

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): 01/01/2009 to 12/31/2009**
3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Tam Mai-Nguyen**
4. **Grant Contact Person’s Telephone Number: 484-476-2755**
5. **Grant ME Number or SAP Number: 4100047637**
6. **Project Number and Title of Research Project: Project 1, Arsenic Drugs and Hydroxyethyl Disulfide in the Control of Cancer Cell Growth and Response to Therapy**
7. **Start and End Date of Research Project: 01/01/2009 to 12/31/2009**
8. **Name of Principal Investigator for the Research Project: Iraitmoudi S. Ayene, PhD**
9. **Research Project Expenses.**

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

\$111,906.68 (DC: \$67,862.18; IC: \$44,044.50)

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).

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 _____ No X _____

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:
	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> Nonfederal source (specify: _____)		\$	\$
	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> Nonfederal source (specify: _____)		\$	\$

	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> Nonfederal source (specify: _____)		\$	\$
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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 R01 grants using the data/results generated from the projects funded by the Health Research Grants.

First grant application is to test this approach in improving radiation and chemotherapies in leukemia and breast tumor models in mouse.

The second grant application is to determine the additional mechanisms of arsenicals induced tissue injury in human normal cells.

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

We will now test this approach in leukemia and breast tumor models in mouse.

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				
Unknown				
Total	1			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1			

Unknown				
Total	1			

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			
Black				
Asian				
Other				
Unknown				
Total	1			

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

Yes _____ No _____

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 _____ No _____

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

I joined the Lankenau Institute for Medical Research in September, 2006. This health research project helped me to continue my laboratory research in novel drug discovery for the improvement of cancer therapy. Further, the results generated from this project will help me to submit two new NIH R01 grants. One is in collaboration with Dr. Thomas Stamato, LIMR Professor, which will be submitted in June, 2010. The other one is in collaboration with a biotech company that will be submitted in October, 2010.

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 _____ No _____

If yes, please describe the collaborations:

Dr. Albert DeNittis, Chief of Radiation Oncology, Lankenau Hospital, has become a collaborator because of the health research funds. He is in charge of the projects that deal with the improvement of radiation therapy.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. 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 (β) should not print as boxes (\square) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project goals, objectives and specific aims as contained in the grant application's strategic plan:

Arsenic trioxide (Trisenox) is a Food and Drug Administration (FDA) approved drug for treatment of acute promyelocytic leukemia in the US. This compound has shown significant improvement in the outcome of leukemia patients (1-6). The efficacy of arsenic trioxide as an anti cancer drug for other types of cancer may be limited due to its detoxification by intracellular glutathione (7-12). Our preliminary results have demonstrated that hydroxyethyl disulfide (HEDS), a non toxic oxidant, increases the response of glucose deprived cancer cells to radiation and certain chemotherapeutic agents by oxidizing glutathione (GSH). HEDS has no significant effect in cells with functioning oxidative pentose cycle, which uses glucose to detoxify HEDS (13, 14). 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 response) improvement in the response of breast tumor xenograft to etoposide. Although a significant percentage of cancer cells in solid tumor may be glucose deprived, which can be targeted by HEDS, increasing the vulnerability of the rest of the tumor cells that contains glucose to HEDS will improve tumor response to cancer therapy. We have previously demonstrated that phenylarsine oxide (PAO), another arsenic compound, inhibits the detoxification of HEDS by rodent cells (15). In this project, we will determine the combined use of non toxic HEDS and arsenic compounds that includes but is not limited to the FDA approved arsenic trioxide (As_2O_3) in controlling the growth and response of cancer cells to chemotherapeutic agents and radiation.

Hypotheses to be tested:

We hypothesize that arsenic compounds in combination with HEDS will inhibit the function of DNA repair protein Ku and glutathione, which are responsible for radiation and cisplatin resistance, in human cancer cells. Additionally, this combined treatment with arsenic compounds and HEDS will improve the response of human cancer cells to radiation and chemotherapeutic agents.

Specific aims related to the above Hypotheses:

Specific Aim 1: In this specific aim, we will determine whether arsenic compounds are effective in inhibiting the detoxification of HEDS by human cancer cells. This will be tested in human cancer cells for various concentrations of arsenic compound on the detoxification 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 arsenic compounds are effective in increasing HEDS mediated oxidation of GSH and proteins, and loss of Ku protein function. This will be tested in human cancer cells for various concentrations of arsenic compound on the

oxidation of GSH by 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 will also be determined by assays routinely used in our lab.

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

PROJECTS CARRIED OUT FOR SPECIFIC AIM 1:

In specific Aim 1, we proposed to determine whether arsenic trioxide is effective in inhibiting the detoxification of HEDS by various human cancer cells. We have selected to focus on two of the most commonly used human colon cancer cells (HCT116, HT29) to determine the impact of our approach not only on cells with wild type p53 (HCT116) but also on cells with mutant p53 (HT29), which is found in more than 50% of human tumors. The advantages of using these cells are that they are both isolated from colon but with different p53 status and radiation response. HT29 cells are more resistant than HCT116 to radiation and certain chemotherapeutic agents.

