Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution: Lankenau Institute for Medical Research**

2. **Reporting Period (start and end date of grant award period): 1/1/12-12/31/12**

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Tam Nguyen**

4. **Grant Contact Person's Telephone Number: 484-476-2755**

5. **Grant SAP Number: SAP# 4100057664**

6. **Project Number and Title of Research Project: Project 1 - 2-Deoxyglucose and Hydroxyethyl Disulfide in Improving the Response of Human Colon Cancer Cells to Radiation**

7. **Start and End Date of Research Project: 1/1/12-12/31/12**

8. **Name of Principal Investigator for the Research Project: Iraimoudi S. Ayene, PhD**

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 136,955.21 (DC: $83,017.21; IC: $53,938)

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Ayene	Principal Investigator	25.47%	$29,601.66
Li	Research Lab Associate	70.96% (1/12-7/12)	$21,809.34
Zhang	Biomedical Research Assistant I	75% (7/12-12/12)	$15,199.15

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Prendergast	Co-Investigator	0%
DeNittis	Collaborator	5%
Jefferson	Co-Op student from Drexel	50%
Mandelbaum	Student	20%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		0

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Oxidative pentose cycle in arsenic induced skin carcinogenesis	☒ NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	June, 2012	$2,062,500	$ Not funded
	☐ NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)			$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We will apply for at least two new NIH RO1 grants using the data/results generated from the projects funded by the Health Research Grants.

First grant application is to use these new technologies (OxPhos and CellCountEZ developed in our lab) for screening and characterizing better glucose metabolic inhibitors to target cancer cells and improve the tumor response to radiation and chemotherapeutics.

The second grant application is to determine the use of these technologies to predict toxicity and tumor response in cancer patients.

12. Future of Research Project. What are the future plans for this research project?

We will synthesize and test the efficacy of 2-DG related compounds in tissue culture and animal tumor models.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	1			
Female	1			
Unknown				
Total	**2**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	2			
Unknown				
Total	**2**			

	Undergraduate	Masters	Pre-doc	Post-doc

White	1			
Black	1			
Asian				
Other				
Unknown				
Total	**2**			

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes____X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    This health research project helped Dr. Ayene to continue his laboratory research in novel drug discovery for the improvement of cancer therapy. Further, the results generated from this project will help him to submit two new NIH R01 grants. One is in collaboration with Dr. Paul Gilman, Chief of Cancer Center, Lankenau Hospital that will be submitted in June, 2013. The other is in collaboration with Dr. George Prendergast, LIMR that will be submitted in November, 2013.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

    Yes___X_____ No_____

    If yes, please describe the collaborations:

Four physicians (Dr. Albert DeNittis, Dr. Fucan, Dr. Bulgarelli and Dr. Ing) have become collaborators because of the health research funds. Dr. DeNittis, Chief of Radiation Oncology, Lankenau Hospital, is in charge of the projects that deal with the improvement of radiation therapy. Dr. Fucan, a physician scientist from Guadelope, will be collaborating with Dr. Ayene on the use of the technology developed by the research grant in diabetes. Dr. Bulgarelli from Physician Recommended Nutraceuticals will be collaborating with Dr.

Ayene on the use of his technologies in stroke prevention studies. Dr. Ing from Main Line Health will be collaborating with Dr. Ayene on the use of his technology in obesity.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS

Project Title and Purpose

2-Deoxyglucose and Hydroxyethyl Disulfide in Improving the Response of Human Colon Cancer Cells to Radiation – Hydroxyethyl disulfide (HEDS) inhibits the function of DNA repair protein and glutathione resulting in a better response of oxidative pentose phosphate cycle deficient or glucose deprived cancer cells to radiation. However, it is not efficient in improving the response of glucose containing cancer cells to radiation due to its detoxification by the oxidative pentose phosphate cycle, which requires glucose for its function. Increasing the susceptibility of the glucose containing tumor cells to HEDS is clinically relevant since all tumor cells are not glucose deprived. We will determine the effects of HEDS and 2-deoxyglucose, a competitive inhibitor of pentose cycle, in increasing the radiation response of glucose containing human cancer cells.

Project Overview

Preliminary results have demonstrated that hydroxyethyl disulfide (HEDS), a non-toxic oxidant, increases the response of glucose deprived cancer cells to radiation by inhibiting the function of DNA repair protein and glutathione. HEDS has no significant effect in cells with a functioning oxidative pentose phosphate cycle, which uses glucose for the detoxification of HEDS. These results have raised the possibility to target therapy resistant hypoxic cancer cells since hypoxic cells are also glucose deprived in solid tumors. Our preliminary studies have demonstrated that HEDS treatment showed a significant (50% better) improvement in the response of breast tumor xenograft to DNA damaging agents. Although a significant percentage of cancer cells in solid tumors may be glucose deprived, which can be targeted by HEDS, increasing the susceptibility of the rest of the glucose containing tumor cells to HEDS will further improve tumor response to cancer therapy. 2-deoxy-D-glucose (2-DG), a small molecule inhibitor of the oxidative pentose phosphate cycle that uses glucose to detoxify HEDS, is currently used for the positron emission tomography imaging of tumors in patients since it specifically accumulates in tumors. The better retention time of 2-DG specifically in tumors compared to normal tissues has led to a series of investigations for its use in cancer therapy. Although there was promising preclinical evidence for its antitumor property, recent studies have demonstrated that 2-DG needs to be used up to 20mM in vitro to increase the sensitivity of cancer cells to radiation. In this project, we will determine the combined use of non-toxic 2-DG and HEDS in improving the response of human colon cancer cells to radiation. All of our studies will be carried out in human colon cancer cells with various concentrations of HEDS and 2-DG. A state-of-the-art high pressure liquid chromatography and electrochemical detection and other biochemical assays will be used to determine whether i) 2-DG is effective in inhibiting the detoxification of HEDS, ii) 2-DG is effective in increasing HEDS mediated oxidation of glutathione and proteins and inhibition of the function of DNA repair protein Ku and iii) the combined treatment of HEDS and 2-DG increases the response of human colon cancer cells to radiation.

Specific Aim 1: In this specific aim, we will determine whether 2-DG is effective in inhibiting the detoxification of HEDS by human cancer cells. This will be tested in human colon (HCT116, HT29) cancer cells for various concentrations of 2-DG on the detoxification of different concentrations of HEDS. A state of the art high pressure liquid chromatography (HPLC) and electrochemical detection will be used to quantify the conversion of HEDS into mercaptoethanol (detoxification of HEDS) in these cells.

Specific Aim 2: In this specific aim, we will determine whether 2-DG is effective in increasing HEDS mediated oxidation of GSH and proteins, and loss of Ku protein function. This will be tested in human colon, cancer cells for various concentrations of 2-DG on the oxidation of GSH by different concentrations of HEDS. A state of the art high pressure liquid chromatography (HPLC) and electrochemical detection and biochemical assays will be used to quantify the oxidation of glutathione in these cells. The oxidation of protein thiols and loss of function of DNA repair protein Ku will also be determined by Ellman's reagent and ELISA assays routinely used in our lab.

Specific Aim 3: In this specific aim, we will determine whether 2-DG increases the cytotoxicity of HEDS or HEDS increases the cytotoxicity of 2-DG in human cancer cells. In addition, we will determine whether the combined treatment of HEDS and 2-DG increase the response of human cancer cells to radiation. These will be tested in human colon cancer cells for various concentrations of 2-DG and different concentrations of HEDS. The cytotoxicity will be determined by clonogenic, proliferation and cell survival assays.

Expected Research Outcomes and Benefits

Glutathione and DNA repair proteins play a major role in the resistance of cancer cells to various therapies including radiation. Our previous studies have shown that hydroxyethyl disulfide (HEDS) improves the response of cancer cells deprived of glucose by targeting DNA repair protein and glutathione, which play a major role in radiation resistance. Although this has clinical relevance since glucose deprivation is common in solid tumors that cause resistance to radiation, the efficiency of HEDS as a radiation sensitizer could be much improved by targeting glucose containing cancer cells. Our proposed studies will determine the efficacy of combined treatment of HEDS and 2-deoxy-D-glucose (2-DG) in improving the response of cancer cells to radiation. 2-DG is known to reduce the glucose level in cancer cells resulting in the inhibition of the oxidative pentose cycle that is involved in the protection of DNA repair protein and glutathione. The combined treatment of HEDS and 2-DG is expected to increase the response of cancer cells to radiation by targeting DNA repair protein and glutathione even in glucose containing cancer cells. The use of 2-DG to induce a low glucose tumor microenvironment is advantageous since it is currently used in the clinic for positron emission tomography (PET) imaging of tumors. Additionally, 2-DG is not toxic to normal tissues since it specifically accumulates in tumor. This approach is the first ever to increase the response of cancer cells by targeting DNA repair protein and glutathione using non-toxic 2-DG in combination with HEDS. In conclusion, our approach is innovative since it consists of an experimental test of a novel hypothesis that inhibition of detoxification of HEDS, inhibition of functions of glutathione and DNA repair proteins by combined treatment of HEDS and 2-DG can improve the response of cancer cells to radiation.

Summary of Research Completed

The goal of this project is to determine the efficacy of combined treatment of 2-DG and HEDS in increasing the sensitivity of human cancer cells with normal glucose to radiation. Our preliminary studies indicated that glucose deprived cells or cells impaired with oxidative pentose phosphate cycle (OPPC) activity are susceptible to HEDS mediated radiation sensitization. However, this approach does not enhance the response of those cells with normal glucose level to radiation since HEDS is detoxified by oxidative pentose cycle, using glucose as a substrate.

