....	16
Table 3 : Future Experimental Treatment Groups .....	25



## **LIST OF ABBREVIATIONS/ACRONYMS/NOMENCLATURE**

C57Bl/6 : Immunocompetent Mouse

DFMO: Difluoromethylornithine

FDA : Food and Drug Administration

GW5074 : Raf Inhibitor (3-(3,5-Dibromo-4-hydroxybenzyliden)-5-iodo-1,3-dihydroinol-2-one)

H&E : Hematoxylin and Eosin Stain

hOTC2 : Human organic cation transporter 2

IHC : Immunohistochemistry

Ki67 : Nuclear Proliferation Protein Marker

L3.6pl cells : Human Pancreatic Cell Line

ODC : Ornithine Decarboxylase

Panc02 : Mouse Pancreatic Cell Line

PTI : Polyamine Transport Inhibitor

## **CHAPTER ONE: INTRODUCTION**

### Problem Statement:

Tumor cells need high levels of polyamines in order to proliferate. Tumor cells obtain polyamines by either manufacturing them or transport of polyamines from the tissue environment. Difluoromethylornithine (DFMO) successfully inhibits cells from being able to manufacture polyamines. However, there is limited information regarding polyamine transport mechanisms and research into blocking the transport of polyamines into the cells.

### Hypothesis:

Polyamine Transport Inhibitors (PTIs) when combined with DFMO will show a synergistic effect on tumor cell proliferation and/or apoptosis.

### Specific Aim 1:

Refine a model to study PTI and its effect on tumor viability and protein expression/phosphorylation in order to optimize the efficacy of DFMO and PTI therapy

Hypothesis of Specific Aim 1:

Different mechanisms of polyamine pathway inhibition may be needed in order to observe increased additivity of the combination effects on tumor viability.

Specific Aim 2:

Evaluate efficacy of PTIs alone or in combination with DFMO using immunohistochemistry (IHC) markers c-Myc, Raf and ODC.

Hypothesis of Specific Aim 2:

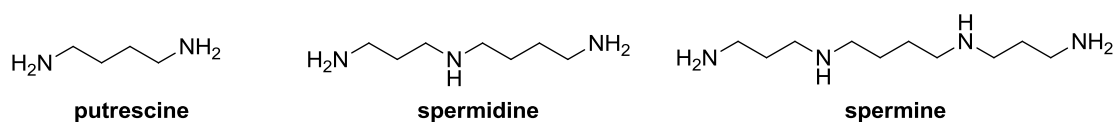
Expression of protein markers by techniques such as immunostaining can be used to evaluate the efficacy of the PTI / DFMO interaction and their combined effect on tumor viability.

## CHAPTER TWO: BACKGROUND

The pancreas is a gland which has two primary functions. The first is that it secretes pancreatic juice which both neutralizes the acidic chyme and assists with the digestion of lipids and proteins immediately after exiting the stomach. It also functions as part of the endocrine system and secretes the hormones which regulate blood sugar levels. (4) Pancreatic cancer is difficult to diagnose early due to the lack of definitive symptoms and the anatomical location of the organ itself. Pancreatic cancer is also hard to treat because usually by detection the cancer has advanced extensively throughout the organ. Pancreatic cancer is the fourth leading cause of cancer death in the United States. If diagnosed, individuals have less than 6% survival rate over five years. (1) Treatments for pancreatic cancer involve surgery, through partial or full removal of the organ, radiation and chemotherapy, and targeted therapy. (4)

Targeted surgery is not always possible, since typically by detection the cancer has already spread throughout the organ. Removal of the pancreas has serious detrimental effects on the quality of life of the patient and is not always a reliable course of action especially if the cancer has already started to metastasize. (4) Even if successful, such a procedure requires lifelong dietary restrictions, digestive system supplementation and strict hormonal control. Radiation and chemotherapy are not typically successful in treatment of pancreatic cancer. (5) Targeted therapy of cancerous cells within the pancreas through different combinations of drugs has shown some promise. (1) Although still investigational, inhibiting the cells' ability to obtain polyamines, a required component to their proliferation, may be a novel drug targeting strategy. (6) (7)

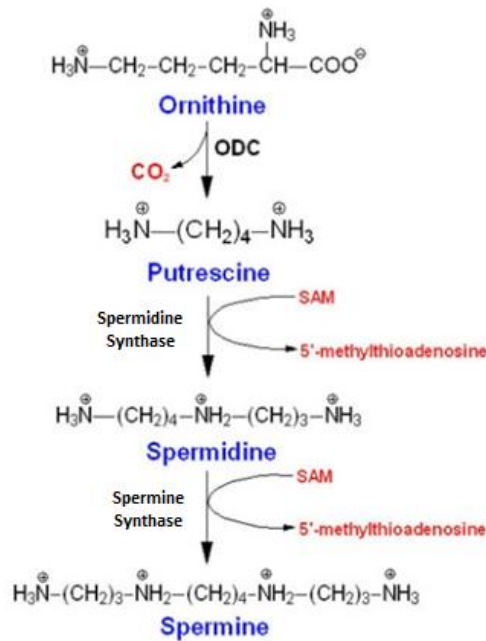
Polyamines are organic compounds that contain two or more amino groups, rather than the single amino group associated with the N-terminus of amino acids. They are required for cell proliferation and differentiation. (8) The three polyamines that eukaryotic organisms require are putrescine, spermidine and spermine, their structures are shown in Figure 1. Elevated levels of polyamines in tumors have been established in several cancers, including colorectal and pancreatic cancers. The role of polyamines in the initiation or progression of these cancers has not been established. (1) There are two methods in which cells can obtain polyamines. The first is that they can manufacture them directly. The second method is that they can obtain polyamines from the environment through transport across the cell membrane. Without adequate levels of polyamines, cancer cells initiate apoptosis, or programmed cell death. (9) Methods that block or neutralize either the synthesis or the transport method of obtaining polyamines has been a focus of research in order to force cancer cells to enter the apoptotic pathway.



**Figure 1 : Polyamine Structures for the Three Polyamines Eukaryotes Require**

The first method by which cells obtain polyamines is through direct synthesis. Ornithine decarboxylase (ODC) is the rate limiting enzyme for polyamine synthesis and is inhibited by DFMO. (1) DFMO is a drug that has been FDA approved and can be given over long periods of times at low doses without causing detectable side effects. (10) The amino acid ornithine is decarboxylated by ODC into the first polyamine, putrescine (Figure 1). Subsequently a propyl-

amine group is added to putrescine by spermidine synthase to form the second polyamine, spermidine. A second propyl-amine is added to spermidine through spermine synthase, resulting in the third polyamine, spermine. An illustration of the mechanism is shown below in Figure 2. Assessment of tumor progression can be done in several ways.

