

# West Chester University of Pennsylvania

## Annual Progress Report: 2009 Formula Grant

### Reporting Period

July 1, 2010 – June 30, 2011

### Formula Grant Overview

The West Chester University of Pennsylvania received \$4,080 in formula funds for the grant award period January 1, 2010 through June 30, 2011. Accomplishments for the reporting period are described below.

### Research Project 1: Project Title and Purpose

#### *DNA Polymerase Expression of Human Colon Cell Lines following Chemotherapeutic Treatment*

Many of today's cancer treatments include chemicals which damage a cell's DNA resulting in the inability of the cell to replicate and survive. For the chemotherapeutic treatments to be more effective, the relationship between DNA polymerase levels and the various drugs needs to be determined. This project would help define that relationship by studying the effect of chemotherapeutic drug on DNA polymerase levels in normal and carcinoma human colon cell lines.

### Anticipated Duration of Project

1/1/2010 - 12/31/2011

### Project Overview

The main objective of this project is to determine the levels of specialized polymerases within normal and carcinoma colon cell lines with and without treatment of cyclophosphamide, doxorubicin, and methylnitrosourea. The lines to be used include normal (LS123) as well as carcinoma (LoVo, HCT-8, HCT-15, HCT-116, and HT-29) cells. The level of expression of DNA polymerases will be determined at the protein level using Western Blot analysis. If time and expenses allow, expression at the RNA level will also be performed using the Quantitative Polymerase Chain Reaction procedure

In order to achieve the specific objectives of this project, several activities need to be completed including maintenance and treatment of the cell lines, isolation of protein (and possibly RNA) from the cells, and analysis of the collected samples. The data then needs to be analyzed to determine the levels of the various samples collected.

## **Principal Investigator**

Erin Gestl, PhD  
Assistant Professor of Biology  
West Chester University of Pennsylvania  
282 Schmucker Science N  
750 S. Church St.  
West Chester, PA 19383

## **Other Participating Researchers**

None

## **Expected Research Outcomes and Benefits**

Two different comparisons of polymerase expression levels will be made during this project. The first is to compare normal and cancerous colon cell lines to determine if there are any changes. Since many treatments involved chemicals that affect polymerase function, it is also possible that cells undergoing treatment may produce a higher level of certain DNA polymerases than untreated cells. With an increased understanding of the expression levels of polymerases, the goal would be able to design more effective chemotherapeutic regimens.

## **Summary of Research Completed**

### Cell Maintenance and Cell Growth Curves

The cells were grown in 100 mm dishes with sufficient media and grown at 37C in a 5% CO<sub>2</sub> environment. When they grew to confluence, the cells were removed with trypsin, pelleted, resuspended, and a fraction of the suspension was added to a new plate with new media. Before treatments a cell dose-response curve was determined by adding known concentrations of cells with media to a 96 well plate and determining the effect of the chemotherapeutic treatment after a few days. The cells were added to each well and allowed to grow for 1 day. Then they were treated for 1 hour, after which the treatment was removed, the cells were washed, and new media was added. The experiment was set up according to the Figure 1. Cell growth/death was assayed from days 1-4 using Dojindo's cell counting kit 8. An example of the results is shown in Figure 2. Using this data, concentrations of 1mM as a threshold dose, 2.5mM as a 50% effective dose, and 0mM as a control were selected for use in larger scale treatments.

### Chemotherapeutic Treatments and Sample Isolation

Treatments of five different colorectal cell lines (HCT-8, HCT-15, HCT-116 40-16, HCT-116 386, HCT-116 379.2) were treated with Methyl-nitrosourea (MNU) at concentrations of 1 mM as a threshold dose, 2.5 mM as a 50% effective dose, and 0 mM as a control were completed, as well as Doxorubicin at concentrations of 12.5, 5, 2.5, 1.25 and 0 uM. DNA, RNA, and proteins were purified and collected using Qiagen multiprep DNA, RNA and extraction kit. This kit allowed us to extract the DNA, RNA and protein from the same cell pellet which will allow us to determine better cause and effect relationships. The DNA and RNA concentrations after the

extractions were determined using a nanodrop spectrometer (Table 1), and the protein concentrations were determined by the D<sub>C</sub> Protein Assay kit.

### Sample Analysis

Western blot analysis has begun to determine polymerase expression at the protein level, while primers for SYBR Green quantitative reverse-transcriptase polymerase chain reaction (Q-RT-PCR) have been tested and confirmed for analysis of gene expression at the transcript level. The genes being analyzed include polymerase delta 1, polymerase delta 2, polymerase beta, polymerase eta, polymerase iota, polymerase kappa, and PCNA.

Figure 1. Diagram of Chemotherapeutic Dose-Response Curve with Colorectal Cell Lines.