We have completed experiments to determine the effects of arsenic trioxide on the detoxification of HEDS in p53 wild type HCT116 and p53 mutant HT29 cells. HEDS is a disulfide dimer of the monomer sulfhydryl compound mercaptoethanol. Disulfides are known to oxidize protein and non protein thiols in cells. Mammalian cells convert HEDS, a thiol oxidant, into mercaptoethanol, a non toxic reductant. The detoxification of HEDS is carried out by most mammalian cells using the oxidative pentose cycle. We have used radiation sensitive p53 wild type (HCT116) and resistant p53 mutant (HT29) human colon cancer cells in vitro to determine the effect of different concentrations of arsenic trioxide on HEDS. Cells grown in a 60 mm dish with normal *DMEM*, 10% FBS, 1% penicillin/streptomycin (P/S) and 20 mM HEPES were treated with different concentrations of arsenic compounds (As_2O_3). The arsenic compound treated cells were then incubated with HEDS. A stock solution of 0.1M HEDS was prepared fresh in glucose free *DMEM* growth medium. HEDS exposure was effected by replenishing cells with 1 (six well plate) and 1.5ml (60mm dishes) of fresh *DMEM* growth medium with glucose, 10% FBS, HEPES and desired concentrations of HEDS. Cells treated with and without HEDS were cooled on ice. To quantify the bioreduction/ME production (detoxification), 0.5 ml of extracellular medium was mixed with 0.5 ml of 100 mM sulfosalicylic acid (SSA) lysis buffer in microfuge tubes and centrifuged at high speed in a Fisher 59A microfuge. These samples were used for the determination of mercaptoethanol produced from the detoxification of HEDS in these cells. Our results have demonstrated that arsenic trioxide inhibited the detoxification of HEDS in both p53 wild type and mutant colon cancer cells (Figures 1 and 2).

PROJECTS CARRIED OUT FOR SPECIFIC AIM 2:

As part of specific aim 2, we proposed to determine whether arsenic trioxide is effective in increasing HEDS mediated oxidation of glutathione (GSH). The oxidation of protein thiols

(PSH) and loss of function of DNA repair protein (Ku) was determined in HCT116 and HT29. During the funding period, we determined the efficacy of arsenic trioxide and HEDS in the modification of GSH in HCT116 and HT29 cells. To quantify the intracellular non protein thiols GSH, the extracellular medium was removed after the incubation of cells with HEDS 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. The intracellular extract was individually transferred to microfuge tubes and centrifuged at high speed in a Fisher 59A microfuge. The supernatant of these samples was used for the quantification of GSH using Ellman's reagent. The results have demonstrated that arsenic trioxide in combination with HEDS decreased GSH in HCT116 and HT29 cells (Figures 3 and 4).

For quantification of PSH, Cancer cells attached to the dishes were incubated with different concentrations of arsenic compound 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 NPSH were washed off the cells. Acid-fixed cells was 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 37° and the absorbancy 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 arsenic trioxide in combination with HEDS increased the oxidation of PSH in HCT116 and HT29 cells (Figure 5).

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 -80°C. 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 4 min, mixed with stop solution and the O.D was read in a microplate reader at 450nm with a reference wavelength of 655nm. The results have demonstrated that arsenic trioxide together with HEDS inhibited the function of Ku protein in both HCT116 and HT29 cells (Figure 6).

The results have demonstrated that arsenic trioxide in combination with HEDS decreased GSH in two different (p53 wild type and mutant) types of human colon cancer cells. Additionally, our studies have also demonstrated that the combined effects of HEDS and arsenic trioxide decreased the total protein thiols and inhibited the function of Ku protein binding to DNA in both HCT116 and HT29 cells.

PROJECTS CARRIED OUT FOR SPECIFIC AIM 3:

In specific aim 3, we proposed to determine whether arsenic trioxide in combination with HEDS improves the response of colon cancer cells to cisplatin and γ radiation. The results have demonstrated that arsenic trioxide in combination with HEDS improved the response of HCT116

and HT29 cells to cisplatin and radiation (Figures 7 - 15). The results have also demonstrated that HEDS improved the response of HCT116 and HT29 cells to arsenic trioxide suggesting that HEDS can regulate arsenic trioxide mediated cytotoxicity in cancer cells.

C. Significance

The results from human cancer cells HCT116 and HT29 demonstrated that arsenic trioxide in combination with HEDS can be used to sensitize colon cancer cells to cisplatin and radiation.