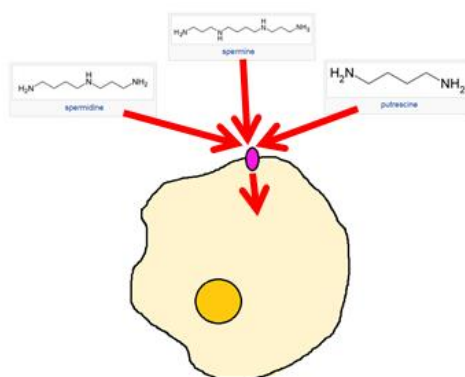


**Figure 2 : Polyamine Synthesis Mechanism (11)**

The most basic method analyzes tumor weights. DFMO has been shown to decrease pancreatic tumor weights in mice by up to 43%, as seen in Figure 5. Other ways to track progression of tumors include using histopathology analysis. Proliferation markers can be seen in cells where cancer cells are actively growing and dividing, whereas apoptosis markers may be observed in cells that are dying. DFMO inhibits tumor progression and modulates ODC

signaling and cell proliferation, resulting in high levels of apoptotic markers and low levels of proliferation markers. (1)

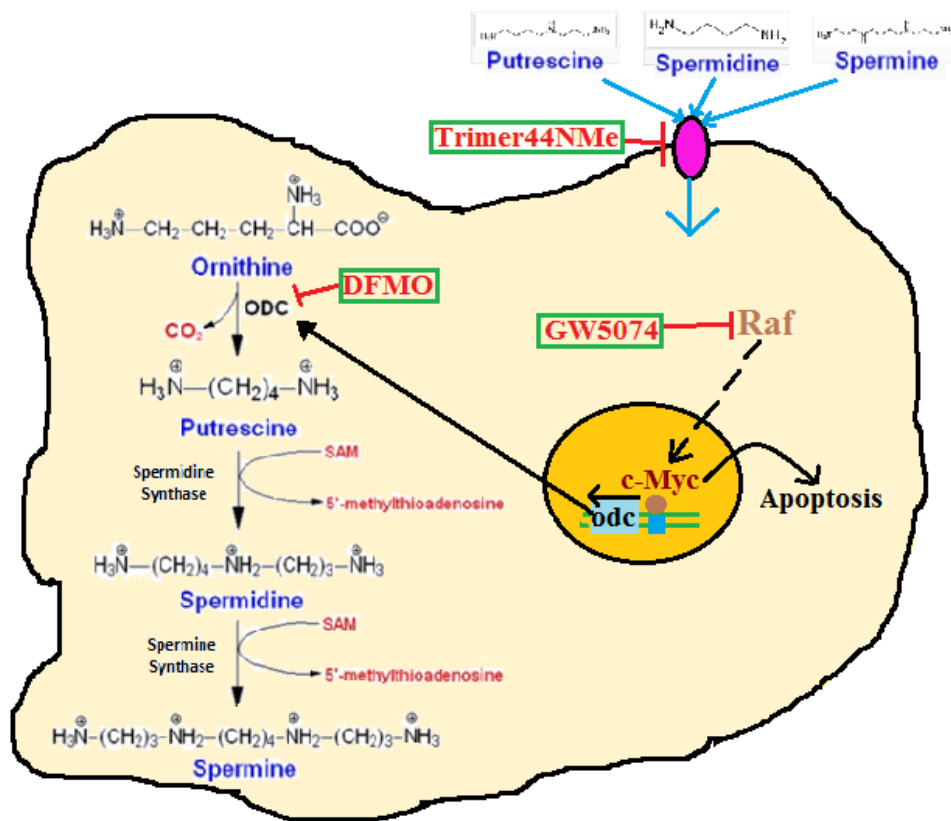
The second method by which cells obtain polyamines is via the transport of them from the environment across the cell membrane via a polyamine transporter and is shown below in Figure 3. Research has demonstrated that there are various recognition sites on the cell membrane which identify polyamines and signal the cells to uptake it through membrane transport mechanisms. For example, cells identify the polyamines putrescine, spermidine and spermine through recognition sites on the cell. One mechanism for uptake is accomplished via binding with human organic cation transporter 2 (hOCT2). (12) Although these receptors have been identified, research into their inhibition has been limited. It is believed that PTI's can be designed to block these receptors and prevent cells from being able to acquire polyamines from the cellular microenvironment. (13) This process when combined with the use of DFMO restricts cellular access to polyamines further than with DFMO alone, increases cell apoptotic markers, and drug treatment has been demonstrated to work *in vitro*.



**Figure 3 : Polyamine Import through a Polyamine Transporter**

Two markers have already been evaluated, the proliferation marker Ki-67 and the apoptotic marker Caspase-3. The Ki-67 marker detects areas of tumors which are considered healthy and growing. Caspase-3 plays a critical role in the apoptotic pathway and reveals parts of the tumor that are either dead or in final stages of apoptosis. The absence of Ki67 and/or presence of Caspase-3 can be used to test the tumor response to new drug treatments (DFMO, PTI & GW5074) that are responsible for an effect on these protein markers. In addition, three proteins have been selected for further study, including ODC, c-Myc, and c-Raf. These proteins have been selected to more thoroughly assess the status of the microenvironment that exists within the tumors. Since ODC is a rate limiting enzyme for cellular production of polyamines, an antibody for ODC has been selected to study the effects that DFMO is having on the cellular microenvironment. The c-Myc oncogene is overexpressed in cancer cells, and its associated c-Myc protein has been selected as a marker to better understand its role within the tumor environment. In addition, c-Myc is a trans-activating transcriptional factor for ODC. (10) Compound GW5074 is an inhibitor of the oncogene c-Raf, which plays a role in the cellular regulation processes' and is overexpressed in tumor cell environments. (14) Since c-Raf is active when phosphorylated at the Serine 338 site, an antibody to evaluate c-Raf(Ser338) has been selected as well. (15) The overall pathway that this thesis is based on is shown below in Figure 4.



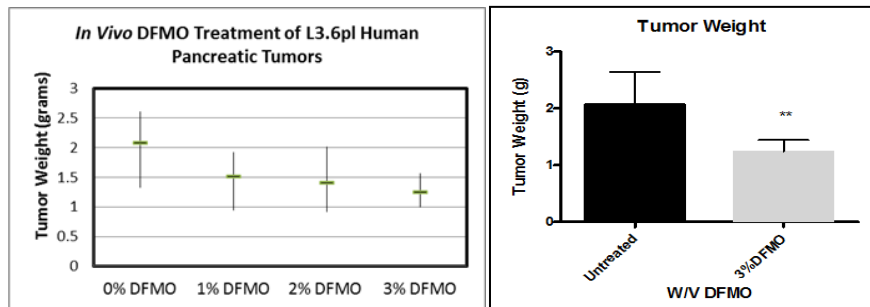


**Figure 4 : Polyamine Synthesis and Transport Pathway with Inhibitors Being Studied**

Immunohistochemistry (IHC) will be conducted to give a visual analysis of the expression of these three proteins, the general concentration, and their location in tumors. This will be conducted for the immune compromised (nude) mouse / L3.6pl experiment since the antibodies are selected and optimized for this experiment.