2-deoxy-D-glucose (2-DG) is known to reduce the glucose level in cancer cells, which is expected to result in the inhibition of the oxidative pentose cycle that is involved in the protection of DNA repair protein and glutathione. The combined treatment of HEDS and 2-DG is expected to increase the response of cancer cells to radiation by targeting glutathione, protein thiols and DNA repair protein even in glucose containing cancer cells. The use of 2-DG to induce a low glucose tumor microenvironment is advantageous since it is currently used in the clinic for positron emission tomography (PET) imaging of tumors. Additionally, 2-DG is not toxic to normal tissues since it specifically accumulates in tumor.

We have carried out several studies to test the efficacy of hydroxyethyl disulfide (HEDS) in improving the response of human colon cancer cells (HCT116 and HT29) to 2-DG mediated inhibition of HEDS detoxification and modulation of cellular thiol redox and radiation response.

As part of specific aim 1, we determined whether 2-DG inhibit the detoxification of HEDS in human colon cancer cells. We used two established human colon cancer cells (HCT116, HT29) obtained from ATCC, USA. All experiments were carried out at a single concentration of cells grown in a 60mm dish or 96 well plates with DMEM medium with 10%FCS, 1% penicillin/streptomycin (P/S) and 20 mM HEPES. These cells were rinsed four times with DMEM without glucose (hereafter called as DMEM-G) to remove the residual glucose in the dish. DMEM-G rinsed cells were incubated for four hours with one ml of DMEM-G containing different concentrations of normal glucose. After 4hr glucose incubation, cells were exposed to different concentrations of 2-deoxyglucose (2-DG) and hydroxyethyl disulfide (HEDS) at 37°C in a 5% CO_2 incubator. The HEDS detoxification i.e. conversion of HEDS into sulfhydryl by the human colon cancer cells was measured by quantifying the sulfhydryl compound in the extracellular medium produced by bioreduction of HEDS. To quantify the disulfide metabolism, 0.5 ml of extracellular medium was mixed with 0.5 ml of 100mM sulfosalicyclic acid (SSA) lysis buffer in microfuge tubes and centrifuged in a microfuge. The medium extract was used for the analysis of the HEDS detoxification.

In these series of experiments, we have tested the efficacy of 0, 1, 2, 3, 5, 10, 15, 20mM 2-deoxyglucose on the detoxification of different concentrations of HEDS (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, 10mM) in normal medium with glucose. The results demonstrated that at higher than 5mM concentrations, 2-DG inhibited the detoxification of HEDS by only 10 to 30% in human colon cancer cells HCT116 (Figure 1). Based on these results in figure 1, we tested the

efficacy of 5 and 20mM of 2-DG in the detoxification of 5mM HEDS in 0, 1, 2, 3, 4, 5mM concentrations of normal glucose to mimic the microenvironment of solid tumors, which are known to have 0 to 5mM normal glucose in different regions of tumor. The results in figure 2A that compares the effects of 2-DG in 0 vs 5mM glucose showed that the detoxification of HEDS is inhibited by 95% in the absence of glucose alone without 2-DG. Surprisingly, the detoxification of HEDS in 0mM glucose is inhibited by 80% in the presence of 2-DG either at 5 or 20mM suggesting that 2-DG may also be a substrate but a weak one as compared to glucose for glucose metabolism. Similar trend was also observed in the presence of 1mM glucose (Fig. 2B). However, a slight but significant 2-DG concentration dependent decrease in HEDS detoxification was observed in cells treated with 2 to 5mM normal glucose (Figure 2C, D, E). Although similar trend was observed in HT29 human colon cancer cells, the HT29 cells are slightly resistant to 2-DG mediated inhibition of HEDS detoxification (Figure 3A – E).

As part of specific aim 2, we determined whether 2-DG is effective in increasing HEDS mediated oxidation of total non protein thiols. Using similar treatment conditions described above, we estimated the effect of 2-DG and HEDS on intracellular non-protein thiol (GSH). To quantify the intracellular non-protein thiols, the extracellular medium was removed after the incubation of cells with and without HEDS in the presence of 0 to 5mM normal glucose and the cells rinsed three times with PBS. The attached cells were lysed with 0.5 ml of ice cold SSA lysis buffer and 0.5 ml water. After 5 min on ice, the cells were scraped with a teflon spatula, transferred into a microfuge, centrifuged and the supernatant used for analysis.

We tested the effect of 5 and 20mM of 2-DG with and without 5mM HEDS in 0, 1, 2, 3, 4, 5mM concentrations of normal glucose on the intracellular non-protein thiols. The non-protein thiol level was only slightly (20%) affected by 20mM 2-DG alone in HCT116 cells without HEDS at any of the concentrations (0-5mM) of normal glucose (Figure 4A, B, C, D, E). However, the results that compares the effects of 2-DG in 0 vs 5mM glucose showed that the non-protein thiol level was decreased by 90% in the absence of glucose alone without 2-DG but only in the presence of 5mM HEDS. Surprisingly, under these conditions, the non-protein thiol level in 0mM glucose is decreased by only 70% in the presence of 2-DG either at 5 or 20mM in the presence of 5mM HEDS. Similar trend was also observed in the presence of 1mM glucose (Figure 4B). However, the non-protein thiol level was only slightly affected by 2-DG in cells with 2 to 5mM normal glucose even in the presence of HEDS (Figure 4C, D, E). Although similar trend was observed in p53 mutant radiation resistant (HT29) human colon cancer cells, these cells are slightly resistant to HEDS mediated depletion of non-protein thiol (Figure 5A, B, C, D, E).

Although the results with 2-DG alone or in combination with 5mM HEDS did not show any significant effect on intracellular non-protein thiols and likely to have similar effect on intracellular protein thiols, we still determined the effect of 5 and 20mM 2-DG with and without 5mM HEDS in 0 and 5mM normal glucose on the intracellular protein thiols in HCT116 and HT29 cells.

For quantification of protein thiols, cancer cells attached to the dishes were incubated with 2-DG and HEDS. These cells were then washed three times with PBS and then treated with SSA. This treatment precipitates cellular macromolecules in place without loss of protein. The acid was

removed and cells washed two more times with 5 ml SSA, which was removed by aspiration. Under these experimental conditions, only the non-protein thiols were washed off the cells. Acid-fixed cells were then incubated with 1 ml of 100 mM phosphate buffer, pH 7.4, containing 1.5 mM dithiobis nitrobenzoic acid (DTNB) for 15 min at 37º and the absorbance read at 412 nm. Protein thiols were quantified using an absorption coefficient for reduced DTNB of 13,600 at 412 nm. The results from these experiments have demonstrated that the protein thiol was not significantly affected even at 20mM 2-DG alone in p53 wild type radiation sensitive (HCT116) cells without HEDS at 0 or 5mM of normal glucose (Figure 6). In contrast the protein thiol level was decreased by 30% in the absence of glucose alone without 2-DG but only in the presence of 5mM HEDS (Fig. 6). Similar trend was observed in p53 mutant radiation resistant HT29 human colon cancer cells (Figure 7).

As proposed in the specific aim 2, we also determined the effect of 2-DG with and without HEDS in 0 and 5mM normal glucose on the DNA repair protein Ku in these cells. The DNA binding efficiency of nuclear Ku was quantified by Ku DNA repair kits from Active Motif, USA. The nuclear proteins were prepared by membrane disruption using Active Motif hypotonic buffer, detergent and complete lysis buffer and centrifugation as per the manufacturer's instructions. The nuclear proteins were stored at –80ºC. Protein concentration was quantified by Biorad Reagent. Nuclear protein (1µg) mixed with AM6 binding buffer was incubated in an eight well strip provided by Active Motif for 1hr at RT, washed and incubated with Ku70 antibody for 1hr. After incubation, the wells were washed, incubated with developing solution for 4min, mixed with stop solution and the O.D was read in a microplate reader at 450nm with a reference wavelength of 655nm. Consistent with the data on intracellular non protein and protein thiols, HEDS significantly decreased the function of Ku protein in HCT116 cells incubated with 0mM normal glucose without any significant effect in 5mM normal glucose either in the presence or absence of 2-DG at the highest concentration used (Fig. 8). Similar trend was observed in p53 mutant radiation resistant HT29 human colon cancer cells (Figure 9).

Although the results showed that 2-DG is not effective in decreasing the bioreduction of HEDS at higher concentration (2 - 5mM) of normal glucose in the medium, longer exposure time for HEDS than the 2hrs exposure used in the above experiments may exhibit a better effect on the thiol level. As part of specific Aim 3, we, therefore, tested the combined effect of 2-DG and HEDS on the radiation response of cells after 2hrs pre and post (4hrs total) radiation incubation with HEDS in HCT116 and HT29 cells incubated with 2-DG for 24hrs in normal glucose medium.

Wild type p53 radiation sensitive (HCT116) and p53 mutant radiation resistant (HT29) cells treated with HEDS and/or 2-DG in the presence of normal glucose were exposed to radiation (0, 1, 2, 4, 6, 8, 12, 16Gy) according to protocols described above in specific aim 2. Although we proposed to use colony, proliferation and metabolic assays to determine the effect of HEDS and 2-DG mediated redox (PSH & GSH) modification on cell death in these cancer cells, we have used mostly cell proliferation and metabolic assay since these assays are simple with better sensitivity. For proliferation assay, the survival was measured by counting total number of cells in each dish using a Coulter counter six days after treatment. For the metabolism based cell survival assay, we used CellCountEZ (LIMR Development Inc.), a novel cell survival assay developed in our laboratory, to quantify the metabolically active cells 6 days after the treatment.

These assays are not only sensitive to measure the surviving fraction after irradiation at doses as low as 1Gy but also quantified the differences in radiation response between the radiation resistant and sensitive cells at all doses of radiation used in these experiments (Figure 10A, B). These results demonstrated that these assays could be reliably used to determine the radiation sensitizing effects of drugs. We therefore used these two assays for all the experiments to determine the effects of 2-DG and/or HEDS in normal concentration of glucose.