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 had shown that arsenic trioxide is more effective in cancer cells when treated in combination with HEDS. Second, our results showed a significant improvement in the response of human cancer cells to radiation when combined with arsenic trioxide and hydroxyethyl disulfide. Third, our results showed better response of human cancer cells to cisplatin when combined with arsenic trioxide and hydroxyethyl disulfide. Additionally, our studies have also demonstrated that phenylarsine oxide, another arsenic compound, was highly toxic (results not shown) compared to arsenic oxide and hence may not be useful for cancer therapy. Most importantly, this approach is not only effective in p53 wild type cells but also for p53 mutant cells, which are known to present in more than 50% of the tumors and are resistant to various therapies.

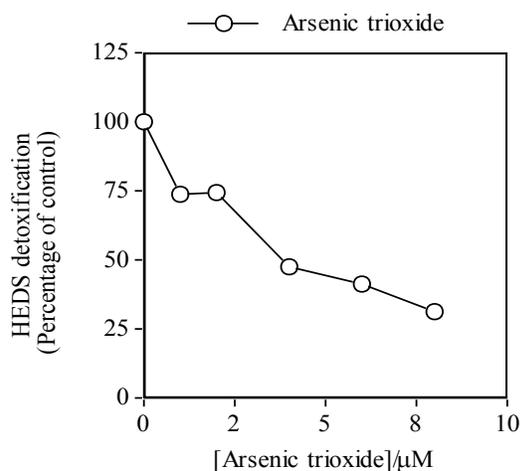


Figure 1. Quantification of detoxification of HEDS by p53 wild type HCT116 human colon cancer cells by medium based assay that measures the conversion of HEDS into a non-toxic mercaptoethanol (ME). These results have demonstrated that arsenic trioxide inhibited the detoxification of HEDS up to 70% of the untreated control at concentrations as low as 8 μM.

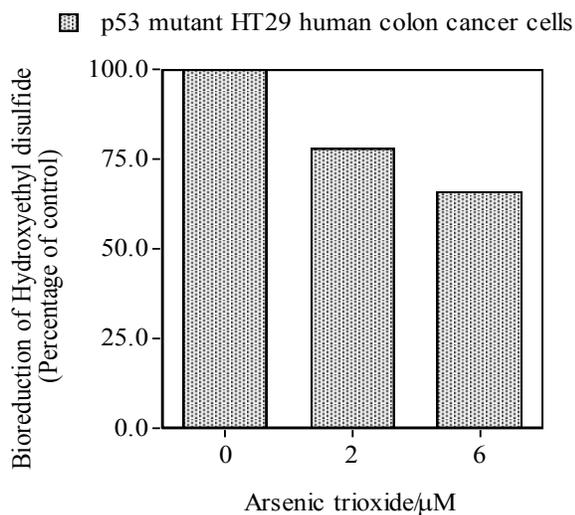


Figure 2. Quantification of detoxification of HEDS by p53 mutant HT29 human colon cancer cells by medium based assay that measures the conversion of HEDS into a non-toxic mercaptoethanol (ME). These results have demonstrated that arsenic trioxide inhibited the detoxification of HEDS up to 40% of the untreated control at concentrations as low as 6 μM.

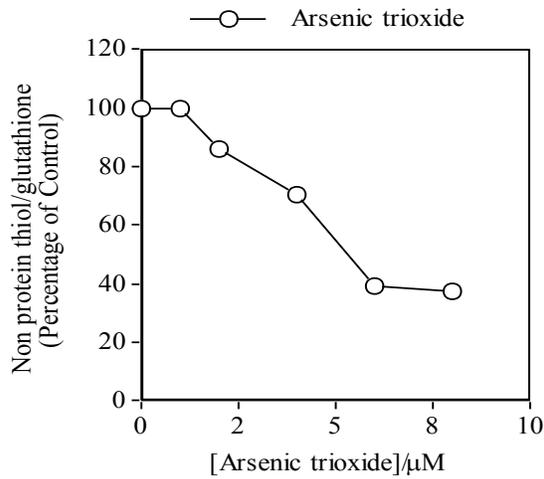


Figure 3. Quantification of intracellular dithiobis nitrobenzoic acid reactive non-protein thiols in p53 wild type HCT116 human colon cancer cells treated with different concentrations of arsenic trioxide in combination with HEDS. The results have demonstrated that arsenic trioxide in combination with HEDS decreased non protein thiols in HCT116 cells up to 60% at concentrations as low as 8 μM .