## CHAPTER THREE: PRELIMINARY DATA

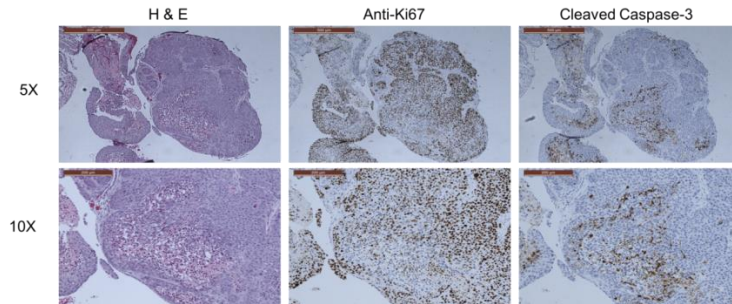
A preliminary experiment has been conducted using immune deficient nude mice. Tumors were induced by injecting  $0.5 \times 10^6$  human pancreatic L3.6pl tumor cells into the mouse pancreas. After a week, treatments were started. The percentage of DFMO in drinking water for subsequent treatments was determined by analyzing tumor weights on four groups of mice as shown below in Figure 5. The tumor weights using 1% DFMO were approximately 30% less than the untreated group. Although the 3% DFMO showed that tumor weights were even further reduced by 40% less than the untreated group, statistically there wasn't a significant advantage to use of more than 1% DFMO in future combination experiments. In fact it may be possible to conduct further experiments to optimize less DFMO concentration, if needed.



**Figure 5 : DFMO Percentages and Tumor Weight for the L3.6pl *In Vivo* Model (16)**

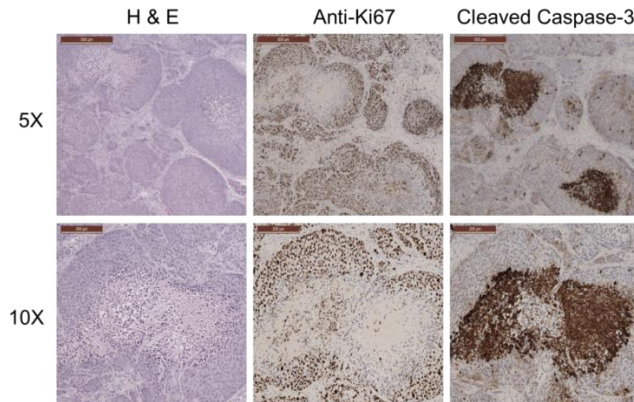
Histological markers for Ki67 and Caspase-3 are shown below in Figure 6, Figure 7, and Figure 8. It can be seen that the proliferation marker Ki67 (brown color in the cell nucleus) is more prevalent in the untreated tumor vs. the DFMO treated model, while the apoptotic marker

Caspase-3 (also brown in color) is more prevalent in the DFMO treated as compared to the untreated tumors. The 5X and 10X in the figures indicate the magnification that the images were taken at for the same region of the tumors.

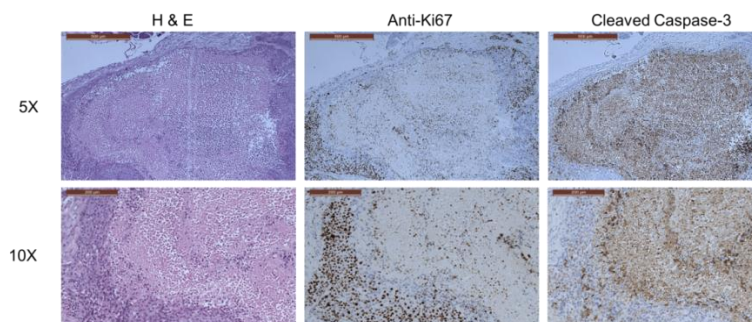


**Figure 6 : Untreated L3.6pl Tumor Histological Markers for Baseline Proliferation and Apoptosis**

**(16)**

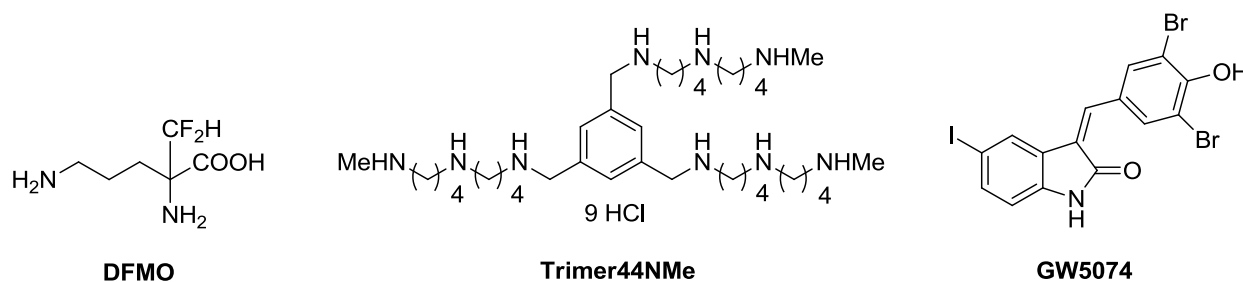


**Figure 7 : 1% DFMO Treated L3.5pl Tumor Histological Markers for Potentially More Apoptosis and Less Cell Proliferation (16)**



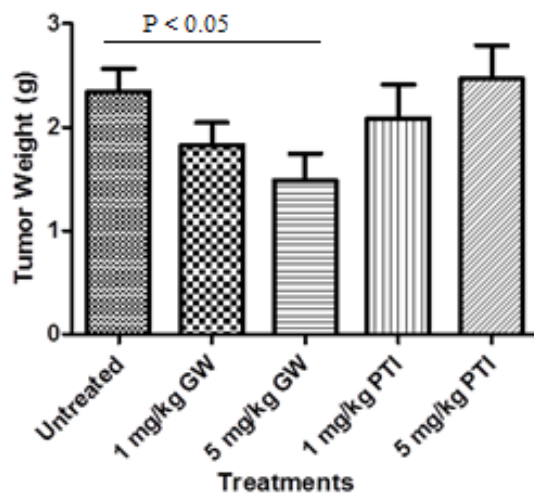
**Figure 8 : 3% DFMO Treated L3.6pl Tumor Histological Markers for Potentially More Apoptosis and Less Cell Proliferation (16)**

The same methodology used to determine the concentrations of DFMO was applied to determine the concentrations of PTI and GW5074 in nude mice with L3.6pl pancreatic tumor cells. The PTI used was Trimer44NMe, which is a benzene substituted with three N-methylhomospermidine motifs. It has shown promise by inhibiting the uptake of spermidine in L3.6pl cells *in vitro*. (7) The chemical structures of DFMO, Trimer44NMe and GW5074 are shown in Figure 9.