The results from the experiments, using Coulter Counter assay, demonstrated that up to 20mM 2-DG did not sensitize either the p53 wild type or mutant cells to radiation. Figures 11A shows the effects of different doses of radiation (0, 1, 2, 4, 8 and 12Gy) on p53 wild type radiation sensitive HCT116 cells in the presence of 0, 5 and 20mM 2-DG. The results have demonstrated that 2-DG at concentrations as high 20mM and incubated for 24 hours did not affect the radiation response of this cell type suggesting that the 30% inhibition of glucose metabolism alone by 20mM 2-DG is not adequate to alter the radiation response of cells in the presence of normal concentrations of normal glucose. Similar effect was also observed in human colon cancer HT29 cells (Figure 12A).

Consistent with the Coulter counter assay, the CellCountEZ also showed no overall sensitizing effect of 2-DG in wild type radiation sensitive HCT116 cells at 5 mM 2-DG but with a slight sensitization at 20mM 2-DG at doses of radiation less than 4Gy (Figure 11B). In contrast, the p53 mutant cells did not exhibit better response to radiation at either 5mM or 20mM 2-DG, which is consistent with a lower decrease (30%) in the detoxification of HEDS by 2-DG (Figure 12B). Although these results seem to indicate some sensitizing effect by 2-DG alone, it is not significant enough to suggest that it is a potent radiation sensitizer to have any clinical significance.

We previously demonstrated that hydroxyethyl disulfide alone at 0.7 and 3.3mM sensitized both these cells to radiation in the absence of normal glucose with a corresponding decrease in non protein thiol, protein thiol and Ku protein binding. We therefore determined whether HEDS in combination with 2-DG could sensitize these cancer cells to radiation even in the presence of normal glucose since results showed a 40 and 20% decrease in the detoxification of HEDS in p53 wild type and mutant respectively. However, our results only demonstrated a slight sensitization for HCT116 cells at the highest concentration of 2-DG used in combination with 1mM HEDS by both these assays (Figure 13A, B). However, no sensitizing effect was observed in the p53 mutant cells which is consistent with a much lower decrease (20%) in the detoxification of HEDS by 20mM 2-DG in this cell type (Figure 14A, B).

We, therefore, tested the combined effect of 2-DG in combination with a higher concentration of 5mM HEDS to determine if p53 mutant cells can also be sensitized similar to that observed for p53 wild type cells at 1mM HEDS in combination with 2-DG. The results demonstrated no significant additional sensitization by 2-DG and 5mM HEDS in this cell type suggesting that 2-DG is not a potent glucose metabolic inhibitor to elicit sensitization by HEDS (Figure 15A, B).

Significance

The results from human colon cancer cells HCT116 and HT29 demonstrated that 2-DG is not a potent inhibitor of glucose metabolism since concentration as high as 20mM is required to get a

20 to 30% inhibition of glucose metabolism as measured by the bioreduction of HEDS. Although recently published results from our laboratory clearly demonstrated that altered glucose metabolism by glucose depletion in combination with HEDS is the best approach to improve the response of cancer cells to radiation, failure to exhibit similar efficacy by 2-DG only suggests that 2-DG is not a potent glucose metabolic inhibitor. These important findings in conjunction with our previously published reports emphasize the necessity of identifying potent glucose metabolic inhibitors to target cancer cells and to improve response of these cells to radiation and chemotherapeutic agents.

Conclusions

The projects carried out during this one year grant period have produced several important findings that will have significant impact in cancer therapy. First, we have shown that thiol redox regulated by glucose metabolism plays a major role in thiol redox modifier mediated radiation response. Second, we have demonstrated the importance of targeting glucose metabolism mediated thiol redox in cancer cells for improved response to radiation. Additionally, we have shown the potential mechanisms for the resistance of cancer cells to 2-DG mediated radiation sensitization. These findings also demonstrated the importance of identifying a better alternative to 2-DG.

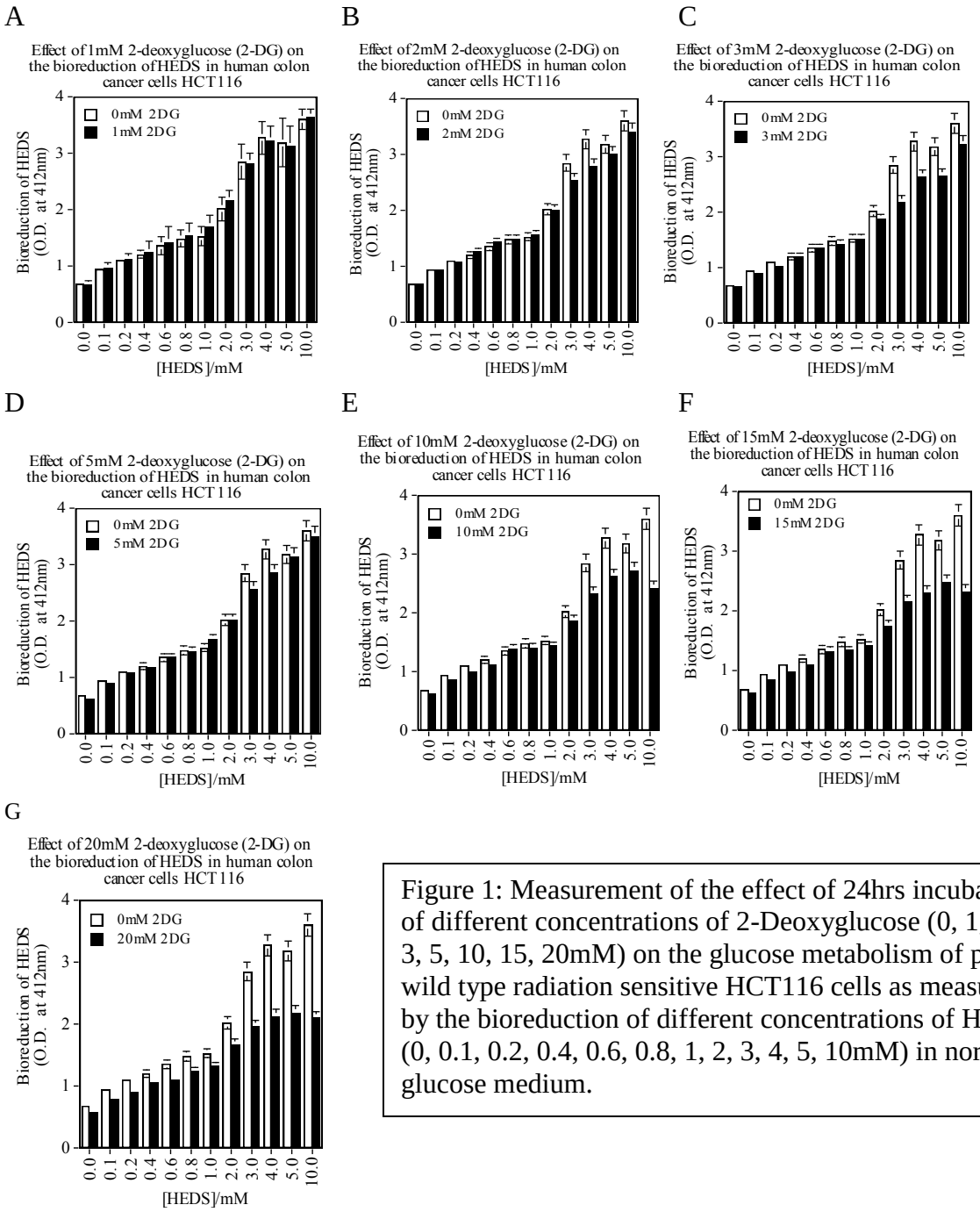

Figure 1: Measurement of the effect of 24hrs incubation of different concentrations of 2-Deoxyglucose (0, 1, 2, 3, 5, 10, 15, 20mM) on the glucose metabolism of p53 wild type radiation sensitive HCT116 cells as measured by the bioreduction of different concentrations of HEDS (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, 10mM) in normal glucose medium.