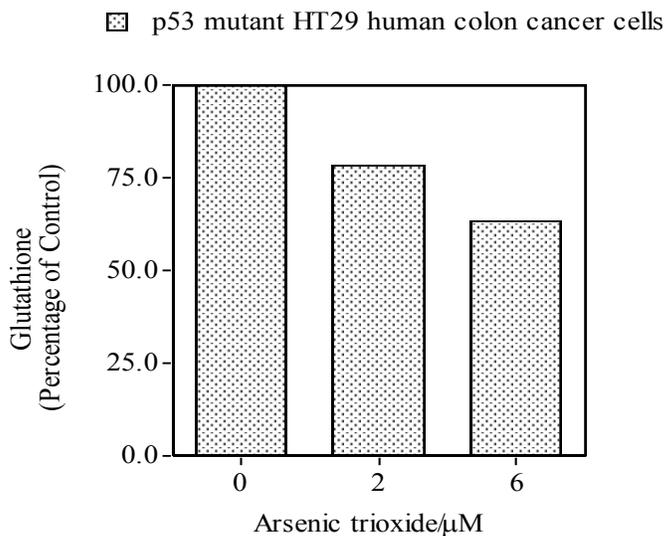


Figure 4. Quantification of intracellular dithiobis nitrobenzoic acid reactive non-protein thiols in p53 mutant HT29 human colon cancer cells treated with different concentrations of arsenic trioxide in combination with HEDS. The results have demonstrated that arsenic trioxide in combination with HEDS decreased non protein thiols in HT29 cells up to 40% at concentrations as low as 6 μM .

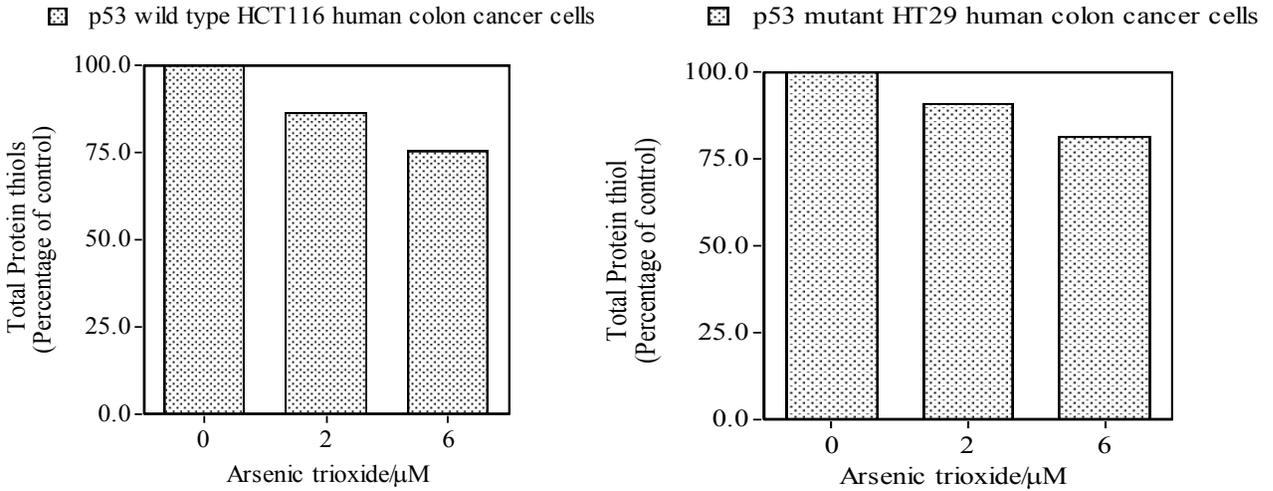


Figure 5. Quantification of intracellular protein thiols in p53 wild type HCT116 and p53 mutant HT29 human colon cancer cells treated with different concentrations of arsenic trioxide in combination with HEDS. The results have demonstrated that arsenic trioxide in combination with HEDS decreased non protein thiols in both these cells up to 30% at concentrations as low as 6μM.