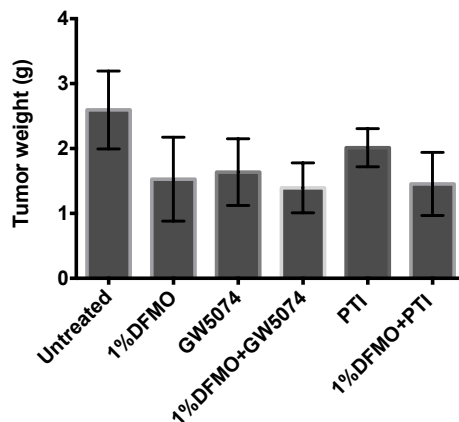


**Figure 9 : Structures of DFMO (17), Trimer44NMe (7) and GW5074 (17)**

The trimer44NMe PTI was tested and the determined maximum tolerated dose was 5 mg/kg body weight. The GW5074 dosage of 1mg/kg was used, which is within the concentration that was recommended by the drug manufacturer of 0.5mg/kg – 10mg/kg. (18) Both the PTI and GW5074 were administered intraperitoneally via injection, whereas the DFMO was administered through the drinking water. Figure 10 shows the results which demonstrate that increasing the concentration of GW5074 effects tumor weights. However, the same was not seen for the trimer44NMe PTI.



**Figure 10 : L3.6pl Ex Vivo Tumor Weights in Response to trimer44NMe (PTI) and GW5074 Treatments (16)**



**Figure 11 : Nude Mice with L3.6pl Derived Tumor Exhibit Anti-Growth Responsiveness to Single and Double Treatments Compared to Untreated Conditions (16)**

The experiment in Figure 11 used 1mg/kg of GW5074 and equimolar concentrations of the trimer44NMe (PTI, 1.85 mg/kg) for the single and combination treatments. The molecular weight of the trimer44NMe PTI (as its nonahydrochloride salt) is 962.19 g/mol and the molecular weight of the GW5074 compound is 520.94 g/mol, respectively. The 1mg/kg of GW5074 was equivalent on a molar basis to a 1.85 mg/kg dose of the trimer44NMe PTI and is below the maximum tolerated dose of 5mg/kg for the trimer 44NMe PTI.

## **CHAPTER FOUR: MATERIALS AND METHODS**

### Tissue Preparation

After the experiment was completed tumor tissues were harvested, placed in 10% neutral-buffered formalin (Leica Surgipath) for preservation, and then processed for further study. After the tissue was fixed for at least four hours, and more often greater than 24 hours, it was ready for processing. Processing was needed in order to infiltrate the tissue with paraffin wax so that it could be uniformly sectioned during slide preparation. This was accomplished with a Leica ASP300 S Vacuum Tissue Processor, through a programmatic series of steps that involved soaking the tissue in cycles of ethanol, xylene and paraffin wax. Upon completion of processing, the infiltrated tissue was placed in a mold filled with paraffin through the use of a Leica EG1150 Tissue Embedding Center, and the blocks were then allowed to harden for at least 24 hours prior to use. Once tissue had been embedded in paraffin, the block could be stored at room temperature.

### Slide Preparation

Slides were prepared by placing the paraffin embedded tissue block on a Leica RM2235 Manual Rotary Microtome. The microtome allows for consistent and fine sectioning of tissue. Layers of tissue, 5 microns thick, were cut and floated in a 40°C hot water bath. The hot water bath allowed for the paraffin to soften but not melt completely. Sections were then guided onto a poly-l-lysine coated glass microscope slide (Thermo/Fisher Scientific), while in the water bath. This type of slide allows for tissue to adhere to the surface of the glass. Once the tissue was

placed on the slide, they were then heated to 65°C for thirty minutes to dry the slides and ensure that the tissue properly adhered.

### Antibody Optimization

Prior to running an experiment, selected primary antibodies were optimized for dilution. Too much antibody used for staining purposes may bind non-specifically as well as result in extensive background staining. Too little antibody may result in light or no staining at all. Antibody slides from the untreated control group were selected for the optimization procedure. Depending on the antibody, various levels of dilution were selected based on the data sheets provided by the manufacturer. The antibodies used and dilution levels are listed below in Table 1.

**Table 1 : Dilutions Selected to Optimize Antibodies for Experimental Staining**

<b>Antibody (source)</b>	<b>Dilutions</b>
ODC (Origene)	1:50 1:100 1:250
c-Myc (Abcam)	1:250 1:500 1:1000
c-Raf (Bioss)	1:100 1:250 1:500

Tissue staining can vary depending on time and concentration of agents. Consistency between each slide can be better maintained by using an automated method. Slides were placed in the Leica Bond Max immunostainer. This instrument prepares slides for staining by removing



the paraffin wax from the tissue sections and treating with an epitope retrieval agent (either Citrate or EDTA). After preparation, staining the tissue for the appropriate antibody and counterstaining the tissue with hematoxylin to show the cell nuclei was conducted. For both the optimization and experiment, each slide was individually bar coded and scanned into the instrument to dictate the desired antibody, as well as the type of epitope retrieval agent using the Leica Bond software. Microscope slides were prepared for coverslipping after antibody staining. The tissue sections were dehydrated by placing the slides in an increasing concentration of ethanol followed by xylene. This procedure was immediately followed by applying Cytoseal-60 (Richard-Allan Scientific) and a coverslip. The slides were then imaged on a Leica DM 2000 microscope with digital DFC 295 camera and Leica imaging software.

### Slide Staining

Once antibody dilution levels were determined, the Leica Bond Max immunostainer was used to conduct staining on all treatment groups for each desired antibody. The dilution levels and epitope retrieval used for this experiment are shown in Table 2.