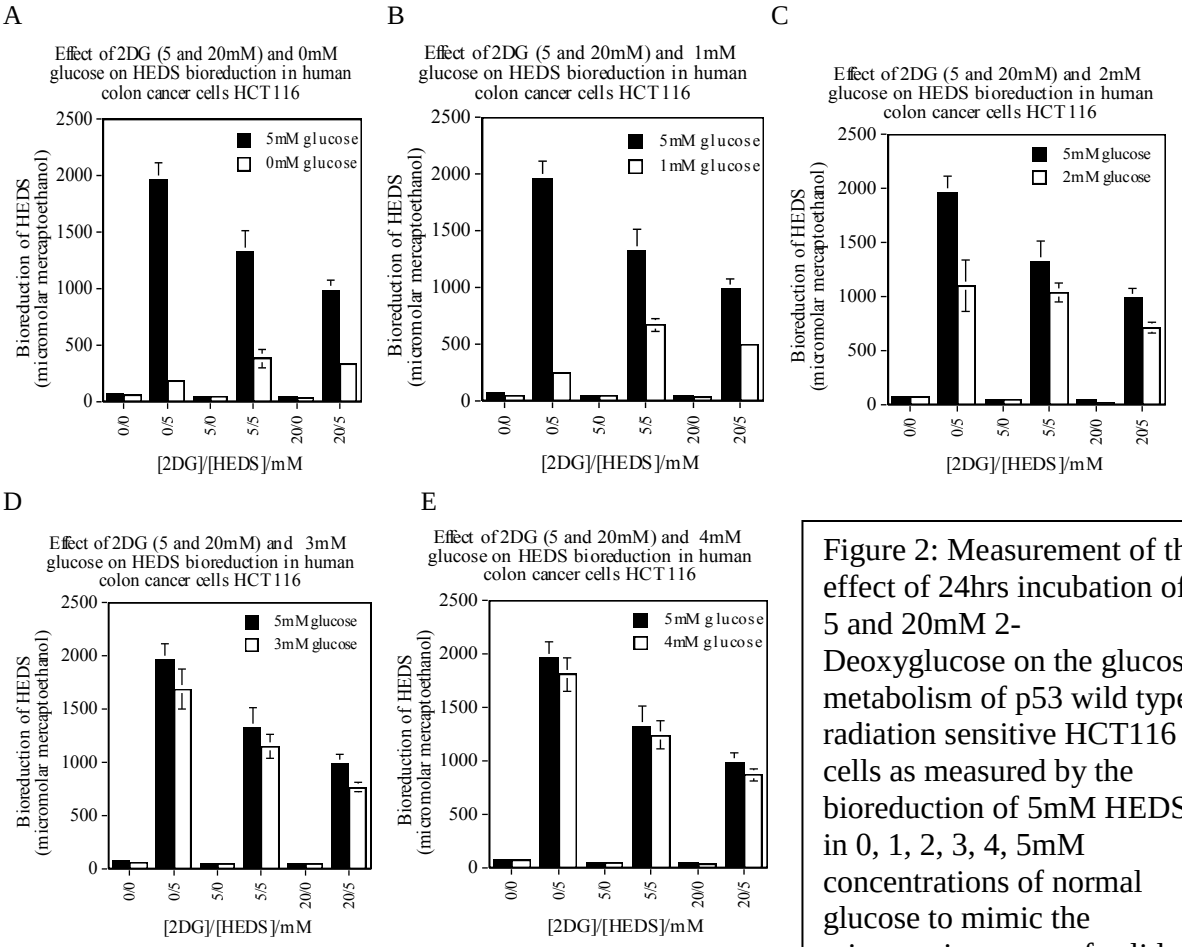

Figure 2: Measurement of the effect of 24hrs incubation of 5 and 20mM 2-Deoxyglucose on the glucose metabolism of p53 wild type radiation sensitive HCT116 cells as measured by the bioreduction of 5mM HEDS in 0, 1, 2, 3, 4, 5mM concentrations of normal glucose to mimic the microenvironment of solid tumors.

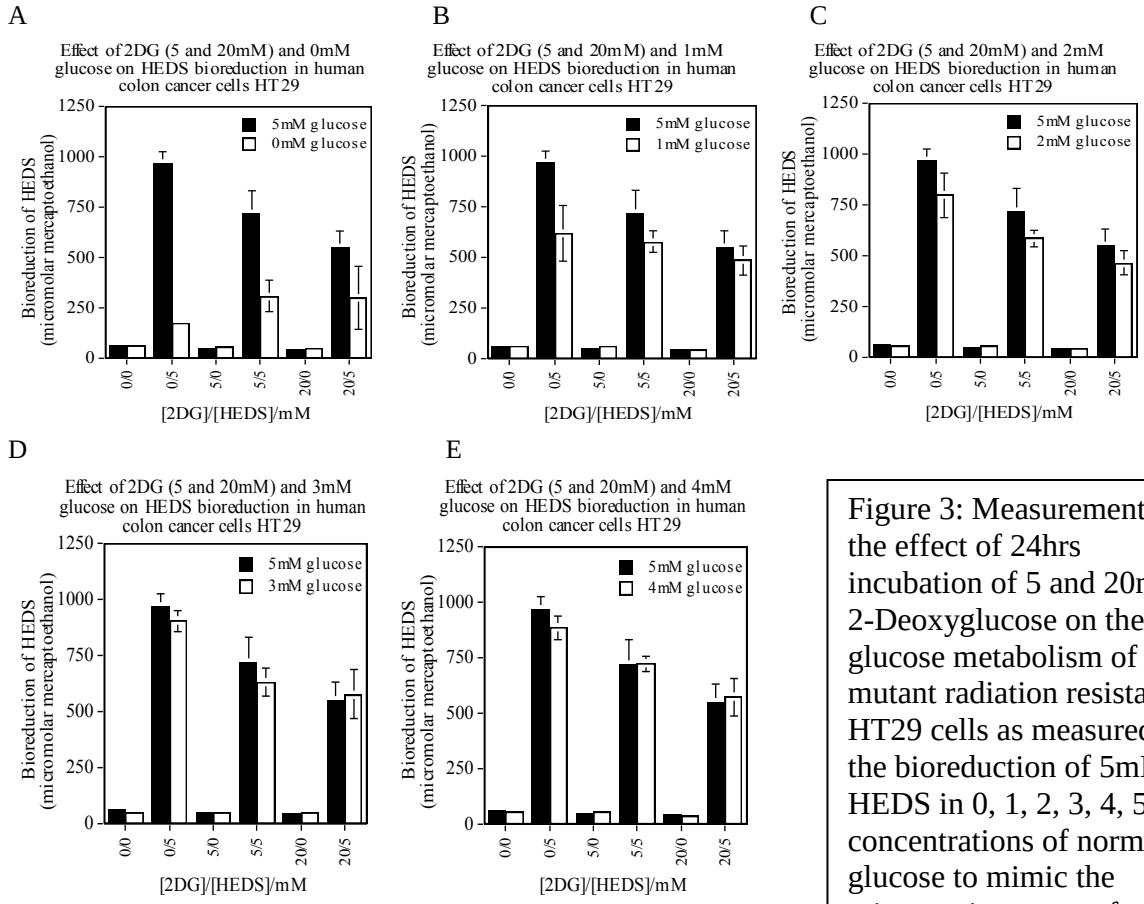

Figure 3: Measurement of the effect of 24hrs incubation of 5 and 20mM 2-Deoxyglucose on the glucose metabolism of p53 mutant radiation resistant HT29 cells as measured by the bioreduction of 5mM HEDS in 0, 1, 2, 3, 4, 5mM concentrations of normal glucose to mimic the microenvironment of solid tumors.

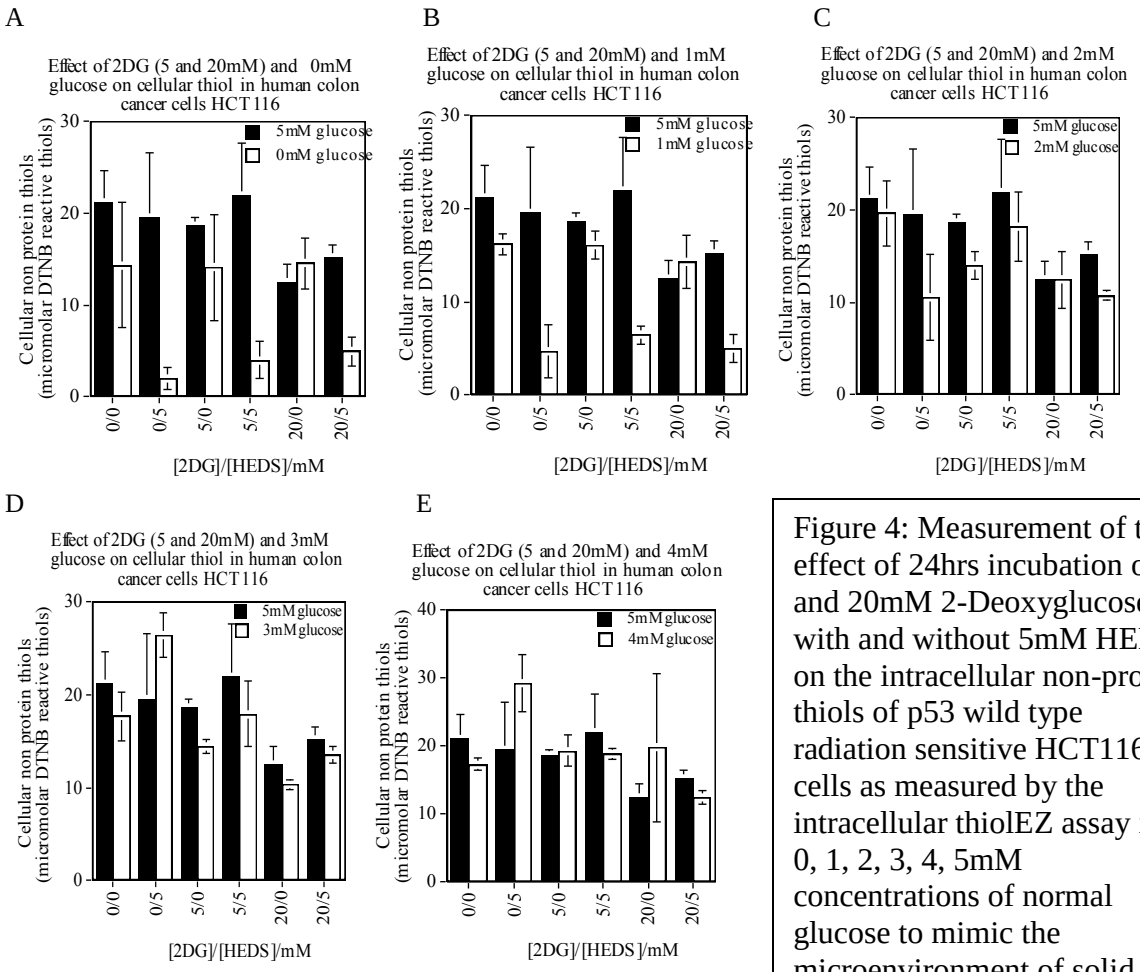

Figure 4: Measurement of the effect of 24hrs incubation of 5 and 20mM 2-Deoxyglucose with and without 5mM HEDS on the intracellular non-protein thiols of p53 wild type radiation sensitive HCT116 cells as measured by the intracellular thiolEZ assay in 0, 1, 2, 3, 4, 5mM concentrations of normal glucose to mimic the microenvironment of solid tumors.