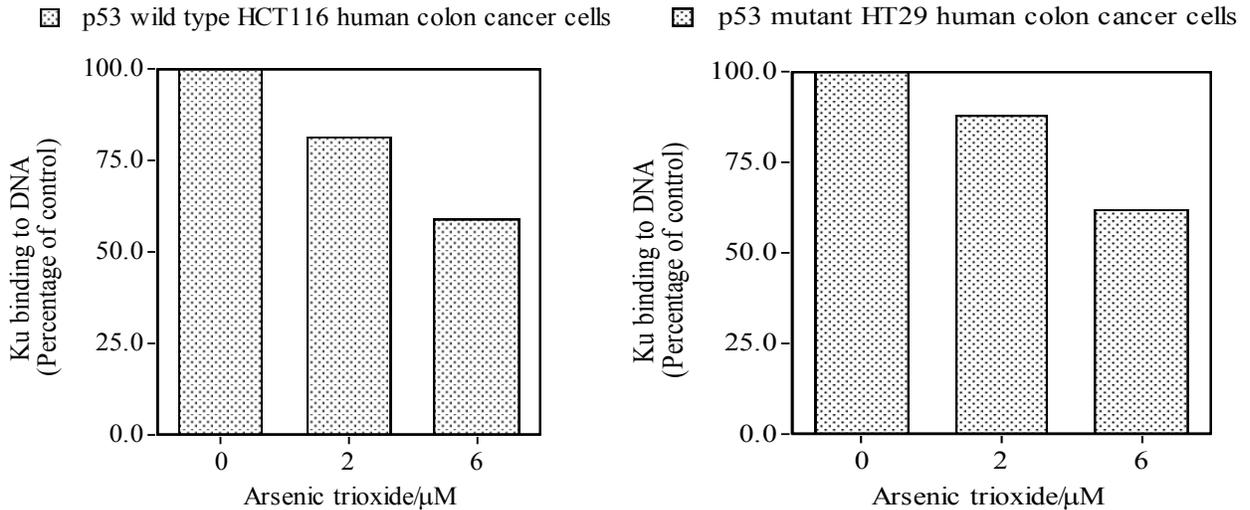


Figure 6. Quantification of Ku protein function in p53 wild type HCT116 and p53 mutant HT29 human colon cancer cells treated with different concentrations of arsenic trioxide in combination with HEDS. The results have demonstrated that arsenic trioxide in combination with HEDS decreased Ku protein function in both these cells up to 40% at concentrations as low as 6μM.

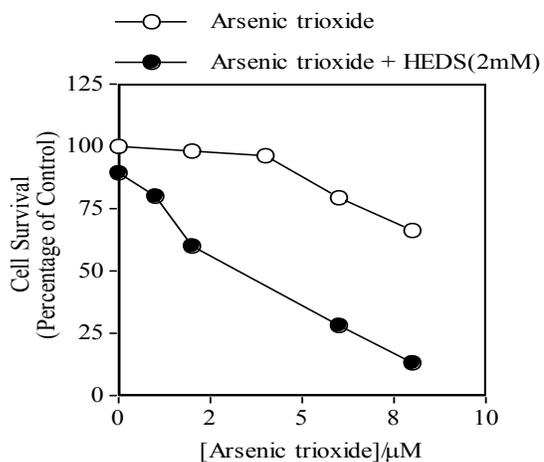


Figure 7. Effects of HEDS (2mM) and arsenic trioxide (0-8 μM) on the growth of HCT116 human colon cancer cells as measured by cell proliferation assay. The results have demonstrated that HEDS improved the response of HCT116 cells from 2 to 3 fold at all concentrations of arsenic trioxide.

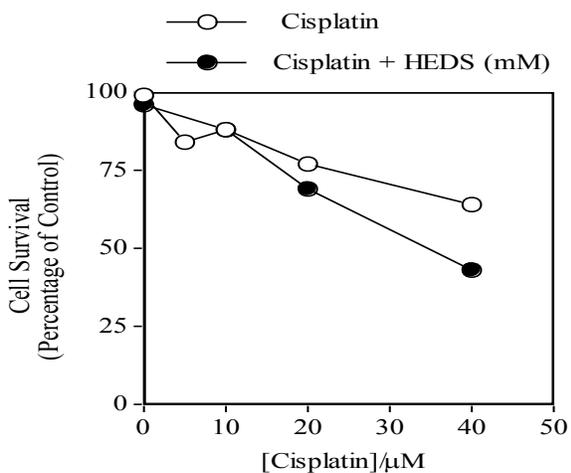


Figure 8. Effects of HEDS (2mM) and cisplatin (0-40 μM) on the growth of HCT116 human colon cancer cells as measured by cell proliferation assay. The results have demonstrated that HEDS did not improve the response of HCT116 cells to cisplatin at lower concentrations (5, 10, 20 μM) of cisplatin. A slight increase in response to cisplatin was observed at 40 μM cisplatin.

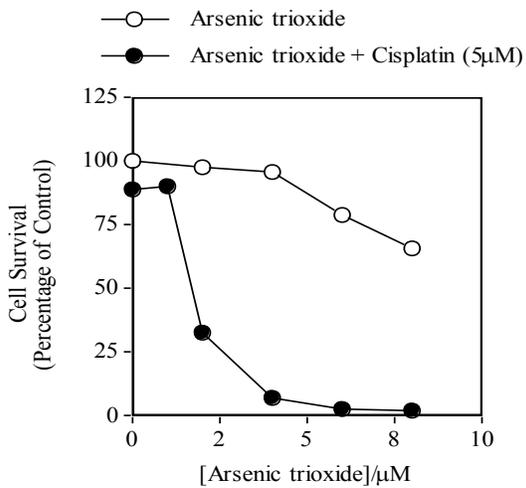


Figure 9. Effects of cisplatin (5μM) and arsenic trioxide (0-8μM) on the growth of HCT116 human colon cancer cells as measured by cell proliferation assay. The results have demonstrated that arsenic trioxide at concentrations as low as 2 μM improved the response of HCT116 cells to 5 μM cisplatin.