**Table 2 : Selected Antibody Dilution and Epitope Retrieval**

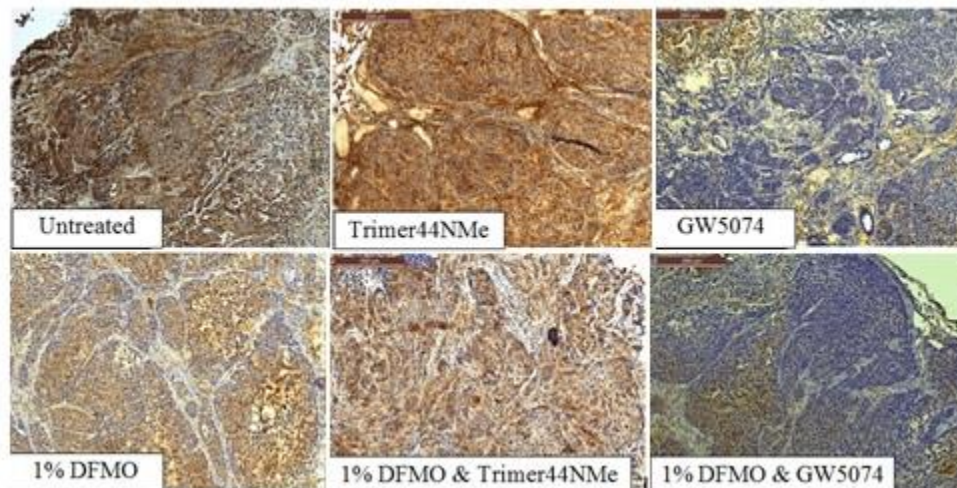
<b>Antibody (source)</b>	<b>Dilution</b>	<b>Epitope Retrieval</b>
ODC (Origene)	1:50	Citrate
c-Myc (Abcam)	1:50	Citrate
c-Raf (Bioss)	1:100	EDTA

The 1:50 dilution was selected for c-Myc even though this was below the low end used in optimization due to light staining seen. Again slides were dehydrated, cover slipped, and then imaged. Once images were obtained Microsoft word was used to create the composite images which are shown and discussed below in chapter five.

## CHAPTER FIVE: RESULTS AND ANALYSIS

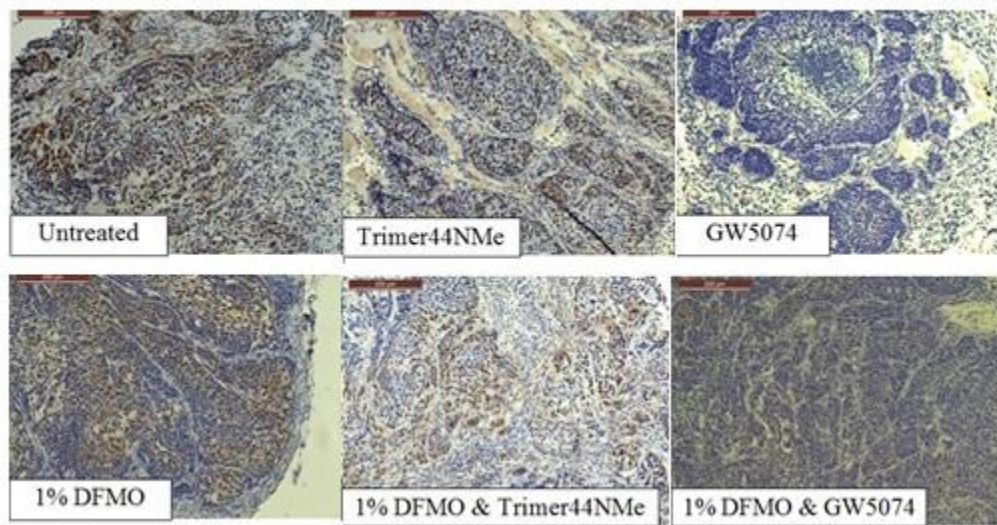
### Immunohistochemistry

Immunohistochemistry was conducted on L3.6pl tumors and the stained slides were imaged for each of the treatment groups to determine protein levels for c-Raf, c-Myc and ODC. Phosphorylated c-Raf was evaluated because it is a protein kinase that mediates the MAPK protein signaling cascade. Images obtained at 10X magnification from the six treatment groups are shown in Figure 12 below. The groups that were treated with GW5074 show a significant decrease in phosphorylated c-Raf. This is expected since GW5074 acts as an inhibitor to Raf. The groups that were not treated with GW5074 did not show decreased levels of phosphorylated c-Raf.



**Figure 12 : Decreased Phosphorylated c-Raf Immunohistochemistry Detected in GW5074 Treatment Groups**

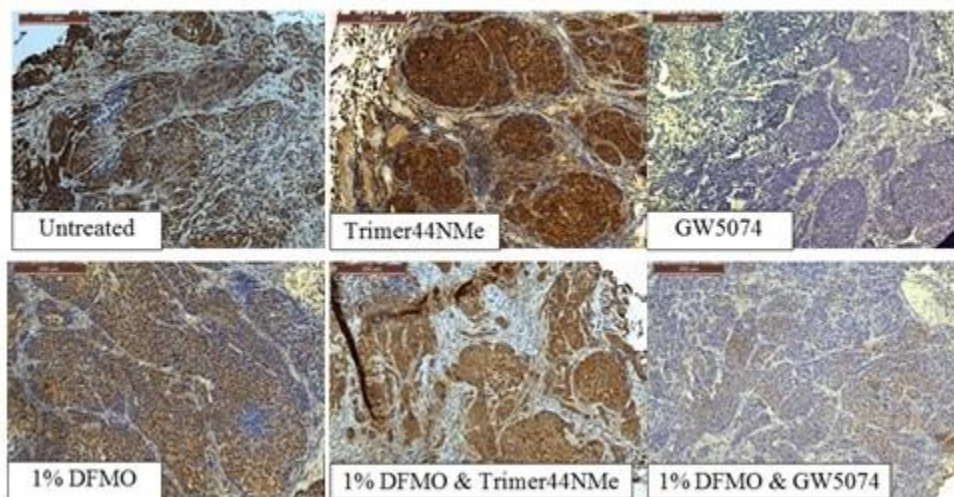
The transcription factor c-Myc was also evaluated at 10X magnification and results are shown below in Figure 13. Since Raf is upstream of c-Myc (see Figure 3), it is expected that there will be a decrease of c-Myc levels in groups that were treated with the c-Raf inhibitor, GW5074. This is the trend that was observed. Other treatment groups that had been treated with DFMO showed a slight decrease in the expression of c-Myc, although not as profound as those treated with GW5074. It has been observed that DFMO plays a role in the down regulation of c-Myc in certain cell types. (19) It is possible that that could be happening with the L3.6pl cells used.



**Figure 13 : c-Myc Immunohistochemistry Showed Decreased Expression that was Most Apparent in Response to GW5074**

The enzyme ODC was also evaluated through immunohistochemistry. The results of the ODC staining at 10X magnification are shown below in Figure 14. As with the c-Raf and c-Myc staining, ODC levels are considerably lower in the treatment groups that were given GW5074.