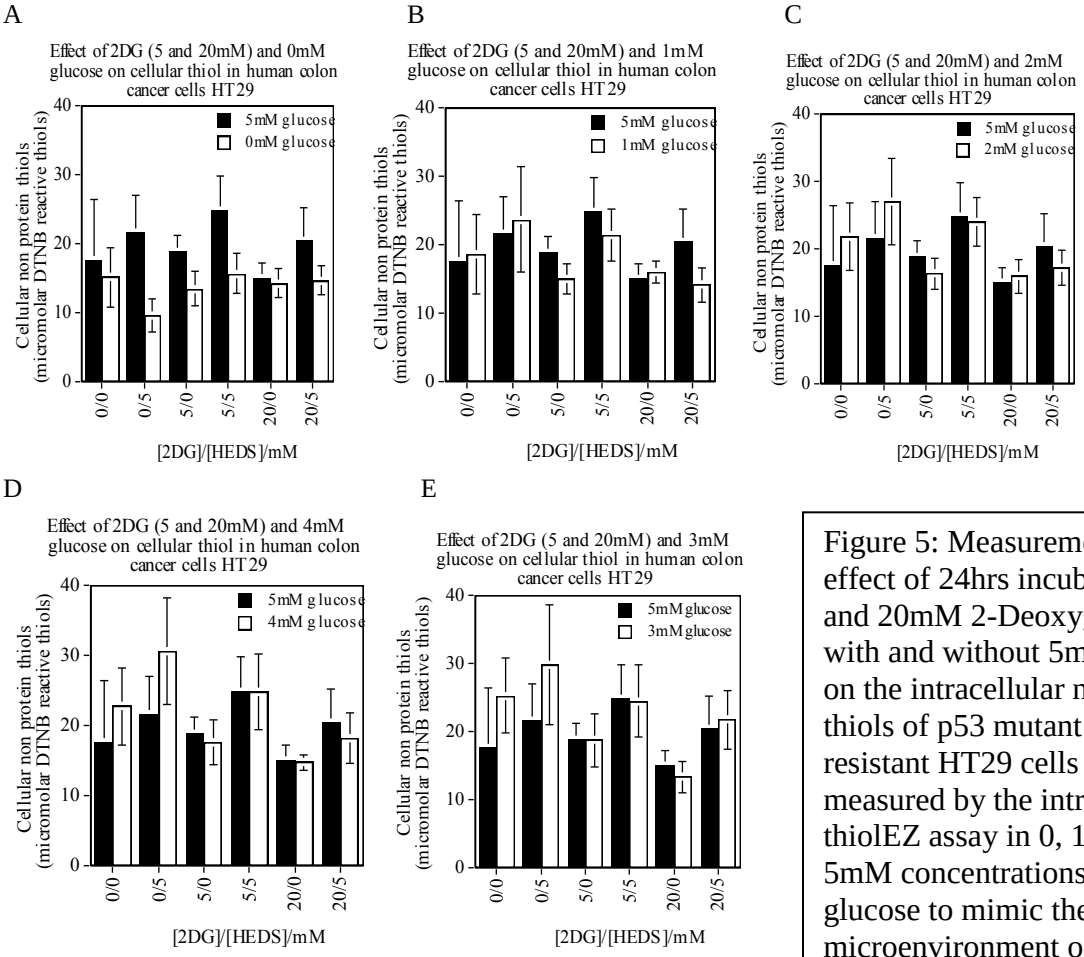

Figure 5: Measurement of the effect of 24hrs incubation of 5 and 20mM 2-Deoxyglucose with and without 5mM HEDS on the intracellular non-protein thiols of p53 mutant radiation resistant HT29 cells as measured by the intracellular thiolEZ assay in 0, 1, 2, 3, 4, 5mM concentrations of normal glucose to mimic the microenvironment of solid tumors.

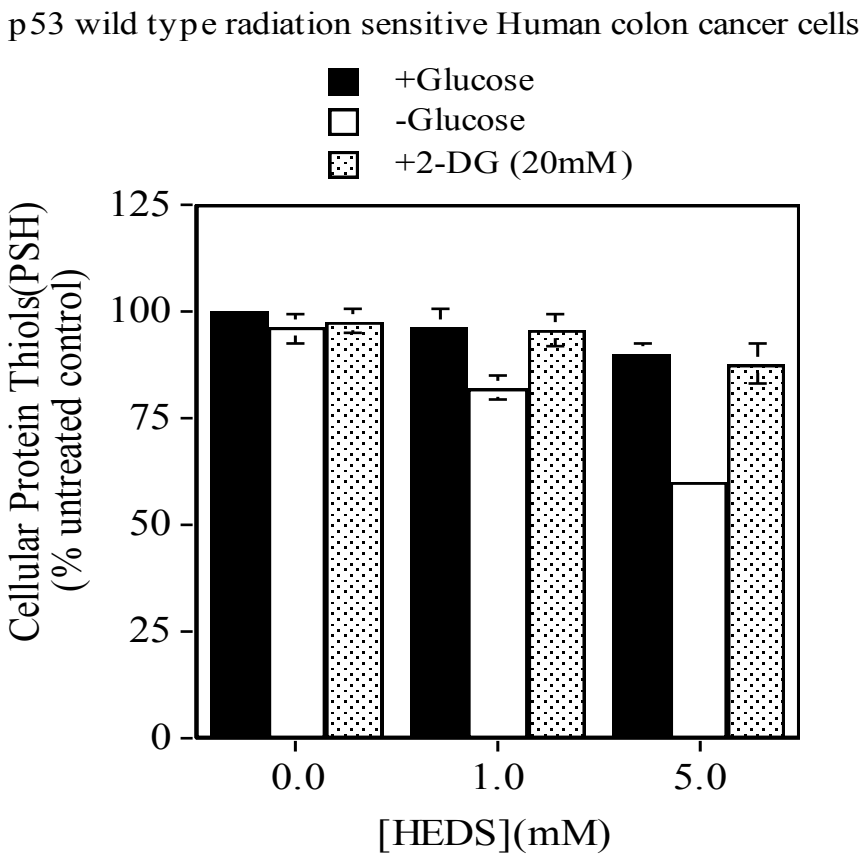

Figure 6: Measurement of the effect of 20mM 2-Deoxyglucose (24hrs incubation) with and without HEDS (1, 5mM, 2hrs incubation) on the intracellular protein thiols of p53 wild type radiation sensitive HCT116 cells in 0 (-Glucose) and 5mM (+Glucose) concentrations of normal glucose.

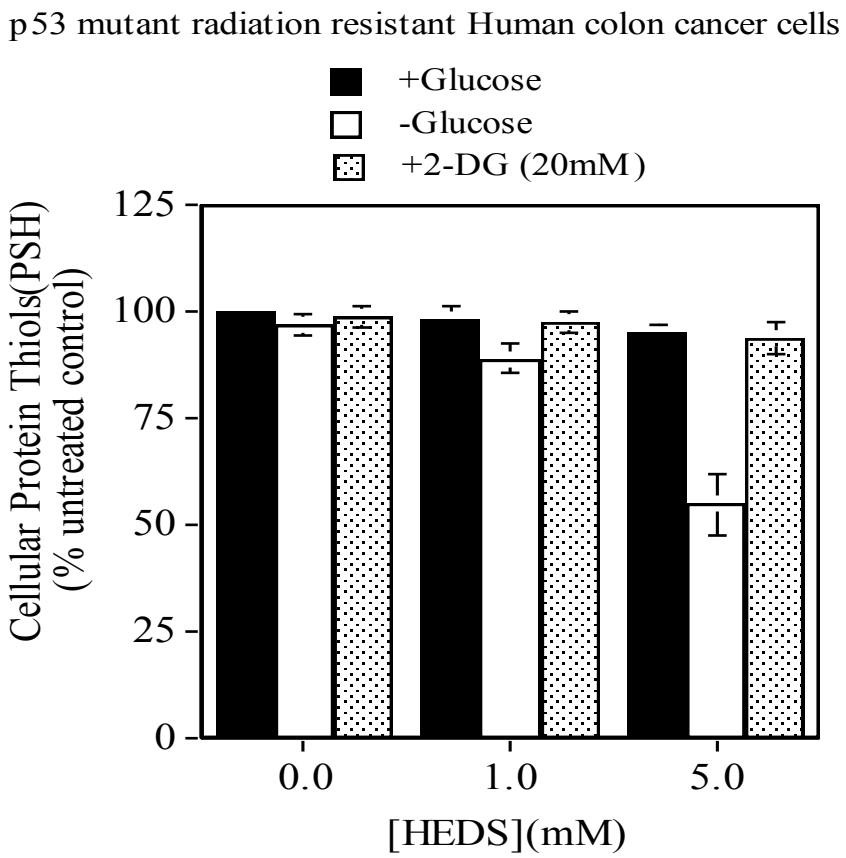

Figure 7: Measurement of the effect of 20mM 2-Deoxyglucose (24hrs incubation) with and without HEDS (1, 5mM, 2hrs incubation) on the intracellular protein thiols of p53 mutant radiation resistant HT29 cells in 0 (-Glucose) and 5mM (+Glucose) concentrations of normal glucose.