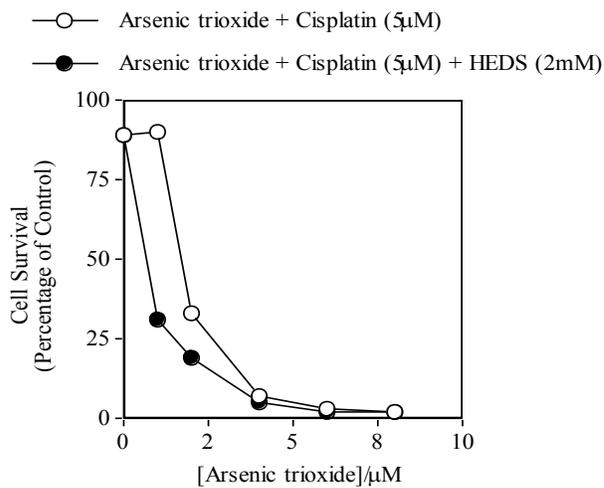


Figure 10. Effects of combined treatment of HEDS (2mM), arsenic trioxide (0-8μM) and cisplatin (5 μM) on the growth of HCT116 human colon cancer cells as measured by cell proliferation assay. The results have demonstrated that HEDS improved the response of HCT116 cells to combined treatment of arsenic trioxide and cisplatin.

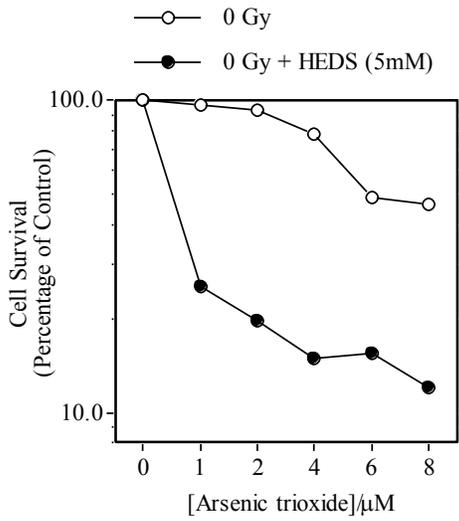


Figure 11. Effects of higher concentration of HEDS (5mM) and arsenic trioxide (0-8 μM) on the growth of HCT116 human colon cancer cells as measured by cell proliferation assay. The results have demonstrated that 5mM HEDS improved the response of HCT116 cells from 3 to 7 fold at all concentrations of arsenic trioxide.

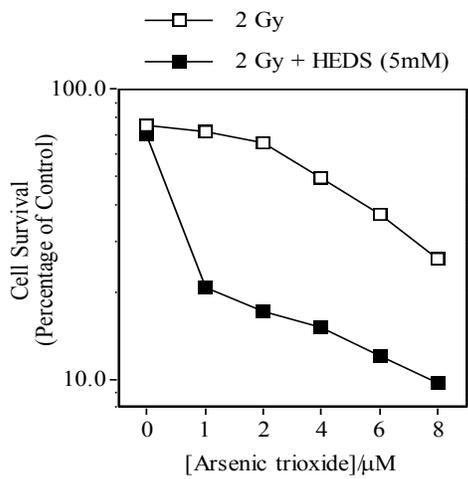


Figure 12. Effects of combined treatment of HEDS (5mM), arsenic trioxide (0-8 μM) and low dose radiation (2Gy) on the growth of HCT116 human colon cancer cells as measured by cell proliferation assay. The results have demonstrated that HEDS improved the response of HCT116 cells to combined treatment of arsenic trioxide and low dose 2 Gy radiation.

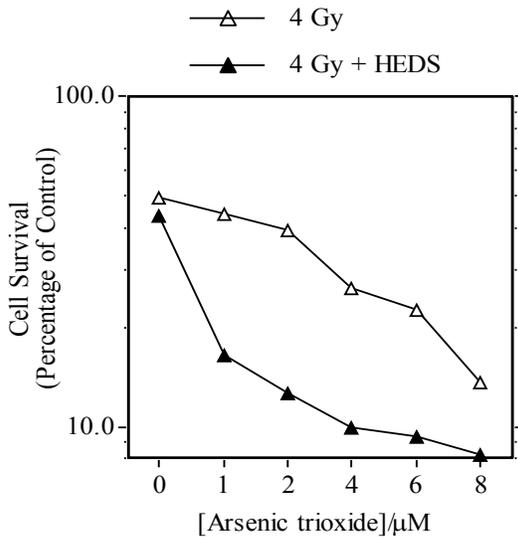


Figure 13. Effects of combined treatment of HEDS (5mM), arsenic trioxide (0-8 μM) and high dose radiation (4Gy) on the growth of HCT116 human colon cancer cells. The results have demonstrated that HEDS improved the response of HCT116 cells to combined treatment of arsenic trioxide and high dose 4 Gy radiation.