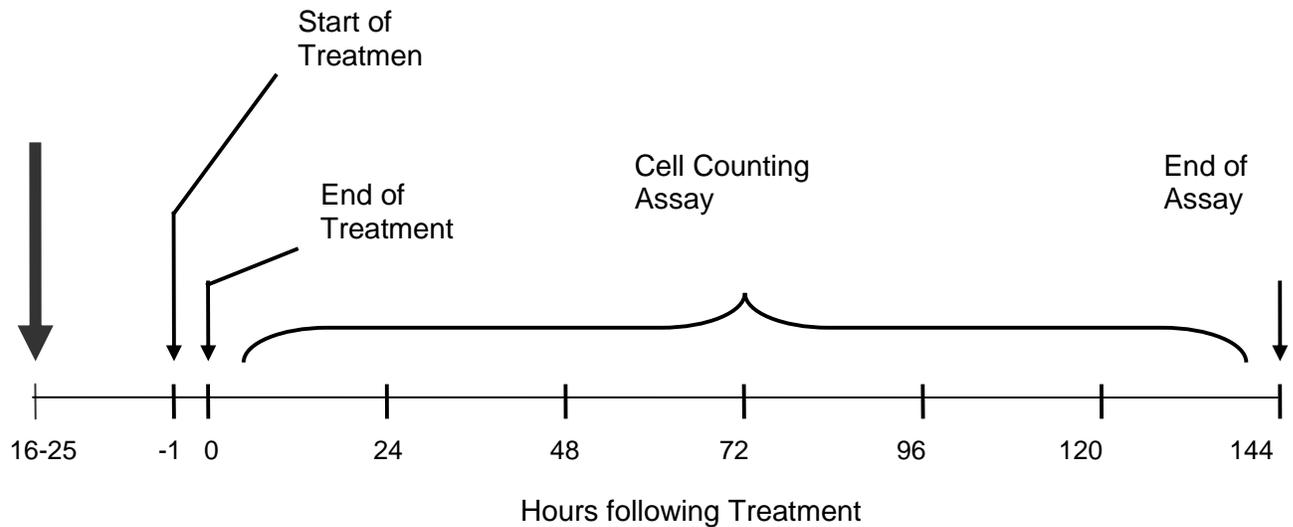


Figure 2. Example of Dose-Response curve using the HCT-8 cells.

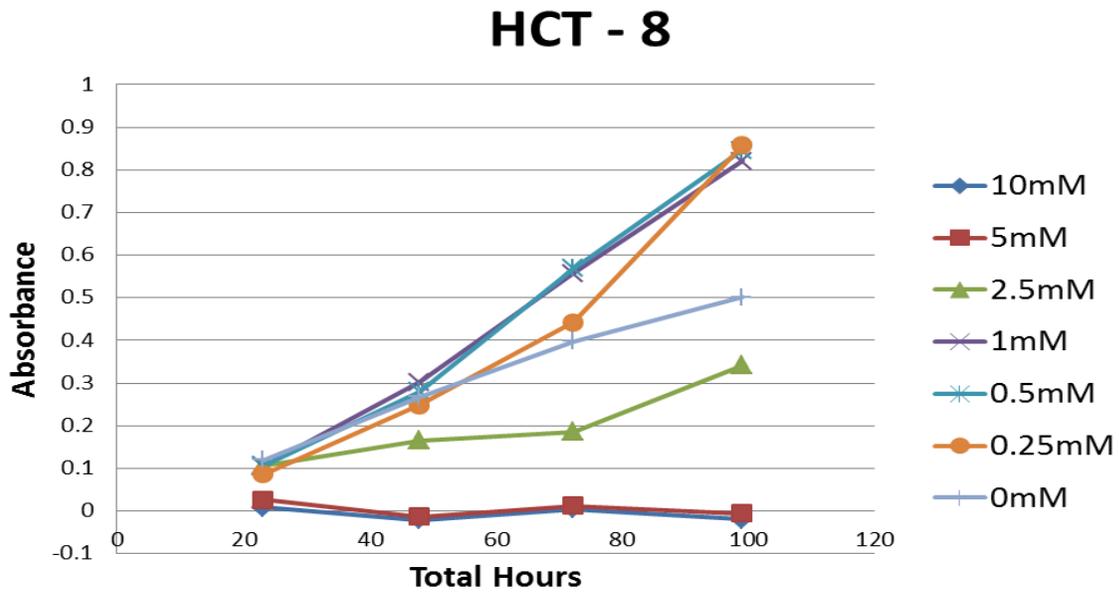


Table 1. DNA and RNA concentrations from Colorectal cells following Treatment

<u>MNU Treatment:</u>			<u>Doxorubicin Treatments</u>		
	<u>DNA</u>	<u>RNA</u>		<u>DNA</u>	<u>RNA</u>
	Concentration (ng/ug)	Concentration (ng/ug)		Concentration (ng/ug)	Concentration (ng/ug)
HCT-116 379.2			HCT-116 379.2		
0mM	189.6	376.6	0μM	232.1	2766.1
1mM	301.4	821.3	0.234μM	659.3	2114.6
2.5mM	393.9	752.6	0.468μM	117.9	1002.9
			0.938μM	368	1590.9
HCT-116 386			2.34μM		
0mM	306.1	384.3			
1mM	421.8	1814.6	HCT-116 386		
2.5mM	264.7	2407.7	0μM	229.7	1636.9
			0.234μM	312.3	3212.7
HCT-116 40-16			0.468μM		
0mM	536.3	1031.2	0.938μM	369.9	1249.6
1mM	314.7	1214.3	2.34μM	704.7	1428.4
2.5mM	327.5	1297.9			
			HCT-116 40-16		
HCT-8			0μM		
0mM	242.9	1158	0.234μM	292	2994.5
1mM	248.7	1164.8	0.468μM	313.9	1666.1
2.5mM	222	2697.4	0.938μM	332.2	1251.2
			2.34μM	417.5	3145.1
				230.9	3230.5
HCT-15					
0mM	163.7	717.1			
1mM	118.6	661.5			
2.5mM	240.8	1826.2			