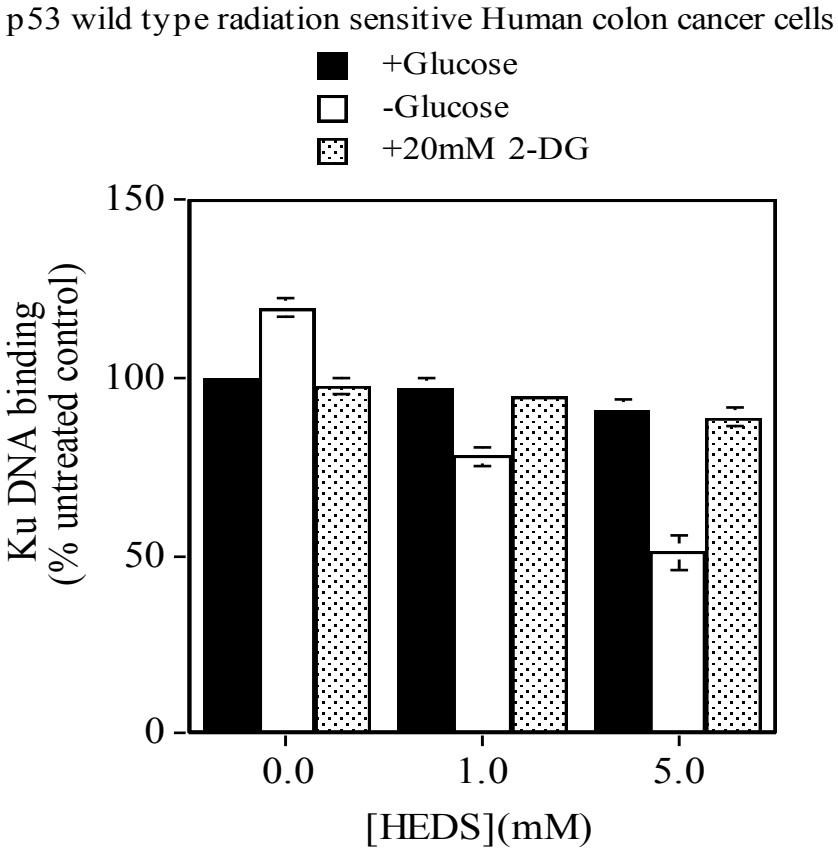

Figure 8: Measurement of the effect of 20mM 2-Deoxyglucose (24hrs incubation) with and without HEDS (1, 5mM, 2hrs incubation) on the DNA repair protein (Ku) function of p53 wild type radiation sensitive HCT116 cells in 0 (-Glucose) and 5mM (+Glucose) concentrations of normal glucose.

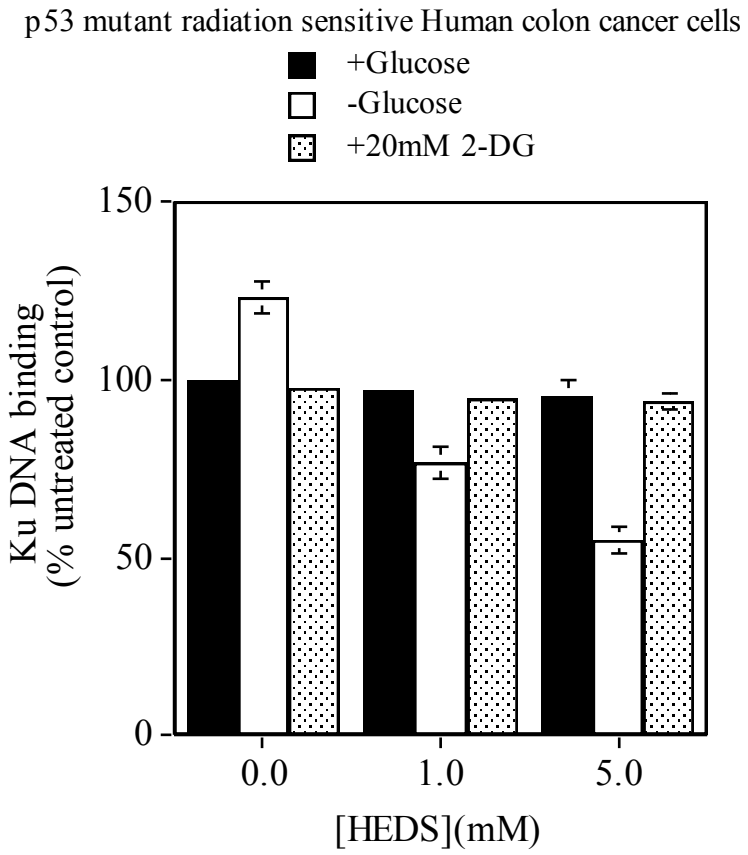

Figure 9: Measurement of the effect of 20mM 2-Deoxyglucose (24hrs incubation) with and without HEDS (1, 5mM, 2hrs incubation) on the DNA repair protein (Ku) function of p53 mutant radiation resistant HT29 cells in 0 (-Glucose) and 5mM (+Glucose) concentrations of normal glucose.

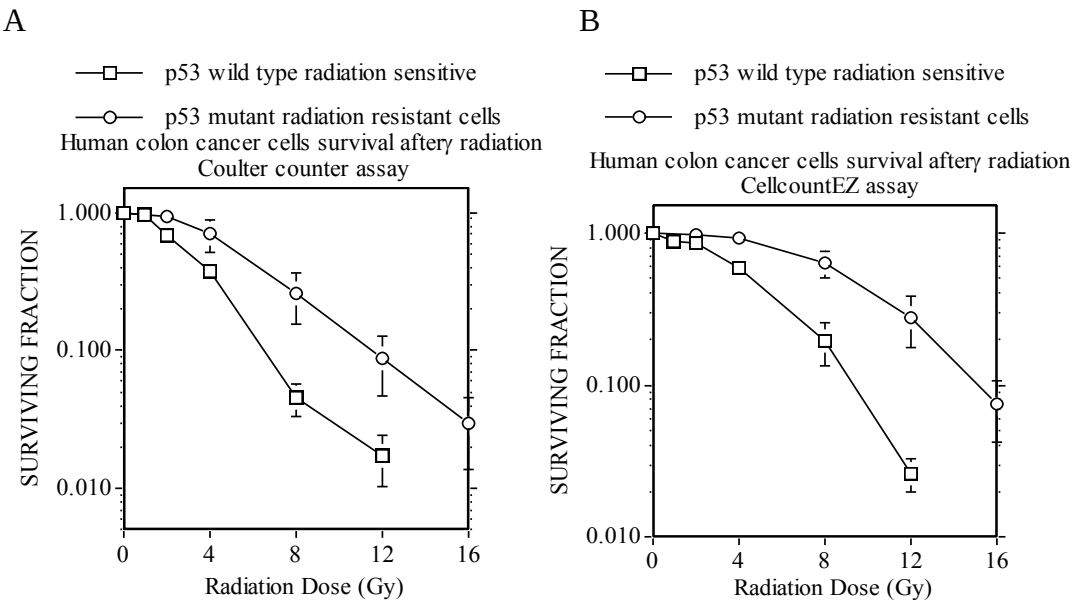

Figure 10: Application of CellCountEZ assay in comparison to the cell proliferation assay (Coulter Counter) to quantify the radiation response of p53 wild type radiation sensitive (HCT116) and p53 mutant radiation resistant (HT29) cells.

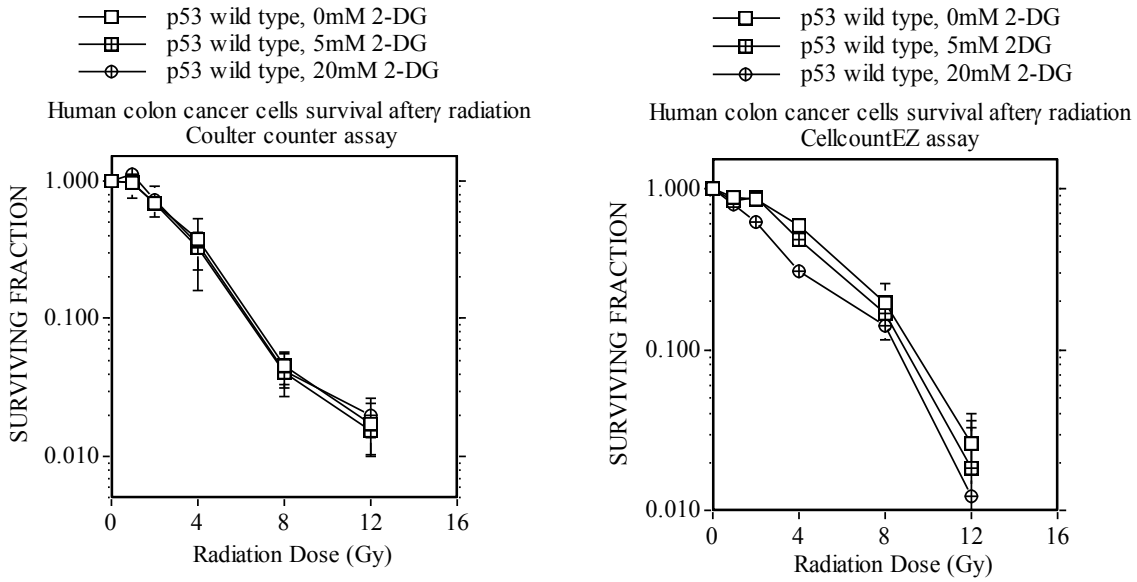

Figure 11: Effect of 24hrs incubation of 2-Deoxyglucose (0, 5 and 20mM) on the radiation response of p53 wild type radiation sensitive (HCT116) cells in normal growth medium containing normal concentration of normal glucose as measured by CellCountEZ and coulter counter assays.