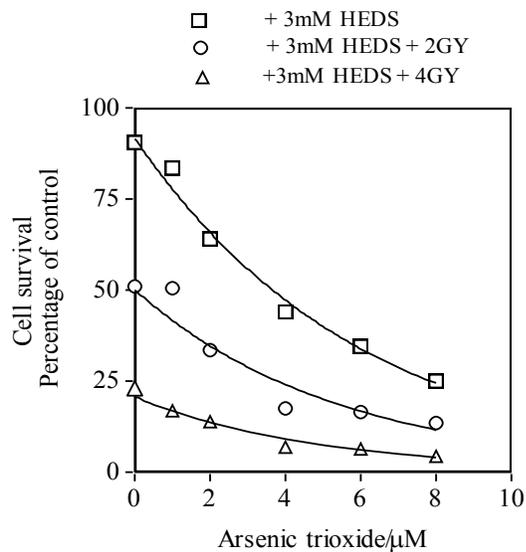
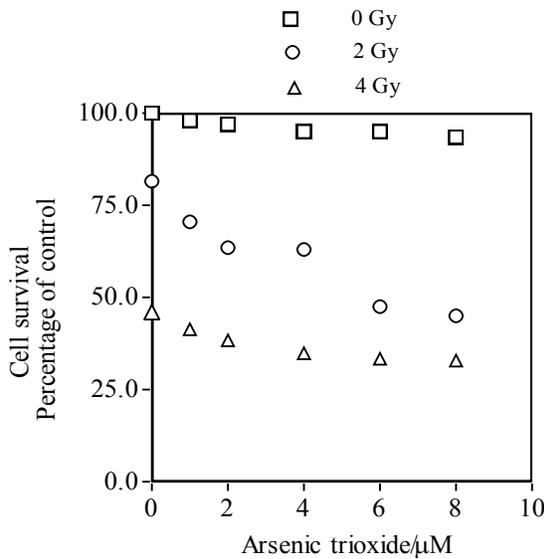


Figure 14. Effects of combined treatment of HEDS (3mM), arsenic trioxide (0-8 μM) and radiation on the growth of HT29 human colon cancer cells. The results have demonstrated that HEDS improved the response of HT29 cells to combined treatment of arsenic trioxide and radiation.

p53 mutant human colon cancer cells HT29

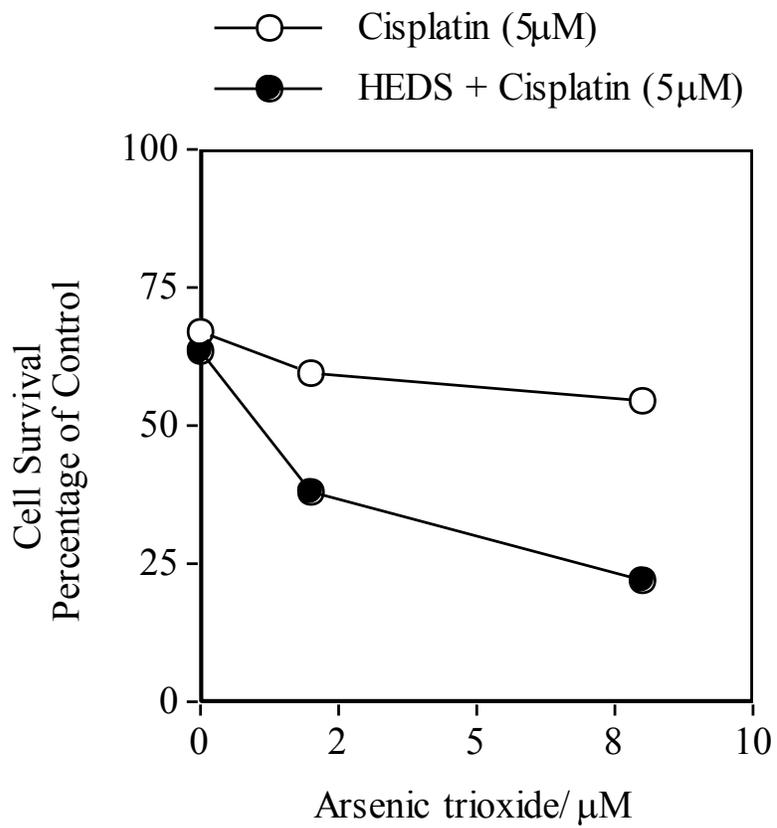


Figure 15: Effects of combined treatment of HEDS (2mM), cisplatin (5μM) and arsenic trioxide (0-8 μM) on the growth of HT29 human colon cancer cells. The results have demonstrated that HEDS improved the response of HT29 cells to combined treatment of arsenic trioxide and cisplatin.