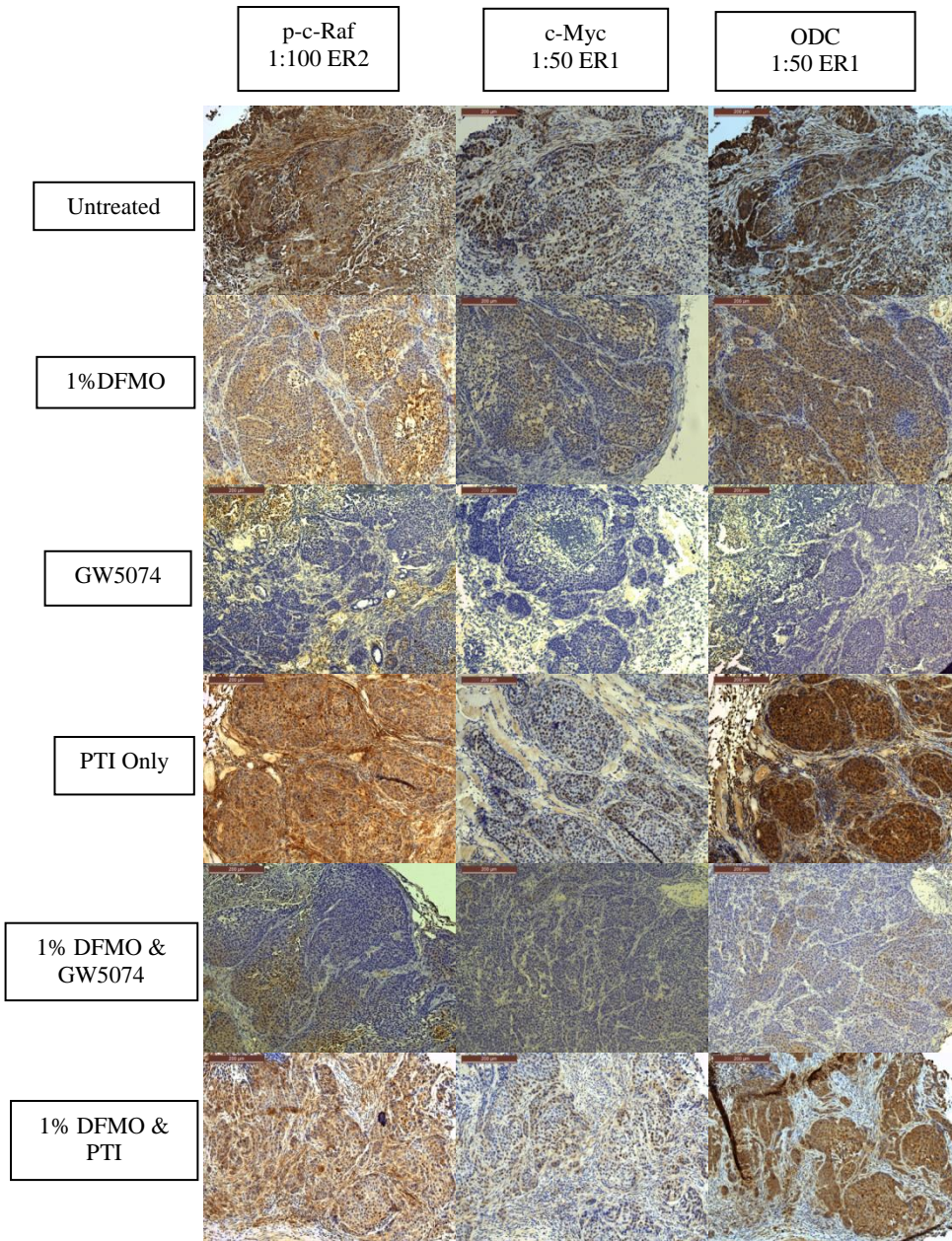
Again, this is expected because c-Myc is a transcription factor which affects the expression levels of ODC. The group treated with DFMO alone still shows staining comparable to the untreated group. This is also expected since DFMO only inhibits the ODC enzyme from synthesis of putrescine, but does not stop expression. As a result ODC is being expressed in groups treated with DFMO, however it may accumulate in its inactive form. (19). Unlike c-Raf, there is no known commercial antibody to detect specific phosphorylation of ODC. The treatment groups with the PTI show even higher levels of ODC expression. This is most likely due to the fact that the cellular capability to import polyamines has been inhibited, and these cells now depend completely on the synthesis of ODC to manufacture polyamines. As a result of this dependency, ODC is shown to be upregulated in the tumors that have been treated with the PTI. The group that is treated with DFMO and PTI shows a decrease in levels of ODC from those treated with the PTI alone, demonstrating that this is a synergistic effect when both DFMO and the PTI are used together.



**Figure 14 : ODC Immunohistochemistry Exhibited Decreased Expression in Response to GW4074**



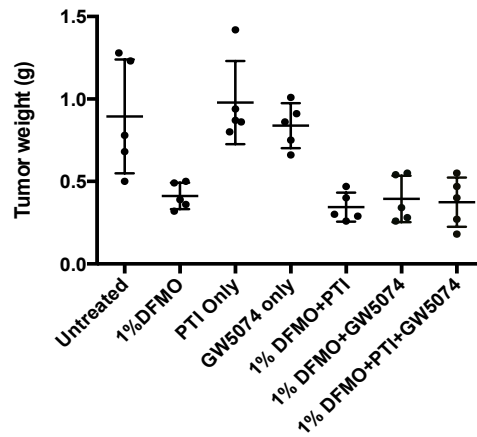
In Figure 15 a composite side by side image is provided for comparison between different antibody staining of each group. All images were taken of the same region of the tumor for each treatment group.



**Figure 15 : Composite Image of All Staining Images**

## CHAPTER SIX: FUTURE EXPERIMENT

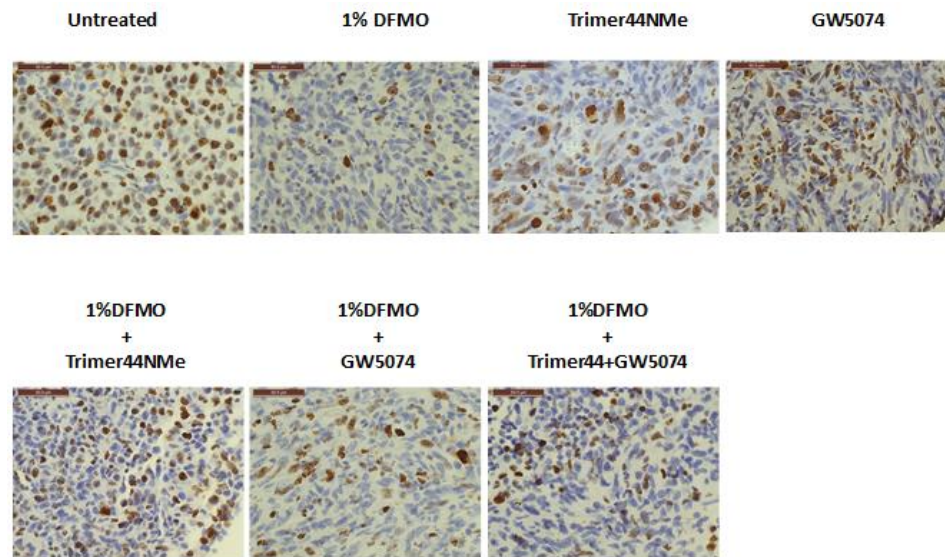
Although not yet examined for protein markers for c-Raf or ODC, the research laboratory also has a pancreatic model whereby mouse Panc02 pancreatic tumor cells were injected into immune competent C57Bl/6 mice. In this experiment seven groups of mice were studied. The treatment and results on tumor weights are shown below in Figure 16. It can be seen that in groups treated with 1% DFMO, tumor weights were reduced by approximately 50%. Tumor weights were even further reduced in the groups that were treated jointly with 1% DFMO and PTI. Finally, groups treated with 1% DFMO, PTI and GW5074 exhibited the smallest tumors..