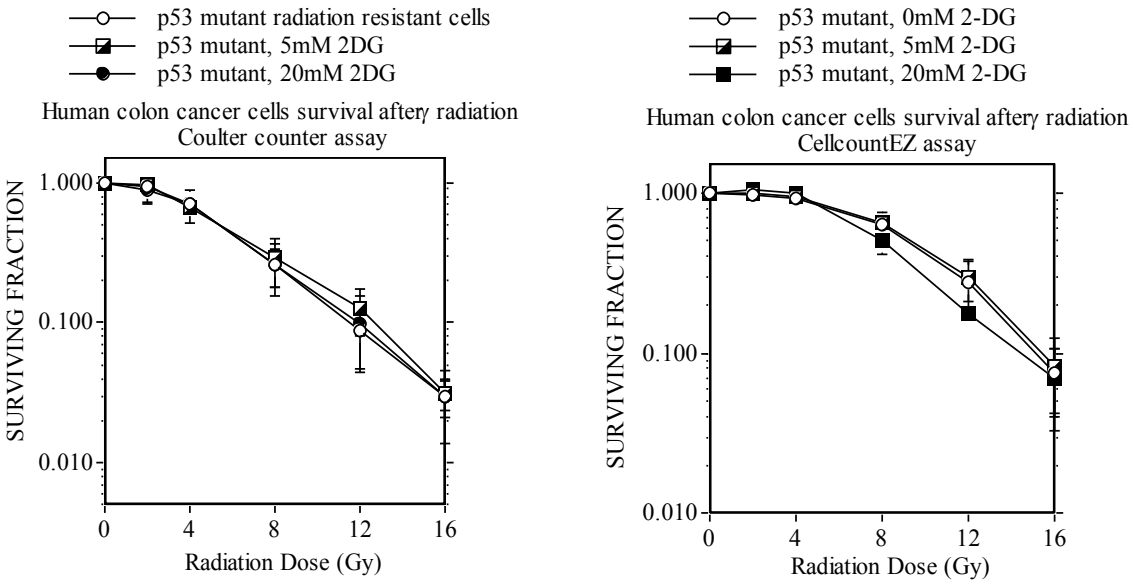

Figure 12: Effect of 24hrs incubation of 2-Deoxyglucose (0, 5 and 20mM) on the radiation response of p53 mutant radiation resistant (HT29) cells in normal growth medium containing normal concentration of normal glucose as measured by CellCountEZ and coulter counter assays.

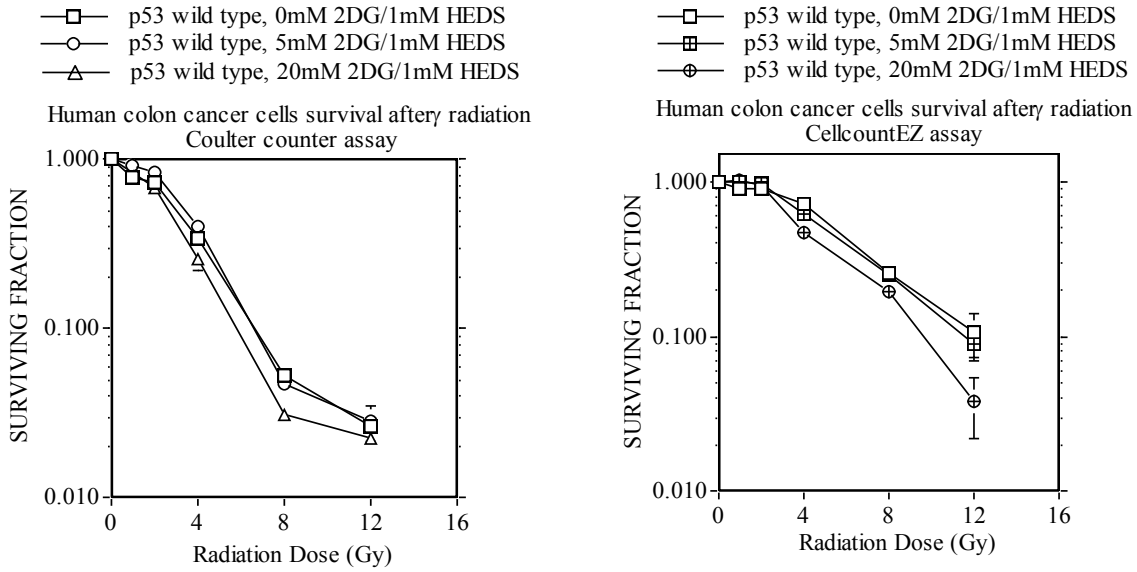

Figure 13: Effect of 24hrs incubation of 2-Deoxyglucose (0, 5 and 20mM) in combination with 2hrs pre- and 2hrs post- irradiation incubation with 1mM HEDS on the radiation response of p53 wild type radiation sensitive (HCT116) cells in normal growth medium containing normal concentration of normal glucose as measured by CellCountEZ and coulter counter assays.

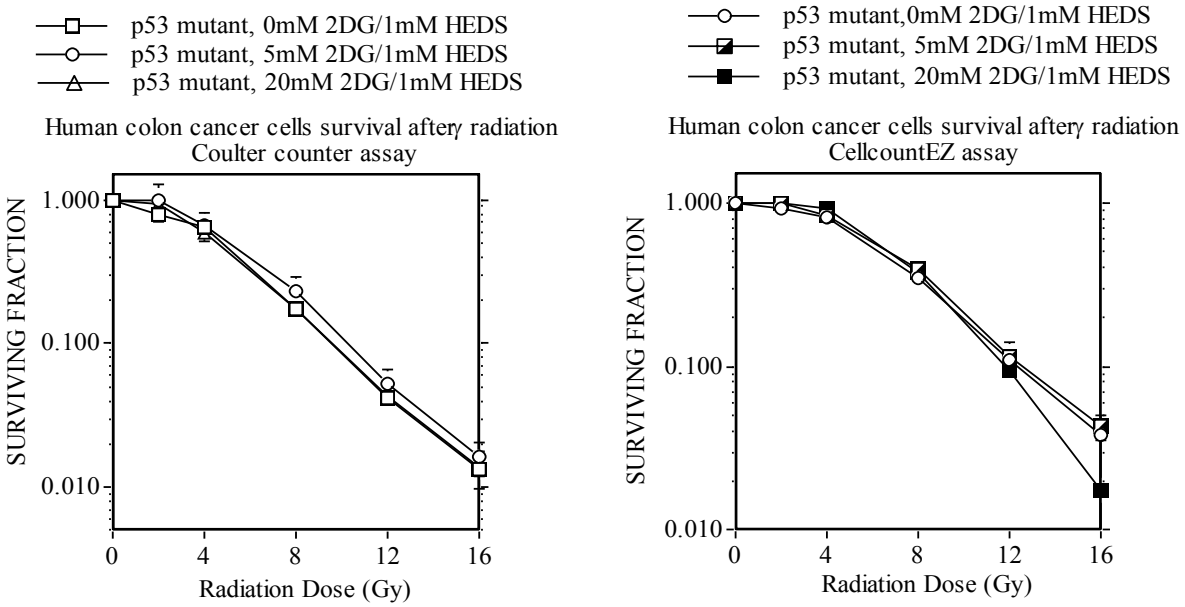

Figure 14: Effect of 24hrs incubation of 2-Deoxyglucose (0, 5 and 20mM) in combination with 2hrs pre- and 2hrs post- irradiation incubation with 1mM HEDS on the radiation response of p53 mutant radiation resistant (HT29) cells in normal growth medium containing normal concentration of normal glucose as measured by CellCountEZ and coulter counter assays.

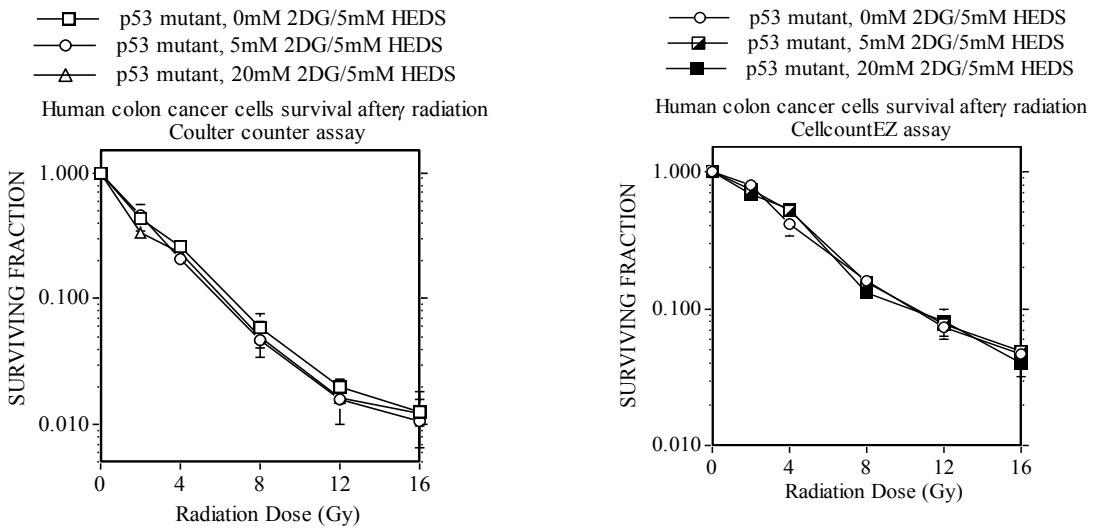

Figure 15: Effect of 24hrs incubation of 2-Deoxyglucose (0, 5 and 20mM) in combination with 2hrs pre- and 2hrs post- irradiation incubation with 5mM HEDS on the radiation response of p53 mutant radiation resistant (HT29) cells in normal growth medium containing normal concentration of normal glucose as measured by CellCountEZ and coulter counter assays.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics

_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include

the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. A bioactive probe of the oxidative pentose phosphate cycle: Novel strategy to reverse radioresistance in glucose deprived human colon cancer cells.	Li, Ward, Zhang, Dayanandam, DeNittis, Prendergast, Ayene	Toxicology in Vitro	May, 2012	☐Submitted ☐Accepted ☒Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

This research project for the first time identified that 2-DG is not a potent inhibitor of glucose metabolism and does not sensitize human colon cancer cells to radiation. It is an important finding since the use of this drug and further improvement of such strategy in cancer therapy has been hindered by the lack of conclusive evidence for the efficacy of 2-DG as a glucose metabolic inhibitor and radiation sensitizer. A novel test developed in our lab has enabled us to demonstrate that 2-DG is not a potent inhibitor of glucose metabolism or a radiation sensitizer. These findings will help the program officials at NIH and other agencies to allocate funds for the screening of better inhibitors of glucose metabolism for use in radiation and chemotherapy. Additionally, using this technology, we have also demonstrated that the antioxidant capacity of humans can be quantified using human blood. This finding is also very important since it could be used to determine the efficacy of compounds like 2-DG in humans.