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
 No

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

Yes
 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

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
 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, listed in the table, in a PDF version 5.0.5 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.				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published
2.				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published
3.				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published

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

Yes No

If yes, please describe your plans:

This research project produced, for the first time, that a combination of arsenic trioxide and hydroxyethyl disulfide increased the response of human colon cancer cells to radiation or cisplatin. Additionally, the results have also identified the potential mechanisms for the increased response to radiation or cisplatin. We will submit two manuscripts (one on radiation and the other on cisplatin) to peer reviewed journals.

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.

Arsenic trioxide (Trisenox) is a Food and Drug Administration (FDA) approved drug for treatment of acute promyelocytic leukemia in the US. This compound has shown significant improvement in the outcome of leukemia patient. The efficacy of arsenic trioxide as an anti cancer drug for other types of cancer may be limited due to its detoxification by intracellular glutathione. Our results have demonstrated that hydroxyethyl disulfide (HEDS), a non toxic oxidant, could be used to increase the response of human colon cancer cells to arsenic trioxide suggesting the potential application of HEDS in improving the efficacy of arsenic trioxide in cancer therapy. More importantly, HEDS and arsenic trioxide combination could also be used to improve the conventional therapies such as radiation and cisplatin.

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.*

Education

Madras University, Madras, India	B. Sc.	1978	Chemistry
Jawaharlal Nehru University, New Delhi, India	M. Sc.	1980	Life Sciences
Jawaharlal Nehru University, New Delhi, India	Ph.D.	1986	Biochemistry

Current Position

2007-Present Associate Professor, Lankenau Institute for Medical Research, Wynnewood, PA

Selected peer-reviewed publications (out of 30 publications).

1. 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 Biochemistry and Function*, 27, 93-101, 2009.
2. 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. *Journal of Cellular Biochemistry*, 103, 123-135, 2008.
3. 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-1 α stabilization under moderate hypoxia. *Journal of Biological Chemistry*, 282, 36790-36796, 2007.

4. Ayene IS, Koch CJ and Krisch RE: DNA strand breakage by bivalent metal ions and ionizing radiation. *International Journal of Radiation Biology*, 83, 195-210, 2007.
5. 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.
6. 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.
7. 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.
8. Ayene IS, Stamato TD, Mauldin SK, Biaglow JE, Tuttle SW, Jenkins SF and Koch CJ: Mutation in the glucose-6-phosphate dehydrogenase gene leads to inactivation of Ku DNA end binding during oxidative stress. *J. Biol. Chem.*, 277, 9929-9935, 2002.
9. Ayene IS, Koch CJ, Tuttle SW, Stamato T, Perez ML and Biaglow JE: Oxidation of cellular thiols by hydroxyethyl disulfide inhibits DNA double strand break rejoining in G6PD deficient mammalian cells. *Int. J. Radiat. Biol.* 76, 1523-1531, 2000.
10. 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.
11. 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.
12. 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.
13. 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.
14. Krisch RE, Ayene IS, Sullivan M, and Koch CJ: A cell-like OH scavenging environment alters the charge dependence of radiation protection by thiols. *Radiat. Res.* 148: 498- 499, 1997.
15. 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.
16. 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.
17. Ayene IS, Koch CJ, and Krisch RE: Modification of radiation-induced strand breaks by glutathione: Comparison of single and double strand breaks in SV40 DNA. *Radiat. Res.* 144: 1-8, 1995.
18. Ayene IS, Koch CJ and Krisch RE: Role of scavenger derived radicals in the induction of double strand and single strand breaks in irradiated DNA. *Radiat. Res.* 142: 133-143, 1995.
19. 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.
20. Fisher AB, Dodia C, Ayene IS and Al-Mehdi A: Ischemia/reperfusion injury to the lung. Cellular, Biochemical, and Molecular Aspects of Reperfusion Injury. *Annals of the New York Academy of Sciences*, 723: 197-207, 1994.

21. Srivastava PN, Ayene IS and Misra VS: Chemical Radioprotection: Thiols (Review). Proc. Indian Natn. Sci. Acad., B60: 197-216, 1994.

Ongoing Research Support

RO1 CA 109604 Ayene (PI)

4/1/05 – 2/28/11

National Cancer Institute

Oxidative Pentose Cycle in Hypoxic Cancer Cell Response

Principal Investigator: Iramoudi S. Ayene

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.

Completed

“Oxidative damage to DNA repair pathways”

Role: Co-Investigator

Principal Investigator: Cameron J. Koch, Ph.D., University of Pennsylvania

Agency: National Cancer Institute

Type: R01 (CA92108)

Period: 12/1/02-12/30/06

The objective is to test the effects of oxidative damage to DNA repair pathways in normoxic G6PD deficient CHO cells.

PA Department of Health

1/1/08-12/31/08

Targeting Radiation Resistant Hypoxic cells in lung tumor

Principal Investigator: Iramoudi S. Ayene

The objective is to increase the response of lung tumor xenograft to radiation by targeting radiation resistant hypoxic cells using a novel inhibitor of DNA repair pathway.