**Figure 16 : C57Bl/6 Mice with Panc02 Derived Tumor Exhibit Response to 1% DFMO alone and in combination with PTI and/or GW5074 (16)**

Histological markers for Ki67 in each of the groups studied are shown at 40X magnification below in Figure 17. It can be seen that there is significant Ki67 present in the

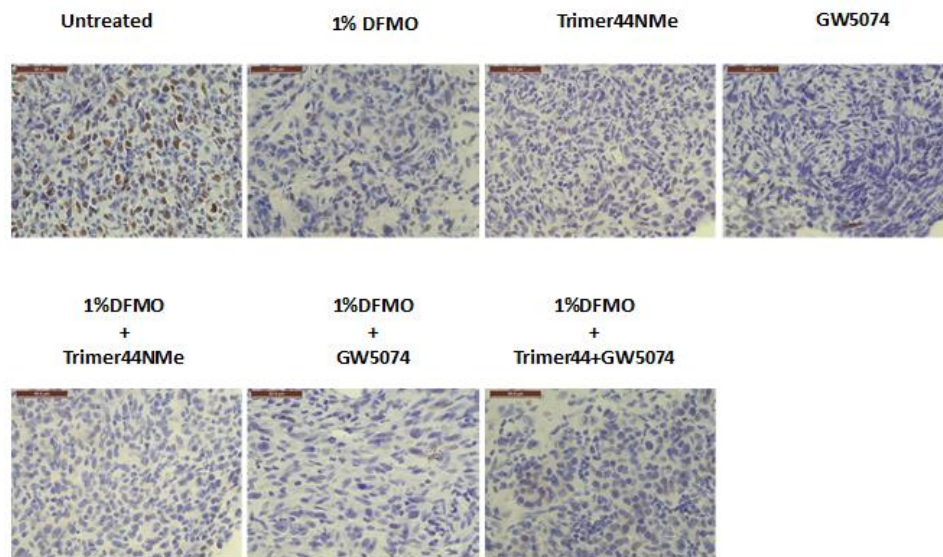
untreated group compared to the treated groups. Only GW5074 by itself failed to show a significant reduction of Ki67.



**Figure 17 : Panc02 Tumors have Decreased Staining Against Ki67 Proliferation Marker in the Presence of Hematoxylin-Nuclear Stain (16)**

Histological markers for c-Myc in each of the groups are shown at 40X magnification below in Figure 18. It can be seen that there is significant c-Myc present in the untreated group compared to the treated groups.





**Figure 18 : Panc02 Tumors have Decreased Staining Against c-Myc Nuclear Marker with in the Presence of Hematoxylin-Nuclear Stain (16)**

The next phase in this series of experiments to study PTIs when combined with DFMO is to follow up with the orthotopic mouse model with Panc02 (mouse cell line) and also to test tumorigenicity of Panc02 in the Nude immunocompromised mouse model. A determination needs to be made if the concentration of DFMO can be lowered in order to better assess and identify if there truly is synergy in combination treatments with DFMO and PTI's. As a result all experiments previously conducted, the Panc02 cell line may need to be repeated with 0.5% DFMO and 0.2% DFMO. The experiment also needs to assess the effect of survival of animals with combination treatments, over those treated with single agents alone, and to delineate the effect that the immune system may have on tumor cell proliferation and/or apoptosis when combined with PTIs and DFMO.

In these experiments seven groups of five mice orthotopically injected with a mouse pancreatic tumor cell line Panc02 will be treated as shown in Table 3 below. These mice will receive between 0.2-1% of DFMO in drinking water. They will be injected intraperitoneally with PTI and GW5074. GW5074 will be delivered at a dosage of 1mg/kg body weight, while PTI will be delivered at an equimolar dosage. They will be injected five days per week for two weeks, which is modeled after the C57Bl/6 experiment previously conducted. Upon completion of the experiment tumor efficacy will be analyzed based on various markers, including Ki67, Caspase-3, ODC, c-Myc, and c-Raf.

**Table 3 : Future Experimental Treatment Groups**

<b>Mouse Group</b>	<b>DFMO</b>	<b>PTI</b>	<b>GW5074</b>
1			
2	X		
3		X	
4			X
5	X	X	
6	X		X
7	X	X	X

*Table 1: Experimental Treatment Groups. Treatment groups that receive DFMO will be injected with 1%, 0.5% and 0.2% DFMO depending on the respective experiment.*

These results will then be compared to the C57Bl/6 (Panc02) experiment to better understand the role that the immune system is having on the synergistic effects in the various treatment groups. This experiment will demonstrate if low dose of DFMO when combined with PTI can have an additive effect on tumor viability. The experiment will be terminated when the health assessment indicates tumor stress.

## CHAPTER SEVEN: CONCLUSION

Pancreatic cancer is a serious disease, one in which the survival rate over five years is less than 6%. (1) In order for pancreatic tumors to exhibit uncontrolled proliferation they have high metabolic needs and require large amounts of polyamines to sustain this uncontrolled growth. There are two methods known in which cells obtain polyamines, through direct synthesis and transport. DFMO is a polyamine synthesis inhibitor which has shown some success in reducing tumor weights. GW5074 is an inhibitor of c-Raf, an upstream protein which plays a role in the synthesis of polyamines as well as the apoptotic pathway. Trimer44NMe, a PTI has also shown promise *in vitro*. It is believed that by combining polyamine synthesis inhibitors with polyamine transport inhibitors a synergistic effect on proliferation and/or apoptosis would be demonstrated.