The following papers will be submitted with in the next three to four months.

1. Li J, Ward KM, Zhang D, Jefferson P, Prendergast GC and Ayene IS: A novel bioactive probe to determine the validity of 2-deoxyglucose as a glucose metabolic inhibitor and radiation sensitizer: Potential for screening glucose metabolic inhibitors. (Will be submitted in May/June to Nature Methods).

2. Li J, Ward KM, Zhang D, Jefferson P, Wallon M, Gilman P, Prendergast GC and Ayene IS: A bioactive probe for glutathione dependent antioxidant capacity: Implications in measuring biological effects of chemotherapeutic agents in human blood and mammalian cells. (Will be submitted in March/April to Nature Methods).

3. Jefferson P, Li J, Ward KM, Zhang D, Prendergast GC and Ayene IS: OxPhos and CellCountEZ tests demonstrate that 2-deoxyglucose is not a potent glucose metabolic inhibitor nor a radiation sensitizer. (Will be submitted for presentation at the 2013 Radiation Research Society Annual Meeting).

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

2-DG is currently used in PET imaging of cancer patients and believed to have therapeutic benefits in improving the response of cancer cells to radiation and chemotherapy. Although this compound has been tested in tissue culture and tumor models, its efficacy in these models and cancer patients is not conclusive. The rationale and the strategy to use glucose metabolic inhibitor is relevant in targeting the tumor cells since the cancer cells rely on glucose for two important pathways (glycolysis and oxidative pentose phosphate cycle) that are required for proliferation, survival and resistance of cancer cells to therapy. Our study showed that the inconclusive evidence for 2-DG as a chemotherapeutic agent is not because these pathways are not important targets but rather due to the failure of this compound to inhibit glucose metabolism. Our new findings will redirect the scientific community to focus on screening and identifying potent glucose metabolic inhibitors for use in cancer therapy.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Iraimoudi S. Ayene, PhD
Current Position
2007-Present Associate Professor, Lankenau Institute for Medical Research, Wynnewood, PA

Selected peer-reviewed publications (out of 34 publications).

1. Li J, Ward KM, Zhang D, Jefferson P, Prendergast GC and Ayene IS: A novel bioactive probe to determine the validity of 2-deoxyglucose as a glucose metabolic inhibitor and radiation sensitizer: Potential for screening glucose metabolic inhibitors. (2013, Will be submitted in May/June to Nature Methods).
2. Li J, Ward KM, Zhang D, Jefferson P, Wallon M, Gilman P, Prendergast GC and Ayene IS: A bioactive probe for glutathione dependent antioxidant capacity: Implications in measuring biological effects of chemotherapeutic agents in human blood and mammalian cells. (2013, Will be submitted in March/April to Nature Methods).
3. Li J, Ward KM, Zhang D, Dayanandam E, DeNittis AS, Prendergast GC and Ayene IS: A bioactive probe of the oxidative pentose cycle: novel strategy to reverse radioresistance in glucose deprived human colon cancer cells. Toxicol. In Vitro, 27, 367-377, 2013.
4. Li J, Zhang D, Ward KM, Prendergast GC and Ayene IS: Hydroxyethyl disulfide as an efficient novel metabolic assay for cell viability in vitro. Toxicol. In Vitro, 26, 603-612, 2012.
5. Li J, Ayene R, Ward KM, Dayanandam E and Ayene IS: Glucose deprivation increases nuclear DNA repair protein Ku and resistance to radiation induced oxidative stress in human cancer cells. Cell Biochem. Funct., 27, 93-101, 2009.
6. Ayene IS, Biaglow JE, Kachur AV, Stamato TD and Koch CJ: Mutation in G6PD gene leads to loss of cellular control of protein glutathionylation: Mechanism and Implication. J Cell Biochem., 103, 123-135, 2008.

7. Tuttle SW, Maity, A, Oprysko PR, Kachur AV, Ayene IS, Biaglow JE, and Koch CJ: Detection of reactive oxygen species via endogenous oxidative pentose phosphate cycle activity in response to oxygen concentration: Implications for the mechanism of HIF-1α stabilization under moderate hypoxia. J. Biol. Chem., 282, 36790-36796, 2007.
8. Ayene IS, Koch CJ and Krisch RE: DNA strand breakage by bivalent metal ions and ionizing radiation. Int. J Radiat. Biol., 83, 195-210, 2007.
9. Biaglow JE, Ayene IS, Tuttle SW, Koch CJ, Donahue J and Mieyal JJ: Role of Vicinal Protein Thiols in Radiation and Cytotoxic Responses. Radiat. Res. 165, 307–317, 2006.
10. Ayene IS, Ford LP and Koch CJ: Ku protein targeting by Ku70 small interfering RNA enhances human cancer cell response to topoisomerase II inhibitor and γ□ radiation. Mol. Cancer Ther. 4(4), 529-536, 2005.
11. Biaglow JE, Ayene IS, Koch CJ, Donahue J, Stamato TD, Mieyal JJ and Tuttle SW: Radiation response of cells during altered protein thiol redox. Radiat. Res., 159, 484-494, 2003.
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13. Ayene IS, Koch CJ, Tuttle SW, Stamato T, Perez ML and Biaglow JE: Oxidation of cellular thiols by hydroxyethyldisulfide inhibits DNA double strand break rejoining in G6PD deficient mammalian cells. Int. J. Radiat. Biol. 76, 1523-1531, 2000.
14. Biaglow JE, Ayene IS, Koch CJ, Donahue J, Stamato T and Tuttle SW: G6PD deficient cells and the bioreduction of disulfides: Effects of DHEA, GSH depletion and phenylarsine oxide. Biochim. Biophys. Res. Comm. 273, 846-852, 2000.
15. Ayene IS**,** Bernhard EJ, Muschel RJ, McKenna WG, Krisch RE and Koch CJ: DNA as an important target in radiation induced apoptosis of myc and myc plus ras oncogene transfected cells. Int. J. Radiat. Biol., 76, 343-355, 2000.
16. Biaglow JE, Koch CJ, Tuttle S, Manevich Y, Ayene IS, Bernhard EJ, McKenna WG, and Kachur A: The measurement of bioreductive capacity of tumor cells using methylene blue. Int. J. Radiat. Oncol. Biol. Phys., 42, 769-773, 1998.
17. Ayene IS, Sullivan M, Krisch RE, and Koch CJ: Protein derived radicals as sensitizers of radiation induced single and double strand DNA breaks. Radiat. Res. 148: 499-500, 1997.
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19. Zhao G, Ayene IS and Fisher AB: Role of iron in ischemia reperfusion oxidative injury of rat lungs. Am. J. Respir. Cell Mol. Biol. 16: 293-299, 1997.
20. Ayene IS, Koch CJ, and Krisch RE: Simulation of the cellular oxygen effect with an in vitro model system using DNA strand breaks as an endpoint. Radiat. Res. 146: 501-509, 1996.
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23. Krisch RE, Ayene IS, Koch CJ: Interaction of Thiol and Non-Thiol Scavengers in the Modification of Radiation-Induced DNA Damage. In: Radiation Damage in DNA: Structure/Function Relationships at Early Times. (Fuciarelli, AF, & Zimbrick, JD, Eds.), Battelle Press, Ohio, pp. 383-391, 1995.

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Ongoing Research Support

1. R03 CA 159315 7/1/12 - 6/30/14
National Cancer Institute
OPPC targeting to improve pancreatic cancer treatment
Role: Principal Investigator (MPI with Dr. George Prendergast)
The objective is to test the therapeutic potential of hypoxin, a lead compound to exploit the depletion of glucose and oxygen in tumor microenvironment of pancreatic cancer. The hypothesis is that tumor cells deficient in glucose and oxygen will have a decreased level of the oxidative pentose phosphate cycle, which would render such cells selectively sensitive to hypoxin and related compounds.

Completed

1. R01 CA 109604 4/1/05 – 2/28/11
National Cancer Institute
"Oxidative Pentose Cycle in Hypoxic Cancer Cell Response"
Role: Principal Investigator
The objective is to test the role of oxidative pentose cycle in the response of hypoxic cancer cells to radiation, etoposide and doxorubicin. Towards this goal, we will use molecular and biochemical approaches to target oxidative pentose cycle in various cancer cells.

2. R01 (CA92108) 12/1/02-11/30/07
National Cancer Institute
"Oxidative damage to DNA repair pathways"
Role: Co-Investigator
Principal Investigator: Cameron J. Koch, Ph.D., University of Pennsylvania
The objective is to test the effects of oxidative damage to DNA repair pathways in normoxic G6PD deficient CHO cells.