By investigating the expression of protein markers the efficacy of the PTI / DFMO interaction and their combined effect on tumor viability was studied. The protein markers selected for study were c-Raf, c-Myc and ODC. The relationship between the markers being studied and the treatments used was shown in Figure 4. It was believed that by inhibiting c-Raf with GW5074 reduced levels of both c-Myc and ODC would be seen, which was the case. When treated with the PTI, higher levels of ODC were observed, most likely due to the exclusive cellular dependence on synthesis of polyamines. A synergistic effect was observed in the treatment groups that received both a polyamine synthesis inhibitor as well as a polyamine transport inhibitor. Through data obtained from this study future experiments can be modeled

and conducted which will more accurately determine the appropriate levels of DFMO, GW5074 and Trimer44NMe to optimize treatments in advance of clinical trials.

## **APPENDIX: ANTIBODY DATASHEETS**

<b>Name:</b> Anti-ODC1 rabbit polyclonal antibody <b>Product Data Sheet - ANTIBODY</b>	<b>Catalog:</b> TA321499
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<b>Components:</b>	<ul style="list-style-type: none"> <li>• Anti-ODC1 rabbit polyclonal antibody (TA321499)</li> <li>• 1 vial of 20ug myc-DDK tagged ODC1 HEK293T over-expression lysate lyophilized in RIPA buffer (LC400909). (Reconstitute into 20ul of 1x SDS sample buffer before loading; load 5ul per lane as WB control or as desired)</li> </ul>
<b>Amount:</b>	100ul
<b>Immunogen:</b>	Fusion protein corresponding to C terminal 300 amino acids of human ornithine decarboxylase 1
<b>Host:</b>	Rabbit
<b>Isotype:</b>	IgG
<b>Species Reactivity:</b>	Human, Mouse, Rat
<b>Guaranteed Applications:</b>	WB, IHC
<b>Suggested Dilutions:</b>	ELISA: 1:2000-5000, WB: 1:200-1000, IHC: 1:50-200
<b>Concentration:</b>	1mg/ml
<b>Buffer:</b>	PBS pH7.3, 0.05% NaN <sub>3</sub> , 50% glycerol
<b>Purification:</b>	Antigen affinity purification
<b>Storage Condition:</b>	Shipped at -20C. Upon delivery store at -20C. Dilute in PBS (pH7.3) if necessary. Stable for 12 months from date of receipt. Avoid repeated freeze-thaws.

### Target

<b>Target Name:</b>	Homo sapiens ornithine decarboxylase 1 (ODC1), transcript variant 1
<b>Alternative Name:</b>	ODC
<b>Database Link:</b>	<a href="#">NP_002530</a> <a href="#">Entrez Gene 4953 Human</a> <a href="#">Entrez Gene 18263 Mouse</a> <a href="#">Entrez Gene 24509 Rat</a>
<b>Function:</b>	This gene encodes the rate-limiting enzyme of the polyamine biosynthesis pathway which catalyzes ornithine to putrescine. The activity level for the enzyme varies in response to growth-promoting stimuli and exhibits a high turnover rate in comparison to other mammalian proteins. Originally localized to both

*This product is to be used for laboratory only. Not for diagnostic or therapeutic use.*

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# Anti-c-Myc antibody [Y69] ab32072



★★★★☆ 23 Abstracts | 47 References | 18 Images

## Overview

Product name	Anti-c-Myc antibody [Y69]
Description	Rabbit monoclonal [Y69] to c-Myc
Specificity	This antibody is specific for c-Myc.
Tested applications	WB, IHC-P, ICC/IF, IP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human c-Myc as 1-100 (N terminal). The exact sequence is proprietary. (Peptide available as <a href="#">ab166837</a> )
Positive control	<div style="border: 1px solid #ccc; padding: 5px; display: inline-block;">Purchase matching WB positive control: <a href="#">Active human c-Myc full length protein</a> &gt;</div> <p>WB: Jurkat, Raji, K562, THP1, A20 and Raw264.7 cell lysates. ICC/IF: HeLa cells. IHC-P: Human skin carcinoma, diffuse large B cell lymphoma, adenocarcinoma of the colon, lung adenocarcinoma, gastric adenocarcinoma and urinary bladder transitional carcinoma tissues. IP: Jurkat cell lysate.</p>
General notes	<p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated "PUR" on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</p> <p>Produced using Abcam's RabMAb<sup>®</sup> technology. RabMAb<sup>®</sup> technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.</p> <p>This product is available conjugated to Agarose validated in IP usage - <a href="#">ab178457</a></p> <p>A 40 µl trial size is available to purchase for this product.</p> <p>Myc is involved in MAPK-p38 signaling pathway - <a href="#">see the interactive version</a>.</p>

## Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Dissociation constant (K <sub>D</sub> )	K <sub>D</sub> = 3.80 x 10 <sup>-12</sup> M
	<p>LOW AFFINITY 10<sup>-6</sup> 10<sup>-7</sup> 10<sup>-8</sup> 10<sup>-9</sup> 10<sup>-10</sup> 10<sup>-11</sup> 10<sup>-12</sup> 10<sup>-13</sup> HIGH AFFINITY</p> <p>Learn more about K<sub>D</sub></p>
Storage buffer	pH 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y69
Isotype	IgG



**bs-3377R**

[Primary Antibody]

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**Rabbit Anti-c-Raf(Ser338) Polyclonal Antibody**

— DATASHEET —

**Host:** Rabbit  
**Target Protein:** c-Raf Ser338  
**Modification:** Ser338  
**Site:**  
**Clonality:** Polyclonal

**Isotype:** IgG

**Entrez Gene:** [5894](#)

**Swiss Prot:**

**Source:** KLH conjugated synthetic phosphopeptide derived from human c-Raf around the phosphorylation site of Ser338

**Purification:** Purified by Protein A.

**Storage:** Aqueous buffered solution containing 100ug/ml BSA, 50% glycerol and 0.09% sodium azide. Store at -20°C for 12 months.

**Background:** The Raf family of serine/threonine specific kinases is comprised of three members (aRaf, bRaf, and cRaf) that play a critical role in regulating cell growth and differentiation, and couple growth factor receptor stimulation to nuclear transcription factors via the Ras/mitogen activated protein kinase (MAPK) pathway. cRaf kinase (also known as Raf1) is a small GTPase like kinase of 73 kDa, and is a signal transducer of multiple extracellular stimuli that is regulated by several pathways, and that once activated, phosphorylates MEK which in turn phosphorylates ERK. Raf1 is involved in the transduction of mitogenic signals from the cell membrane to the nucleus. It is part of the Ras dependent signaling pathway from receptors to the nucleus.

**Size:** 100ul

**Concentration:** 1ug/ul

**Applications:** WB(1:100-1000)  
IHC-P(1:100-500)  
IF(IHC-P)(1:50-200)

**Cross Reactive Species:** Human  
Mouse  
Rat

Caution: For research use only. Not for human or animal therapeutic or diagnostic use.

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